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Aims and Scope

The Journal of Physiological Sciences publishes peer-reviewed original papers, reviews, short communications, technical notes, and letters to the editor, based on the principles and theories of modern physiology and addressed to the international scientific community. All fields of physiology are covered, encompassing molecular, cellular and systems physiology. The emphasis is on human and vertebrate physiology, but comparative papers are also considered. The process of obtaining results must be ethically sound.

Fields covered:

- Adaptation and environment
- Autonomic nervous function
- Biophysics
- Cell sensors and signaling
- Central nervous system and brain sciences
- Endocrinology and metabolism
- Excitable membranes and neural cell physiology
- Exercise physiology
- Gastrointestinal and kidney physiology
- Heart and circulatory physiology
- Molecular and cellular physiology
- Muscle physiology
- Physiome/systems biology
- Respiration physiology
- Senses

Plenary Lectures

PSJ and JSPFSM co-organized Special Talk Session

Special Lectures

Plenary Lecture1

Olfaction and Stress

(March 28, Thu., 17:30-18:30, Room A)

Plenary Lecture2

Natural Products as Probes of the Pain Pathway: From Physiology to Atomic Structure

(March 29, Fri., 8:50-9:50, Room A)

PL1

Olfaction and Stress

Linda Buck (*Fred Hutchinson Cancer Research Center, USA*)

The sense of smell allows mammals to perceive a multitude of environmental chemicals as having a distinct odor. It also mediates the detection of pheromones and predator odors that elicit innate responses. We are interested in how the olfactory system detects different chemicals and how the nervous system translates those chemicals into diverse perceptions and behaviors. Using a combination of molecular, cellular, and genetic approaches, we have identified families of receptors that initially detect odorants and pheromones in peripheral sense organs, asked how those receptors encode the identities of different chemicals, and investigated how the signals they generate are routed and organized in the nervous system to yield distinct perceptions and instinctive responses. Our work also touches on other neural circuits that affect emotions and innate drives that modulate behavior. (COI: No)

PL2

Natural Products as Probes of the Pain Pathway: From Physiology to Atomic Structure

David Julius (*Department of Physiology, UC, USA*)

We are interested in determining the molecular basis of nociception with an emphasis on identifying molecules that detect noxious (pain-producing) stimuli. We are also interested in understanding how nociception is altered in response to tissue or nerve injury. Our approach has been to identify molecular targets for natural products that mimic the psychophysical effects of commonly encountered somatosensory stimuli, such as heat or cold, and to then ask how these molecules are activated or modulated by noxious stimuli or injury.

We have focused on three members of the TRP channel family (TRPV1, TRPM8, and TRPA1) that are expressed by subpopulations of primary afferent sensory neurons and which have been implicated in the detection of thermal stimuli and/or inflammatory agents. Genetic studies support the idea that the capsaicin receptor (TRPV1) and the menthol receptor (TRPM8) function as detectors of heat and cold, respectively, whereas the wasabi receptor (TRPA1) functions as a detector of environmental and endogenous chemical irritants.

From a signal transduction and therapeutics perspective, there is great interest in understanding how these channels are activated (gated) by physical and/or chemical stimuli. We have used a combination of molecular genetics, natural product biochemistry, and biophysics to address these issues and probe mechanisms of stimulus detection, channel activation, and coding logic of the somatosensory system. (COI: No)

Plenary Lecture3

Looking back on 30 years of autophagy research
-dynamic equilibrium of the cell-

(March 30, Sat., 8:50-9:50, Room A)

PL3

Looking back on 30 years of autophagy research -dynamic equilibrium of the cell-

Yoshinori Ohsumi (*Institute of Innovative Research, Tokyo Institute of Technology(IIR), Japan*)

Every cellular event is achieved through a balance between synthesis and degradation. Now it is realized that degradation process is highly regulated and is equally as important as synthesis. My studies of autophagy begun with simple light microscopy, showing that nutrient starvation induces massive delivery of cytoplasmic components to the vacuole in yeast. EM analysis revealed that the membrane dynamics underlying this process are similar to known macroautophagy in mammals. Taking advantage of the yeast, we were able to rapidly identify many autophagy-defective mutant, revealing 18 *ATG* genes essential for autophagy. These Atg proteins function in concert to form a specialized membrane structure, the autophagosome. The identification of the *ATG* genes, which are conserved from yeast to mammals, completely changed the landscape of autophagy research. Up to now a truly broad range of physiological functions of autophagy have been unveiled. Autophagy plays critical roles not only in nutrient recycling, but also intracellular clearance through the elimination of harmful proteins and organelles, and is relevant to many diseases. We are working to understand the unique membrane dynamics during autophagy by structural and functional analyses of Atg proteins, and also are trying to answer quantitatively when, what and how cytoplasmic constituents are degraded via autophagy in yeast. I will present some of our recent results and discuss their implications for autophagy research. (COI: Properly Declared)

PSJ and JSPFSM co-organized Special Talk Session

Towards the Summit with Sport Science

(March 30, Sat., 18:20-19:10, Room A)

no abstract

Special Lecture1

The Susumu Hagiwara Memorial Lecture

Beyond memory circuit: Origins of metamemory and retrospection in the primate

(March 29, Fri., 17:20-18:10, Room A)

Special Lecture2

Signaling by Mitochondrial Flashes

(March 29, Fri., 17:20-18:10, Room B)

SL1

Beyond memory circuit: Origins of metamemory and retrospection in the primate

Yasushi Miyashita (*RIKEN Center for Brain Science, Japan*)

Research field on memory mechanisms is dramatically growing. In this lecture, I will discuss recent progresses in understanding of hierarchical structures of the cognitive memory system and "metamemory" system that enables retrospection and self-evaluation of our own memory [1-2]. Our investigation started from single neuronal activities in the temporal cortex [3-5]. Then we combined whole-brain searches for neural correlates of metamemory using fMRI and subsequent examinations for causal behavioral impacts of targeted intervention in macaques: inactivation at area 9 and area 10 selectively impaired metacognitive performance on experienced and unexperienced events, respectively, without impairing recognition performance *per se*. It indicates that distinct prefrontal read-out sites supervise recognition networks, but not contribute to the recognition itself. I propose that this functional architecture provides a foundation for our retrospection.

References:
[1] Miyamoto, K., Setsuie, R., Osada, T. & Miyashita, Y. ***Neuron*** 97, 980-989, 2018. [2] Miyamoto, K., Osada, T., Setsuie, R., Takeda, M., Tamura, K., Adachi, Y. & Miyashita, Y. ***Science*** 355, 188-193, 2017. [3] Tamura, K., Takeda, M., Setsuie, R., Tsubota, T., Hirabayashi, T., Miyamoto, K. & Miyashita, Y. ***Science*** 357, 687-692, 2017. [4] Hirabayashi, T., Takeuchi, D., Tamura, K. & Miyashita, Y. ***Science*** 341, 191-195, 2013. [5] Takeuchi, D., Hirabayashi, T., Tamura, K. & Miyashita, Y. ***Science*** 331, 1443-1447, 2011. (COI: No)

SL2

Signaling by Mitochondrial Flashes

Heping (Peace) Cheng, Xianhua Wang (Institute of Molecular Medicine, Peking-Tsinghua Center for Life Sciences, *Peking University, China*)

Mitochondrial flashes (mitoflashes) represent fundamental biochemical and biophysical dynamics of the organelle, involving sudden depolarization of mitochondrial membrane potential ($D\Psi_m$), bursting production of reactive oxygen species (ROS), and accelerated extrusion of matrix protons. The mitoflash is universally present and highly conserved across all the eukaryotic cells and organisms ranging from C elegans to zebrafish and to rodents and humans, with its ancestral activity may be evolutionarily emerged in prokaryotes. Core mitochondrial signals, e,g., matrix Ca^{2+}, basal ROS, and nanodomian matrix protons, act as potent physiological mitoflash regulators, and the rate of occurrence of mitoflashes is highly regulated, varying over orders of magnitude in response to regulatory factors, altered metabolic rate and energy demand, and physiological activities such as synaptic transmission in hippocampal neurons, as well as stresses and diseases. As a signaling entity, mitoflashes are digital, local (single-mitochondrion), and stochastic, and operate in primary frequency-modulatory manner. As such, targeting mitoflash activity may provide a novel means for the control of mitochondrial metabolism and signaling in health and disease.
(COI: No)

Special Lecture3

Tentonin 3, a Mechanosensitive Channel with Baroreceptor Function

(March 29, Fri., 17:20-18:10, Room C)

Special Lecture4

Finding Instructional Balance Using the Educational Triangle

(March 29, Fri., 17:20-18:10, Room D)

SL3

Tentonin 3, a Mechanosensitive Channel with Baroreceptor Function

Uhtaek Oh (*Brain Science Institute, KIST, Korea*)

Mechanosensation is required for tactile sensation, proprioception, hearing, baroreceptor reflex, pain and other physiological functions. Mechanosensation begins with mechanotransduction channels in nerve terminals or receptor cells. With bioinformatics, we identified that TMEM150C/Tentonin 3 confers mechanosensitive currents in DRG neurons with slowly-adapting inactivation kinetics. Tentonin 3 (TTN3) is expressed highly in DRG neurons and activated by mechanical stimuli with distinctly slow inactivation kinetics. Baroreceptors present in carotid sinus and aorta are stretch receptors detecting blood pressure changes. As TTN3 is a mechanosensitive channel, therefore, it is likely that TTN3 acts as a mechanosensitive channel responsible for baroreceptor function. Indeed, TTN3 is expressed in nodose ganglion neurons that innervate in baroreceptors. Neural activity of aortic depressor nerves in response to intra-aortic is markedly reduced in TTN3-/- mice. Ambient blood pressures and heart rates of freely moving TTN3-/- mice were higher. The sensitivity of baroreceptor reflex was markedly reduced in TTN3-/- mice. We also found a clear rescue of blood pressure, heart rate, and baroreceptor reflex sensitivity to the levels of those of wild-type mice when Ttn3 is overexpressed in nodose ganglion in TTN3-/- mice. These results suggest that TTN3 is a mechanosensitive channel responsible for detecting dynamic change in arterial pressures in baroreceptors. (COI: No)

SL4

Finding Instructional Balance Using the Educational Triangle

Robert Graham Carroll (*Office of Medical Education, Brody School of Medicine, East Carolina University, USA*)

The rapid transformation of the educational setting has generated unease and confusion in both teachers and learners. One approach for managing educational change is to understand the interrelationships of three essential components of the educational process: objectives, activities, and assessments. Using the example of medical education, the instructional objectives have shifted from a focus on content and now describe "competencies" as the desired instructional outcome. This shift toward competencies expands the educational goal from teaching in individual to the development of a professional. The second component, the educational activity, is the most malleable of the three and is shaped by the adult learning theory of Malcolm Knowles. The impressive learning gains from "retrieval-based learning" demonstrated by Jeffrey Karpicke provides insights and possibly a mechanism for the superior outcomes associated with active learning approaches. Transformation of assessment now allows this third component of the triangle to better align with the activities of a profession. Practical demonstrations, such as the Entrustable Professional Activities (EPAs) of Ollie ten Cate ensure that determinations of mastery reflect real-world capabilities. Conceiving the learning environment in the form of the educational triangle assists instructors focus on things that truly impact the educational process. (COI: No)

Special Lecture5

Toward the Mysteries of Sleep

(March 30, Sat., 17:20-18:10, Room B)

Special Lecture6

The Beauty of Physiological Mechanisms in Skeletal Muscle Function and Fatigue

(March 30, Sat., 17:20-18:10, Room C)

SL5

Toward the Mysteries of Sleep

Masashi Yanagisawa (*International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*)

Despite the fact that the executive neurocircuitry and neurochemistry for sleep/wake switching has been increasingly revealed in recent years, the mechanism for homeostatic regulation of sleep, as well as the neural substrate for "sleepiness" (sleep need), remains unknown. To crack open this black box, we have initiated a large-scale forward genetic screen of sleep/wake phenotype in mice based on true somnographic (EEG/EMG) measurements. We have so far screened >8,000 heterozygous ENU-mutagenized founders and established a number of pedigrees exhibiting heritable and specific sleep/wake abnormalities. By combining linkage analysis and the next-generation whole exome sequencing, we have molecularly identified and verified the causal mutation in several of these pedigrees (Funato et al. Nature 539: 378-383, 2016). Biochemical and neurophysiological analyses of these mutations are underway. Since these dominant mutations cause strong phenotypic traits, we expect that the mutated genes will provide new insights into the elusive pathway regulating sleep/wakefulness. Indeed, through a systematic cross-comparison of the Sleepy mutants and sleep-deprived mice, we have recently found that the cumulative phosphorylation state of a specific set of mostly synaptic proteins may be the molecular substrate of sleep need (Wang et al. Nature, 558: 435–439, 2018). (COI: No)

SL6

The Beauty of Physiological Mechanisms in Skeletal Muscle Function and Fatigue

Graham Douglas Lamb (*Department of Physiology, La Trobe University, Australia*)

The almost sole task of skeletal muscle is to contract upon demand, accurately and repeatedly, enabling fine or powerful movements as required. Muscle contraction is initiated by action potentials spreading out from the neuromuscular junction along the muscle fibre and then into the fine transverse-tubular system, where it triggers release of calcium from internal stores, the sarcoplasmic reticulum, and this calcium in turn activates the contractile proteins to contract. Optimum performance of the system is achieved by a whole range of exquisite physiological mechanisms that ensure the reliable spread of the excitation and accurate control of calcium release despite the severe constraints set by the physical structure of the muscle fibre and the large ionic and metabolic changes occurring with repeated highly energetic contractions. Even the inevitable decline of muscle performance with repeated activity, which we call 'muscle fatigue', is a highly controlled event closely governed by further specific physiological mechanisms, all acting to ensure the best functional outcome. (COI: No)

Special Lecture7

The importance of understanding fetal physiology for detecting brain injury before birth

(March 30, Sat., 17:20-18:10, Room F)

Special Lecture8

The Sunao Tawara Memorial Lecture

Mitochondria in fetal programming of metabolic syndrome-associated end organ dysfunctions in adults

(March 31, Sun., 9:40-10:30, Room A)

SL7

The importance of understanding fetal physiology for detecting brain injury before birth

Laura Bennet (*Department of Physiology, The University of Auckland, Australia*)

Hypoxic-ischaemic encephalopathy at birth affects around 1-3/1000 live births at term and upwards of 120/1000 live preterm births. Preterm infants in particular are at significant risk for impaired neurodevelopment, which is underpinned by both brain injury and altered development of the neural network. While very preterm babies are most at risk, even late or near-term babies have an increased risk for cognitive and behavioural problems. Importantly, it is increasingly recognised that many adverse events that cause brain injury or impaired brain development, such as hypoxia, can occur well before birth. Clinical evidence suggests, for example, that many cases of cerebral palsy, in children born term and preterm, have their origins in fetal life. Detecting these at risk babies, even before birth, is critical for early implementation of neuroprotection and neurorepair treatments. This talk will present research on how perinatal brain injury evolves after adverse events such as hypoxia and inflammation, and how knowing the timing of an insult is important for effective treatment strategies. The presentation will explore how the fetus adapts to injurious insults and how fetal adaptation can be altered by combinations of insults and clinical therapies. Potential perinatal neuroprotection and repair strategies will be discussed as well as methods for detecting evolving brain injury before birth, such as fetal heart rate, fetal behaviour and fetal circadian rhythms. (COI: Properly Declared)

SL8

Mitochondria in fetal programming of metabolic syndrome-associated end organ dysfunctions in adults

Julie Yh Chan[1]; Yung-Mei Chao[1]; You-Lin Tain[2] ([1]*Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital, Taiwan;* [2]*Department of Pediatric Nephrology, Kaohsing Chang Gung Memorial Hospital, Taiwan*)

Compelling evidence suggests that adult metabolic syndrome (MetS) may originate from a suboptimal intrauterine environment that affects fetal and infant development and alters the risk profile for disease later in life. This process is referred to as fetal programming of adult disease. Our laboratory has demonstrated that cardiac and kidney dysfunctions are associated with MetS in adult offspring to maternal caloric restriction, high fructose or fat diet, and diabetes. The pathologic mechanisms for the fetal programming of MetS-associated end organ dysfunction are multifactorial but altered mitochondrial integrity and function is the key culprit for structural changes and/or functional deficiency in adult offspring. In particular, exposure to maternal high fructose during fetal life profoundly affects mitochondrial nutrient sensing signaling, decreases mitochondrial biogenesis and bioenergetics, and influences mitochondrial dynamics, leading to augmented generation of reactive oxygen species, production of proinflammatory cytokines and promotion of epigenetic changes. Conversely, interventions that protect mitochondrial integrity or functions alleviate the biological changes, tissue damage and functional alterations in offsprings subjected to maternal malnutrition. Together our data indicate that mitochondria play a pivotal role in priming the heart and kidney to oxidative stress that might enhance the risk for MetS-associated cardiovascular disease in adult offsprings. (COI: No)

Special Lecture9

Modeling Human Neurological/Psychiatric Disorders using iPS cells and Transgenic Non-Human Primates

(March 31, Sun., 9:40-10:30, Room B)

SL9

Modeling Human Neurological/Psychiatric Disorders using iPS cells and Transgenic Non-Human Primates

Hideyuki Okano (*Department of Physiology, Keio University School of Medicine, Japan*)

In order to overcome these difficulties for the investigation of human psychiatric/psychiatric disorders, we are taking advantage of iPS cell technologies and transgenic non-human primates for modeling human psychiatric/psychiatric disorders. So far, we have established iPS cells from the patients of about 40 human psychiatric/psychiatric disorders and characterized their pathophysiology. Using iPSC-technology, we have established a large number of in vitro cellular models of sporadic ALS (ALS). These models showed phenotypic differences in their pattern of neuronal degeneration, types of abnormal protein aggregates, cell death mechanisms, and onset and progression of these phenotypes in vitro among cases. We further evaluated multiple-phenotype rescue of these subclassified SALS models using agents selected from non-SOD1 FALS models, and identified ropinirole as a potential therapeutic candidate. Furthermore, for faithfully modeling the human psychiatric/psychiatric disorders *in vivo*, we developed transgenic non-human primates (common marmosets) with germline transmission. In the present talk, we also wish to mention our recent data of generation of common marmoset transgenic models of Parkinson disease. Furthermore, we recently generated knock-out technologies of common marmoset using genome editing technologies for the generation of transgenic marmoset model of autism and psychiatric disorders. (COI: Properly Declared)

Symposia

Local Organizing Committee Symposium

Symposium1

Molecular mechanisms of aging

(Co-organized by the Japanese Society of Anti-Aging Medicine)

(March 29, Fri., 10:00-12:00, Room A)

S1-1

The FGF-Klotho endocrine system and aging

Makoto Kuro-o (*Division of Anti-aging Medicine, Center for Molecular Medicine, Jichi Medical University, Japan*)

Phosphate homeostasis in mammals is maintained by the FGF23-Klotho endocrine axis. In response to phosphate intake, a peptide hormone FGF23 is secreted from the bone and binds to its receptor Klotho expressed in the kidney to increase urinary phosphate excretion, thereby maintaining the phosphate balance. Mice lacking FGF23 or Klotho suffer from hyperphosphatemia due to impaired phosphate excretion, and unexpectedly, from complex phenotypes resembling human aging. Restoration of the phosphate balance by dietary phosphate restriction rescued them from the aging-like phenotypes, suggesting that phosphate is regarded as a factor that accelerates aging. Because the blood is super-saturated solution in terms of phosphate and calcium ions, increase in the serum phosphate level can trigger precipitation of calcium phosphate. Calcium phosphate binds to serum protein fetuin-A and form colloidal nanoparticles called calciprotein particles (CPP). CPP has the activity that induces cell death in vascular endothelial cells, calcification in vascular smooth muscle cells, and innate immune responses in macrophages. CPP levels in the blood are increased in mice lacking Klotho and in patients with chronic kidney disease (CKD), both suffer from accelerated aging. We propose that CPP is a pathogen of aging and thus a potential therapeutic target for age-associated disorders including arteriosclerosis, chronic non-infectious inflammation, and CKD. (COI: Properly Declared)

S1-2

The roles and mechanisms of SASP in aging and cancer

Eiji Hara[1,2] ([1]*Research Institute for Microbial Diseases, Osaka University, Japan;* [2]*Immunology Frontier Research Center, Osaka University, Japan*)

Over the last few decades, it has become apparent that oncogenic proliferative signals are coupled to a variety of growth inhibitory responses, such as the induction of apoptotic cell death or irreversible cell cycle arrest known as "cellular senescence". Thus, both apoptosis and cellular senescence are thought to act as important tumor suppression mechanisms. Unlike apoptotic cells, however, senescent cells remain viable for long periods of time and accumulate with increasing age in various organs and tissues *in vivo*. Moreover, recent studies reveal that although cellular senescence initially functions as a tumor suppressive process through implementation of stable cell cycle arrest, it may eventually promote chronic inflammation through secretion of various pro-inflammatory factors called "senescence associated secretory phenotype (SASP)". It is therefore quite possible that accumulation of senescent cells during the aging process *in vivo* may contribute to age-associated increases of various inflammatory disorders, such as cancer. Here, I introduce our recent work on molecular and cellular biology of cellular senescence, focusing on its positive and negative roles in controlling cancer development. We believe that a better understanding of the molecular mechanisms involved will lead to new strategies for the prevention of aging-associated cancer. (COI: No)

S1-3

Necroptosis promotes the Aging of the Male Reproductive System in Mice and Man

Xiaodong Wang; Dianrong Li; Lingjun Meng; Tao Xu; Yaning Su; Xiao Liu; Zhiyuan Zhang (*National Institute of Biological Sciences, China*)

Necroptosis is a form of regulated necrotic cell death in mammals that is executed by a pseudokinase named mixed lineage kinase-domain like protein MLKL. Upon necroptosis induction mediated by the tumor necrosis factor family of cytokines, a pair of kinases, RIP1 and RIP3, become activated and RIP3 kinase then phosphorylates MLKL at specific sites within its pseudokinase domain to cause MLKL activation and cell death. Recently, our laboratory discovered that male reproductive organs of necroptosis defective mice retain "youthful" morphology and function into advanced age, while those of age-matched wild type mice morphologically and functionally deteriorate. The RIP3 phosphorylation of MLKL, the activation marker of necroptosis, is detected in spermatogonial stem cells and Sertoli cells in the seminiferous tubules in testis of old but not in young wild type mice. Similar observation was also made in old but not in young men. Moreover, feeding of wild type mice with an RIP1 inhibitor prior to the normal onset of age-related changes in their reproductive organs blocked the appearance of signs of aging. However, the offspring of these old male mice showed a variety of birth defects, possibly due to damages still happened in their DNA. We therefore propose that testis aging is controlled by a necroptosis-mediated program that effectively eliminates damaged DNA accumulated during the animal aging process from the reproductive pool of animal species. (COI: No)

S1-4

Significance of NAD/Sirtuins in Non-Communicable Diseases (NCD) and Metabo-Aging

Hiroshi Itoh (*Department of Endocrinology, Metabolism and Nephrology School of Medicine, Keio University, Japan*)

I proposed the clinical concept of "Metabolic domino", which illustrates the whole picture of cause, pathophysiology and complication of metabolic syndrome. Cancers are known to frequently occur in Metabolic domino, and these diseases are collectively called, "Non-Communicable Diseases (NCD). NAD-dependent deacetylases, Sirtuins are responsible for life span expansion, which indicates that energy metabolism and epigenetic gene regulation are significant for longevity ("metabo-aging"). The tissue concentration of NAD and/or its precursor, nicotinamide mononucleotide (NMN) is crucial for Sirtuin activation. We have reported that in diabetic nephropathy, the decrease of Sirt 1 in the proximal renal tubules by high blood glucose is the first event, which triggers the decrease of Sirt1 in podocyte via the decreased delivery of NMN from the tubules to podocytes. The decrease of Sirt1 in podocytes induces up-regulation of Claudin 1 epigenetically and causes albuminuria (Nat Med 2013). Down-regulation of Sirt1 expression by high glucose is mediated by the increased uptake of glucose via SGLT (sodium-glucose co-transporter) 2 and stimulates renal gluconeogenesis (Sci Rep 2018). We also observed that renal proximal tubules-specific iNampt knock-out mice exhibit severe renal fibrosis. NAD/Sirtuins is, thus, the appropriate target for the "pre-emptive medicine" of NCD. The clinical study to investigate the effect of orally-administered NMN has started in Keio University. (COI: Properly Declared)

Local Organizing Committee Symposium

Symposium2

Thermal biology: A new world of life science (whole day symposium) part I

(Co-organized by Grant-in-Aid for Scientific Research on Innovative Areas 'Thermal Biology' of MEXT, Japan)

(March 29, Fri., 10:00-12:00, Room B)

S2-1

Physiological Significance of Thermosensitive TRP Channels

Makoto Tominaga[1,2] ([1]*Division of Cell Signaling, National Institute for Physiological Sciences, Japan;* [2]*Thermal Biology Group, Exploratory Research Center on Life and Living Systems, Japan*)

TRP (transient receptor potential) channels are non-selective cation channels having relatively high Ca^{2+}permeability, and comprise six related protein families (TRPC, TRPV, TRPM, TRPA, TRPML, TRPP) in mammals. One subunit of the TRP channel is composed of six transmembrane domains and a pore region with both amino and carboxyl termini on the cytosolic side. Among the huge TRP super family of ion channels, some have been proven to be involved in thermosensation detecting ambient temperatures from cold to hot. There are now eleven thermosensitive TRP channels (TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM3, TRPM4, TRPM5, TRPM8, TRPA1 and TRPC5) with distinct temperature thresholds for their activation. Structures of TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM4, TRPM8 and TRPA1 have been clarified in the last several years mainly with single particle analysis with cryoEM. We found that some thermosensitive TRP channels make a complex with Ca^{2+}-activated chloride channel, anoctamin1 (ANO1). Interaction between TRPV4 and ANO1 is involved in water efflux in choroid plexus, salivary gland and lacrimal grand epithelial cells. And TRPV1 (TRPA1)/ANO1 interaction was found to be involved in the enhancement of nociceptive signals through further depolarization upon chloride efflux in peripheral sensory neurons. This interaction could be one of the reasons why TRP channels have high Ca^{2+}permeability. (COI: No)

S2-2

Imaging intracellular temperature unveils thermal signaling in single cells

Kohki Okabe[1,2] ([1]*Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan;* [2]*PRESTO, JST, Japan*)

Temperature, a key regulator of biochemical reactions, influences many physiological functions of organisms. Recent progress in intracellular thermometry shows temporal and spatial variation associated with cellular functions, shedding light on an intriguing hypothesis: temperature change inside of a cell is essentially involved in cell functions. Considering this, we have been investigating how intracellular temperature mediates cell functions, which we call intracellular thermal signaling. We first developed a novel method to visualize intracellular temperature distribution using a fluorescent polymeric thermometer. The images of intracellular temperature distribution of COS7 cells indicated an interesting temperature gradient observed between the nucleus and the cytoplasm at the steady-state and the local temperature change provoked by endogenous heat production from mitochondria. Furthermore, the introduction of a simple and artificial heat source using infra-red (IR) laser irradiation allowed transient and quantitative heating in single living cells. By the manipulation of local temperature inside living cells, we have also revealed a unique cellular response including acute translation reprogramming through RNA granule formation, revealing the existence of this intracellular thermal signaling. These results propose a novel principle of intracellular signal transduction, which will be of great significance in thermal biology. (COI: No)

S2-3

Transient intracellular acidification regulates the core transcriptional heat shock response

David Allan Drummond[1]; Catherine G Triandafillou[2]; Christopher D Katanski[1]; Aaron R Dinner[3] ([1]*Department of Biochemistry and Molecular Biology, The University of Chicago, USA;* [2]*Graduate Program in Biophysical Sciences, The University of Chicago, USA;* [3]*Department of Chemistry and the James Franck Institute, The University of Chicago, USA*)

Cellular stress induces rapid expression of genes encoding molecular chaperones. Stress also triggers transient intracellular acidification which, by unknown mechanisms, is broadly associated with increased stress resistance in eukaryotes. Here, using budding yeast as a model, we discover that preventing cells from transiently acidifying during heat shock compromises induction of molecular chaperones and fitness. Both acidification and subsequent restoration of intracellular pH are required for robust chaperone induction, with pH recovery and chaperone production predicting resumption of division in single cells. Failure to acidify specifically suppresses genes regulated by the conserved heat shock transcription factor Hsf1, which is repressed by chaperones under non-stress conditions. The failure of heat to induce the heat shock response without concomitant acidification implicates pH-sensitive stress-sensing proteins in recruiting chaperone repressors to activate Hsf1 under physiological conditions. Our findings reveal a central, causal role for intracellular pH in the eukaryotic transcriptional stress response. (COI: No)

International Scientific Program Committee Symposium

Symposium3

Gastrointestinal microbiome and immunophysiology (CPS, Taiwan)

(March 29, Fri., 10:00-12:00, Room C)

S3-1

Metabologenomic approach reveals the function of gut microbiota in health and disease

Shinji Fukuda[1,2,3,4,5] (*[1]Institute for Advanced Biosciences, Keio University, Japan; [2]PRESTO, Japan Science and Technology Agency (JST); [3]Kanagawa Institute of Industrial Science and Technology (KISTEC-KAST); [4]Transborder Medical Research Center, University of Tsukuba; [5]Metabologenomics, Inc., Japan*)

The gut microbiota form a highly complex ecological community together with host intestinal cells. The so-called gut ecosystem has a profound influence on human physiology, immunology, and nutrition. It has been reported that imbalance in the structure of gut ecosystem could be a risk factor in human disorders including not merely gut-associated diseases but also systemic disorders. However, the molecular mechanisms of the host-microbial crosstalk remain obscure. To this end, we firstly established a highly integrated metagenomic and metabolomic approaches, metabologenomics, and found that acetate produced from carbohydrate metabolism by probiotic bifidobacteria largely contributes to the protection of mice from enterohaemorrhagic *E. coli* O157:H7 lethal infection through enhancement of gut epithelial barrier function. In addition, we showed that butyrate produced from dietary fiber metabolism by microbial order Clostridiales progresses the induction of regulatory T cell differentiation from naïve T cells through epigenetic modification, which suppress colonic inflammation. Moreover, metabologenomic approach revealed that succinate produced from neonate gut microbiota mediates Clostridiales colonization followed by protection against enteropathogenic infection. Taken together, gut microbiota-derived metabolites are considered to be crucial factors to shape host physiological homeostasis. (COI: Properly Declared)

S3-2

Pathophysiology of the gut microbiota in digestive diseases

Sunny Hei Wong[1,2,3] (*[1]Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, China; [2]Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong; [3]Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong*)

Micro-organisms have contributed to the etiology of many diseases, including infectious diseases but also metabolic, inflammatory and neoplastic diseases. Some of these diseases occur in the digestive tract, such as inflammatory bowel diseases, colorectal cancer and gastric cancer. With the advent of sequencing technology and bioinformatics, we now understand our microbiome in an unprecedented resolution; whereas gnotobiotic and transgenic animal studies allow us to dissect the functional role of these microbes. These work have shown a distinct pattern of microbial composition in the gut of diseased patients, and evaluated putative candidate bacteria involved in the pathogenesis. In this talk, I will share on the importance of gut microbiome in these digestive diseases, as well as recent insight into the pathogenic mechanisms from the gut microbiome. I would also discuss potential clinical implications of these recent findings. (COI: No)

S3-3

Microbiota dysbiosis and immune abnormality in colorectal carcinogenesis

Linda Chia-Hui Yu (*National Taiwan University College of Medicine, Taiwan*)

An ecosystem of microorganisms habituates the human intestinal tract which is defined as the gut microbiota, composed of bacteria, archaea, virus, and fungi. The gut microbiota is involved in the maintenance of intestinal homeostasis, and abnormality in the microbial composition (termed dysbiosis) played a crucial role in colorectal tumorigenesis. A two-hit theory that involves aberrant host immune signals recognizing the dysbiotic bacterial contents has been proposed in carcinogenesis. I will discuss the specific roles from the host and bacterial side that lead to colon tumor formation. From the host's side, intestinal epithelial CD14/TLR4-mediated innate signaling after recognizing bacterial lipopolysaccharide was responsible for antagonistic regulation of cell death and proliferation, two of the major hallmarks of cancers. From the microbial side, tumorigenic bacteria with transmissible properties, mucosal-association, and Gram-negative characteristics were identified in experimental models of colitis-associated colon cancer models. The characterization of virulence factors related to epithelial genotoxicity and signaling had provided mechanistic insights into host-microbe interaction for tumor progression. Our understanding of the two-hit mechanistic interplay between host and bacteria will shed light to the development of novel strategies for management of colon cancers. (COI: No)

S3-4

Microbiota biofilm dysbiosis and pathobiont release induced by enteropathogens or in IBD

Andre G. Buret (*Biological Sciences, Inflammation Research Network, Canada*)

Abnormalities in the commensal gut microbiome (dysbiosis) contribute to the pathogenesis of Inflammatory Bowel Disease (IBD) and a variety of other disorders. Functional abnormalities of the dysbiotic microbiota and the formation of pathobionts remain incompletely understood. Gut microbiota live on the intestinal mucus as poly-microbial communities called "biofilms". Beyond abnormalities in their taxonomic representations, a better understanding of how disruptions in these commensal mucosal biofilm communities are regulated will pave the way towards new therapies.

Dietary iron supplementation leads to disease exacerbation and a higher risk of infection in IBD, via unclear mechanisms. Iron uptake is known to facilitate the expression of virulence in bacteria. AIM: to characterize iron-dependent mechanisms that promote pathobiont release from dysbiotic microbiota biofilms induced by enteropathogens, or in IBD, and to test whether a hydrogen sulfide (H_2S)-releasing compound (ATB-429) could selectively correct this microbiota dysbiosis. These findings highlight a new pathogenic mechanism of pathobiont dispersion from microbiota biofilms. Results also demonstrate that ATB-429 inhibits the development of pathobionts from microbiota biofilms through iron chelation, restoring microbiome biofilm and mucus homeostasis, and reducing inflammation, all critical hallmarks of IBD. (COI: No)

Local Organizing Committee Symposium:

Symposium4

Teaching physiology; International perspectives (whole day symposium) part I

(March 29, Fri., 10:00-12:00, Room D)

S4-1

Role that the 'step-by-step study of life sciences' may play in health-related higher education

Masato Shibuya[1,3]; Kaname Higuchi[1,3]; Toshikazu Yamashita[2,3]
([1]*Dept Physiol, Kagawa Nutrition Jr Col, Tokyo Japan, Japan;* [2]*Dept Applied Physiol, Kagawa Nutrition Univ, Saitama Japan;* [3]*Life Science Education Sharing Group, Japan*)

An innovative educational material on human life science was designed, including 3 features: 1) The entire material is presented in very small steps. For example, typical introductory figure on the circulatory system is divided into 20 steps and figures. 2) Straight-forward illustrations and animations are presented making it easier to understand the mechanism of various functions. This was done in part by using different types of arrows with different meanings such as movement, chemical changes, increase and decrease. 3) Simple multi-choice questions are presented allowing learners to self-test their understanding. The materials were installed onto Moodle, making it possible to give online tests using randomized questions and to record/monitor progress. The system and materials were used to enforce self-study and group studying. An anonymous survey showed that the material, the opportunity for self-study, and the class settings were effective and favored by the students. (COI: No)

S4-2

Team-based Learning - the backbone of medical education in LKCMedicine

Fabian C.I. Lim (*Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore*)

Lee Kong Chian School of Medicine (LKCMedicine) is a joint medical school by Nanyang Technological University, Singapore, and Imperial College London.

One unique feature of the curriculum in LKCMedicine is the application of "Team-based Learning" (TBL) as our core pedagogy in the delivery of the curriculum. All lectures in the 5-year programme are delivered using the TBL format, which focuses on self-directed learning and research and the collective knowledge of the team, and class, to arrive at a solution. Such a pedagogy exposes the students to effective problem solving in the working environment i.e., strong foundation in biomedical science, research, team debates and discussions, and the delivery of solutions.

The students download and listen to online lecture materials before attending the TBL session. Each TBL sessions goes from 0830h - 1730h and begins with an individual assessment, followed by team-based assessments using the same set of MCQ questions. The students then put up "burning questions" to seek clarifications on lecture materials or assessment questions /answers, which are opened to the class to answer. The facilitators, comprising clinicians and scientists, will guide the discussions or supplement the information to address these questions in depth. The second half of the day is spend on application exercise conducted at the team-level. All assessments and application exercises are graded as part of the final scores in the semester. (COI: No)

S4-3

The role of Indonesian Physiology Society to improve physiology teaching in Indonesia

Adrianta Surjadhana (*Department of Physiology,Ciputra University, Indonesia*)

The Indonesian Physiology Society (IPS) was built in 1964 at Yogyakarta and is a national professional organization for physiologists. The mission of IPS is to improve physiology education besides research and social services.Our members are medical doctors from the 84 medical schools in Indonesia ,besides that there are members working at the veterinary,dental ,pharmacy and other health sciences .Since Indonesia is a wide spread country with many islands ; which is a geographic problem, also the newer medical schools have few lecturers and less teaching experience.A faculty development program from the IPS to improve the quality of teaching physiology is a must.Activities that help these programs, include to have national IPS meeting for teaching physiology, making national learning objectives for teaching physiology, workshops on teaching, dry lab and computer simulations to replace the wet lab experiments, sending staff to abroad to get fellowships for teaching physiology, student physiology Olympiad, translating English physiology textbooks to Indonesian language.With the use of new technology we have What's App groups for teaching physiology where we could discuss important issues that the students face, difficult concept and also Zoom teleconferences among our members.The IPS is proud to and thankful for the dedicated members who helped the IPS tasks to improve physiology education in Indonesia.

Keywords : Indonesian Physiology Society, faculty development (COI: No)

S4-4

Ethical Teaching: A Dilemma in Medical Education

Arif Siddiqui[1]; Kusal Kanti Das[2] ([1]*Barrett Hodgson University, Pakistan;* [2]*BLDE University, India*)

The ethical underpinnings of professional sessions like meeting, training and workshops cover faculty activities like research ethics, publication ethics and clinical ethics but it hardly discusses issues of Ethical Teaching. An equivalent emphasis on incorporating sound ethical approaches in teaching has yet to be established. Academic sessions specific to teaching physiology also generally focus on the mechanics and philosophy of teaching, but hardly ever touch the issues of Ethical Teaching.

Teachers are the greatest assets of any education system. They stand in the interface of transmission of knowledge, skills and values. It is not that easy to become an 'ethical teacher' as there are very few guidelines or rules that are available which could be followed as principles on ethical teaching. Certain issues pertaining to ethical teaching are based on academic honesty between teachers and student may destroy the fabric of sanctity of medical profession whereas other based on laws, such as sexual harassment and discrimination. Beyond these, there are many issues that medical faculty members may face, as these are quite different by nature as compared to clinical ethics or research ethics. The session is intended to be a resource for educators to help discuss, recognize and analyze situations that could result in public and professional harm. (COI: No)

Symposium5

New Translational Insights on Cardiopulmonary Remodeling

(March 29, Fri., 10:00-12:00, Room E)

S5-1

Calcium-sensing receptor and PDGF signals on vascular remodeling in pulmonary hypertension

Aya Yamamura; Motohiko Sato (*Department of Physiology, Aichi Medical University, Japan*)

Aim: Pulmonary arterial hypertension (PAH) is a progressive and fatal disease of the pulmonary artery. We previously reported that the calcium-sensing receptor (CaSR) is upregulated in pulmonary arterial smooth muscle cells (PASMCs) from idiopathic pulmonary arterial hypertension (IPAH) patients. However, the mechanisms have not yet been elucidated. In the present study, we demonstrate that platelet-derived growth factor (PDGF) promotes CaSR expression and thereafter facilitates vascular remodeling in PASMCs.
Results: The expression of PDGF receptors was stronger in IPAH-PASMCs than in normal-PASMCs. The phosphorylation of PDGF receptors by PDGF was longer lasting in IPAH-PASMCs. The phosphorylation levels of the downstream pathways were also higher in IPAH-PASMCs. The PDGF-induced CaSR upregulation was attenuated by the siRNA knockdown of PDGF receptors, STAT1/3, or imatinib. In monocrotaline-induced pulmonary hypertensive rats, imatinib reduced the CaSR upregulation, thereby improving the pathological state of pulmonary hypertension. The combination of NPS2143 and imatinib acted additively to inhibit the development of pulmonary hypertension.
Conclusion: The present results suggest that enhanced PDGF signaling is involved in the CaSR upregulation, leading to vascular remodeling due to excessive proliferation of IPAH-PASMCs. The crosslink between CaSR and PDGF signals is a novel pathophysiological mechanism contributing to the development of PAH. (COI: No)

S5-2

Relationship between Physical Stimulus and Cardiac Remodeling

Masanari Umemura; Masatoshi Narikawa; Ryo Tanaka; Yoshihiro Ishikawa (*Cardiovascular Research Institute, Yokohama City University Graduate School of Medicine, Japan*)

Mechanical stimulus and humoral factors may contribute to not only enlargement of cardiac myocytes, but also to activation and proliferation of cardiac fibroblasts, and differentiation of cardiac fibroblasts into myofibroblasts, which synthesize larger amounts of extracellular matrix (ECM), resulting in cardiac hypertrophy, excessive accumulation of ECM, and cardiac fibrosis. However, the function and cellular signaling pathway of physical stimulus in cardiac fibroblast remain elusive. We evaluated the effect of hyperthermia and compressive force, *i.e.* hydrostatic pressure (HP) in human cardiac fibroblast (HCFs). Hyperthermia (42 °C) inhibited the TGF-b-induced IL-6 production and α-SMA expression. Moreover, hyperthermia treatment prevented cardiac fibrosis in Ang II infusion mice model. Our results showed that hyperthermia directly inhibited the TGF-b-induced differentiation from fibroblast to the myofibroblast phenotype in HCFs and cardiac fibrosis in mice model. In contrast, we also evaluated the effects of HP using a pressure-loading apparatus in HCFs. High HP (200 mmHg) resulted in phosphorylation of Akt. HP then greatly inhibited glycogen synthase kinase 3 (GSK-3). HP inhibited collagen matrix production in a three-dimensional HCF culture. Our finding suggested that HP under a certain condition suppressed cardiac fibrosis via Akt/GSK-3 signaling in HCFs. We concluded that regulation of physical stimulus may be applicable to the prevention of cardiac fibrosis. (COI: Properly Declared)

S5-3

The Neuro-Mechanical unloading limits the infarct size and prevents subsequent heart failure

Keita Saku (*Department of Advanced Risk Stratification for Cardiovascular Disease, Center for Disruptive Cardiovascular Medicine, Kyushu University, Japan*)

Acute myocardial infarction (AMI) is the major cause of heart failure. It is well known that reducing the infarct size in AMI leads to attenuate the occurrence of subsequent heart failure. Left ventricular (LV) assist device (LVAD) mechanically unloads the heart, thereby decreases myocardial oxygen consumption. Vagal nerve stimulation (VNS) restores normal autonomic balance and exerts powerful anti-infarct effects. We examined whether the combination of LVAD and VNS in AMI limits the infarct size and prevents heart failure in the long term. We allocated 24 dogs into 4 groups, IR (no treatment group, n=7), LVAD (n=6), VNS (n=4) and LVAD+VNS (n=5). We occluded left anterior descending coronary artery for 180 minutes then reperfused. Each treatment was initiated from 90 minutes after the onset of ischemia and terminated to 60 minutes after reperfusion. LVAD+VNS strikingly reduced the infarct size by more than 70% (IR: 13.3±2.5, LVAD: 8.7±2.9, VNS: 5.8±4.7, LVAD+VNS: 2.9±1.3 %, $p<0.05$). In addition, LVAD+VNS fully normalized LV end-systolic elastance and LV end-diastolic pressure. In conclusions, the combination of LVAD and VNS, neuro-mechanical unloading, in AMI synergistically reduces the infarct size and prevents heart failure in the long term. In this session, we will also introduce our approach to translate this therapeutic strategy into clinical by the catheter LAVD (Impella®) and intravenous VNS technic (iVNS). (COI: Properly Declared)

S5-4

Long noncoding RNAs: emerging players in cardiac electrical and structural remodeling

Yong Zhang; Ying Zhang; Lei Jiao; Lina Xuan; Xin Liu; Baofeng Yang (*Department of Pharmacology, Harbin Medical University, China*)

Cardiac remodeling results in poor prognosis because of its association with ventricular dysfunction and malignant arrhythmias. We firstly performed the microarray profiling and bioinformatics analyses of long non-coding RNAs (lncRNAs) in a mouse model of heart failure (HF) characterized by cardiac remodeling. Then we measured the levels of selected lncRNAs with high profiles linking to cardiac dysfunction in clinical samples. We found that circulating level of ZFAS1 was markedly lower in AMI than in non-AMI subjects. While CCRR is decreased in patients with HF. Then we identified ZFAS1 as a inhibitor of SERCA2a by binding to SERCA2a protein. Abnormally increased ZFAS1 in MI impaired cardiac function. Most prominently, we identified a conserved functional domain of ZFAS1, which is much shorter and responsible for its deleterious effects. Moreover, we found CCRR silencing induced arrhythmias by destruction of intercalated discs and gap junctions to slow longitudinal cardiac conduction in healthy mice. CCRR overexpression improves cardiac conduction by blocking endocytic trafficking of connexin43 (Cx43) to prevent its degradation via binding to Cx43-interacting protein CIP85. We identified the functional domain of CCRR, which can reproduce the functional roles of full-length CCRR. Our studies suggest ZFAS1 and CCRR play pivotal roles in cardiac remodeling, provides therapeutic strategy for ameliorating cardiac dysfunction. (COI: No)

Symposium6

Facilitation of Recovery of Motor Function After Paralysis

(Co-sponsored by Uno Hospital)

(March 29, Fri., 10:00-12:00, Room F)

S6-1

CRMP2 Binding Compound, Edonerpic Maleate, Accelerates Motor Function Recovery from Brain Damage

Takuya Takahashi (*Department of Physiology Yokohama City University, Japan*)

Brain damage such as stroke is a devastating neurological condition, which may severely compromise patient quality of life. No effective medication-mediated intervention to accelerate rehabilitation has been established. We found that a small compound, edonerpic-maleate, facilitated experience-driven synaptic glutamate AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid) receptor delivery and resulted in the acceleration of motor function recovery after motor cortex cryoinjury in mice in a training-dependent manner through cortical reorganization. Edonerpic bound to collapsin-response-mediator-protein 2 (CRMP2) and failed to augment recovery in CRMP2-deficient mice. Edonerpic-maleate enhanced motor function recovery from internal capsule hemorrhage in non-human primates. Thus, edonerpic-maleate, a neural plasticity enhancer, could be a clinically potent small compound to accelerate rehabilitation after brain damage. (COI: Properly Declared)

S6-2

Bypassing damaged neural pathways via a neural interface

Yukio Nishimura (*Neural Prosthesis Project, Tokyo Metropolitan Institute of Medical Science, Japan*)

Functional loss of limb control in individuals with spinal cord injury or stroke can be caused by transection of descending pathways those connects cortical to spinal network, although neural circuits locate above and below the impaired site remains their function. I will show an artificial neuronal connection (ANC) that bridges supra-spinal system and spinal network beyond the lesion site restore lost function. The ANC was produced by a computer interface that can detect the neural activity and converted in real-time to activity-contingent electrical stimuli delivered to nervous system. A promising application is to bridge impaired biological connections, a paradigm that was demonstrated for cortically controlled electrical stimulation of paralyzed forearm muscles. ANC have clinical potential for restoring walking ability in patients with spinal cord injury. A clinical application of the ANC is the volitional walking in could be restored by muscle-controlled non-invasive magnetic stimulation to lumbar spinal cord. Patients with severe SCI could regain voluntarily-controlled walking which are initiate, stop walking and change the step cycles through ANC. These paradigms have numerous potential applications, depending on the input signals, the computed transform and the output targets. (COI: Properly Declared)

S6-3

Repetitive facilitation exercise with non-invasive stimulation for recovery of hemiplegia

Seiji Etoh; Megumi Shimodozono; Kazumi Kawahira (*Department of Rehabilitation and Physical Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Japan*)

It is thought that increases in the number of repetitions involved in rehabilitation of hemiplegic limbs are associated with improved functional outcome. The repetitive facilitation exercises (RFE) give sufficient physical stimulation to elevate the level of excitation of the corresponding injured descending motor tracts and it allows the patient to initiate movements of the hemiplegic limbs in response to his/her intention. RFE is more effective than conventional rehabilitation in improving hemiplegic upper limb function.

Neuromuscular electrical stimulation (NMES) over the hemiparetic limbs can facilitate excitation of the affected motor areas and enhance the recovery of motor dysfunction. RFE under NMES is more effective than conventional rehabilitation for lessening arm impairment. Downregulation of the contralesional motor cortex by 1Hz repetitive transcranial magnetic stimulation (rTMS) decrease the transcallosal inhibition from the contralesional to the ipsilesional motor cortex, and thus facilitate recovery after stroke. 1Hz rTMS facilitated the effects of RFE in improving motor function of the affected upper limb. NMES combined with rTMS also facilitate the beneficial effects of RFE. The Direct application of vibratory stimuli (DAViS) that delivered to the spastic muscles induces improvements in clinical spasticity which prevents the functional recovery. RFE with these non-invasive stimulations can be an effective approach for functional recovery of hemiplegic limbs. (COI: No)

S6-4

Predicting motor outcomes for individual patients after stroke

Marie-Claire Smith; Cathy Maree Stinear (*Department of Medicine, University of Auckland, New Zealand*)

Recovery of motor function after stroke is crucial for regaining independence. However, making accurate predictions of an individual patient's motor outcome is difficult when based on clinical assessment alone. Combining clinical assessment, neurophysiological and neuroimaging biomarkers of corticomotor structure and function can help to predict motor outcome after stroke. These combined biomarkers provide clinically useful information for planning the personalised rehabilitation of a patient. Biomarkers can also be used for patient selection and stratification in trials investigating rehabilitation interventions that are initiated early after stroke, potentially improving their sensitivity and efficiency. (COI: No)

International Scientific Program Committee Symposium

Symposium7

From synaptic and network plasticity to behavior (CAPS, China)

(March 29, Fri., 10:00-12:00, Room G)

S7-1

Fear extinction requires ASIC1a-dependent regulation of hippocampal-prefrontal correlates

Tian-Le Xu; Qin Wang; Qi Wang; Wei-Guang Li (*Collaborative Innovation Center for Brain Science, Department of Anatomy and Physiology, Shanghai Jiao Tong University School of Medicine, China*)

Extinction of conditioned fear necessitates the dynamic involvement of hippocampus, medial prefrontal cortex (mPFC), and basolateral amygdala (BLA), but key molecular players that regulate these circuits to achieve fear extinction remain largely unknown. Here, we report that acid-sensing ion channel 1a (ASIC1a) is a crucial molecular regulator of fear extinction, and this function requires ASIC1a in ventral hippocampus (vHPC), but not dorsal hippocampus, mPFC, or BLA. While genetic disruption or pharmacological inhibition of ASIC1a in vHPC attenuated the extinction of conditioned fear, overexpression of the channel in this area promoted fear extinction. Channelrhodopsin-2–assisted circuit mapping revealed that fear extinction involved an ASIC1a-dependent modification of the long-range hippocampal-prefrontal correlates in a projection-specific manner. Gene expression profiling analysis and validating experiments identified the *Fos*, *Npas4*, and *Bdnf* as the potential mediators of ASIC1a regulation of fear extinction. Mechanistically, genetic overexpression of brain-derived neurotrophic factor (BDNF) in vHPC or supplement of BDNF protein in mPFC both rescued the deficiency in fear extinction and the deficits on extinction-driven adaptations of hippocampal-prefrontal correlates caused by the *Asic1a* gene inactivation in vHPC. Together, these results establish ASIC1a as a critical constituent in fear extinction circuits and thus a promising target for managing adaptive behaviors. (COI: No)

S7-2

How does social conflict affect the synaptic plasticity in habenulo-interpeduncular pathway?

Hitoshi Okamoto (*Lab. for Neural Circuit Dynamics of Decision Making, RIKEN Center for Brain Science, Japan*)

Nicotine stabilizes emotion. Although nicotine activates brain acetylcholine (ACh) systems, little is known about the mechanisms how to stabilize emotions. We previously identified two subregions of the dorsal habenula (dHb) in zebrafish that antagonisticallyregulate the outcome of conflict. Silencing of the dHbL or medial subregion of dHb (dHbM) caused a stronger predisposition to lose or win a fight, respectively. These results demonstrated that the dHbL and dHbM comprise a dual control system for conflict resolution of social aggression. In mouse, the dorsal and ventral subregions of the medial habenula (dMHb and vMHb) are the direct evolutionary homolog of the dHbL and dHbM in zebrafish. The neurons in the vMHb projecting to the IPN use both glutamate and acetylcholine as co-neurotransmitters. Here we show that a loss of cholinergic neurotransmission from the ventral part of the medial habenula (vMHb) to the interpeduncular nucleus (IPN) prevented surrender and allowed mice to overcome physically stronger opponents in instantaneous social conflicts. Conversely, mice in which the vMHb-IPN pathway was optogenetically activated tended to stop the fight and yield against even gentler opponents. In this talk, I will put focus on our current study on how the cholinergic transmission is involved in the neural plasticity in the Hb-IPN pathway which is dependent on the outcome of social conflict both in zebrafish and mouse. (COI: Properly Declared)

S7-3

Postnatal refinement of circuit plasticity for spatial navigation

Ying-Shing Chan; Kenneth Lap-Kei Wu; Wei Shi; Qiu-Fen Jiang; Chun-Wai Ma; Daisy Kwok-Yan Shum (*School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong*)

The formation and consolidation of neural networks for a specific function are dependent on maturation of synaptic elements in the circuit. We hypothesize that synaptic efficacy of vestibular neurons undergoes postnatal tuning to sharpen spatial coding for the presentation of relevant behaviors. Whole-cell patch-clamp data from the vestibular nucleus (VN) of neonatal rodents indicated that endocannabinoid, serotonin and orexin could tune the efficacy of GABA transmission. The impact of such neuromodulators on postnatal emergence of vestibular-dependent graviceptive reflex was revealed using specific agonist/antagonist delivered to the neonatal VN; the deficits in spatial cognition so caused lasted well into adulthood. However, similar perturbations in juvenile animals posed no effect. To study whether the observed effects were due to perturbation of GABA circuits, we directly attenuated GABA transmission in the neonatal VN and found similar behavioral deficits. Optogenetic activation of inhibitory VN interneurons in these animals revealed permanent reorganization of inhibitory input to projection neurons. We reason that this underlies derangement of spatial maps in the forebrain of the experimental animals with spatial cognition deficits. Altogether, we show that neuromodulators tune GABA transmission and are instrumental in developmental consolidation of the vestibular circuitry for spatial recognition. *[HKRGC-GRF 762313, 17131816, 17113717; NSFC-RCG N_HKU735/14]* (COI: No)

S7-4

Behavioural Impact of Synaptic Kainate Receptor Protein Levels

Juan Lerma (*Instituto de Neurociencias CSIC-UMH, San Juan de Alicante, Spain*)

Establishing the adequate receptor number and type at synapses is fundamentally important to fine tune neuronal communication and brain plasticity associated to learning and memory. Normally, we tend to think about mutations that alter protein function as a source of disruption of this process that may lead to disease. However, the vulnerability of neurons to modest changes (increases or decreases) in levels of normal proteins is an emergent theme in brain disorders and may provide a new way to understand pathogenesis. Indeed, pervasive brain pathologies can emerge because of variations in the dosage of certain genes and insertions, deletions, inversions and duplications may result in loss or gain of protein function. This seems to be the case of kainate receptors (KARs) and we have addressed this problem in two situations. First, a *de novo* duplication of the chromosome 11q23.3–q24.1 locus in which the gene *GRIK4* (coding for a high affinity KAR subunit) lies has been identified in cases of autism, and more recently, a genome-wide linkage analysis found this gene to be associated to different endophenotypes in schizophrenia. Animals carrying excess of *Grik4* display signs of depression, anxiety and social impairment, closely reflecting the human endophenotypes associated to autism and schizophrenia. The synaptic effects of KAR genes overexpression recapitulate functional circuit activity in humans and may be significant to understand the ethiopathology of human disorders. (COI: No)

Symposium8

Biophysical mechanisms underlying nano-vibrations of the sensory epithelium in hearing organs

(Co-sponsored by the Society for Promotion of International Oto-Rhino-Laryngology)

(March 29, Fri., 10:00-12:00, Room H)

S8-1

Detection of an atypical motion in cochlear sensory epithelium

Takeru Ota[1,2]; Fumiaki Nin[1,2]; Samuel Choi[2,3]; Hiroshi Hibino[1,2]
([1]*Department of Molecular Physiology, Niigata University School of Medicine, Japan;* [2]*AMED-CREST, AMED, Japan;* [3]*Department of Electrical and Electronics Engineering, Niigata University, Japan*)

Sounds are converted into electrical signals by the cochlea of the inner ear. This process is triggered by nanoscale vibrations induced in the sensory epithelium inside the organ. In general, any types of waves are composed of oscillating and/or non-oscillating motions. Whether the latter occurs in the epithelium in response to acoustic stimuli remains uncertain, because of methodological limitations in conventional vibrometers. Here we develop an advanced laser interferometry that simultaneously detects both of the amplitudes of the vibration and mean position shifts of the object, which represent oscillating and non-oscillating motions, respectively. The performance of this called "dual Sinusoidal Phase Modulating (SPM)" method was tested and verified with a piezo-actuator. When a live guinea pig was exposed to acoustic stimuli, the SPM interferometer quantitively recorded the vibration amplitude of the sensory epithelium as described elsewhere. An upward baseline shift of several nanometers was also detected. This reaction was observed with loud sounds of >70 dB, and it was negligible when the animal was dead. A theoretical approach further suggested that the shift protects the epithelium from injury induced by strong stimuli. (COI: No)

S8-2

Sensory tissue motion and hair cell responses in the base of the gerbil cochlea

Elizabeth Sue Olson[1]; Clark Elliott Strimbu[4]; Yi Wang[2]; Nathan C Lin[3]; Elika Fallah[2] ([1]*Departments of Otolaryngology and Biomedical Engineering, Columbia University, USA;* [2]*Department of Biomedical Engineering, Columbia University, USA;* [3]*Department of Electrical Engineering, Columbia University, USA;* [4]*Department of Otolaryngology, Columbia University, USA*)

An important open question of cochlear mechanics is how the cochlear amplifier works at the right frequencies for a given location. This frequency/location tuning of amplification produces the impressive nonlinear enhancement of peak responses in mid- and basal-cochlear locations. Recent measurements of motion responses within the organ of Corti, made with Phase Sensitive Optical Coherence Tomography (PS-OCT), go beyond the already well-studied basilar membrane motion, to observe motions within the organ of Corti. A key motion for understanding cochlear operation is the stereocilia shear motion, and that motion is not yet clearly accessible. A quantity that is closely related to OHC stereocilia shear is the transducer current through OHCs, and we have been measuring that quantity with a thin metal electrode positioned close to the basilar membrane. In published work (Dong and Olson, Biophysical Journal 105, 2013), we described a phase shift of voltage relative to motion. Based on known OHC mechanics, we interpreted the shift as activating the cochlear amplifier. The shift is quite independent of level and reduced endocochlear potential, indicating it is based in passive cochlear mechanics. OHCs are most certainly the motor of cochlear amplification, and new experimental results are beginning to expose the elusive mechanics steering that motor. (COI: No)

S8-3

Dual-mode OCT system for vibrometry in mammalian hearing mechanics at high frequencies

Fangyi Chen[1]; Cuixia Guo[2]; Xiaojie Yang[1]; Yonghong He[2]
([1]*Department of Biomedical Engineering, Southern Univ. of Sci. & Tech., China;* [2] *Graduate School at Shenzhen, Tsinghua University, China*)

Direct measurement of the vibrational properties of either the inner ear or the middle ear is highly demanded for understanding the fundamental hearing mechanics. Phase-sensitive optical coherence tomography, which enables noninvasive vibrometry at the subnanometric scale with optical sectioning capability, has been proved to be an excellent vibrometer in hearing research. To date, the reported OCT systems for vibrometry were developed based on the technique of either time-domain optical coherence tomography (TD-OCT) or spectral-domain optical coherence tomography (SD-OCT). Phase-sensitive SD-OCT is currently more popular because both depth-resolved imaging and vibrometry along the entire imaging depth can be accomplished in parallel. However, performing such vibrometry at high frequencies (*e.g.*, > 50 kHz) is limited by the line-scan rate of the SD-OCT setup. A TD-OCT system is able to measure the vibration at higher frequencies. Such vibrometry, however, is challenged by the absence of the real-time imaging that is crucial for localizing the point of interest (PoI) in the sample. We present a newly developed PS-OCT system involving both the SD-OCT and TD-OCT modes. The SD-OCT is responsible for the depth-resolved imaging, while the TD-OCT works as low-coherence vibrometry. We demonstrate the capability of the developed system in both high-frequency vibrometry and image-guided localization of the PoI by measuring the sound-induced vibration in the middle-ear samples *ex vivo*. (COI: No)

S8-4

In-Vivo Nanomechanics in the Miniaturized Hearing Organ of an Insect

Manuela Nowotny[1]; Jan Scherberich[1]; Jennifer Hummel[1]; Stefan Schöneich[2] ([1]*Institute of Cell Biology and Neurosciences, Goethe University, Germany, German;* [2] *Institute for Biology, University of Leipzig, Germany*)

The capability of auditory receptor cells to sense sound-induced mechanical forces is an evolutionarily conserved function that can be found in all hearing systems from insects to mammals. The mechano-electrical transduction process of acoustic signals from sound-induced vibrations to frequency-specific neuronal responses of sensory cells is based on the opening of mechanosensitive ion channels. Here we investigated the mechanotransduction in an insect (katydid) hearing organ by *in vivo* measurements of sound-induced mechanical oscillation and electrical responses of sensory neurons at the transduction site.

Laser Doppler vibrometry in the front leg ears revealed tonotopically ordered traveling waves covering most of the length of this miniature hearing organ (*crista acustica*). The *crista acustica* is about 1-2 mm long and its geometry allowed us to probe the entire hearing organ by a wide range of acoustic stimuli from 4 to 80 kHz. The traveling waves propagated along the *crista acustica* from the distal (high frequency) to the proximal (low frequency) part. By combining mechanical and neuronal response measurements we show that the stretch-induced ion channel gating in the associated receptor neurons largely depends on the inclination angle of the sound-induced mechanical oscillation in the organ. Our study demonstrates channel-opening and biomechanical filter mechanisms that sharpen the neuronal frequency tuning in insects. (Supported by DFG: NO 841/8-1, 10-1) (COI: No)

S8-5

Nonlinear micromechanics of the organ of Corti in the low-frequency region of the cochlea

Tobias Reichenbach; Nikola Ciganovic (*Imperial College London*)

The organ of Corti in the mammalian inner ear houses the mechanosensitive inner and outer hair cells. Outer hair cells can provide mechanical forces that can amplify a weak sound stimulus, which is then detected by the inner hair cells. However, the micromechanics of how mechanical amplification is achieved by the organ of Corti remains unclear. In particular, the apical organ of Corti is shaped differently from the basal organ, indicating a different micromechanical functioning that remains poorly understood. Here we combine mathematical modeling and laser-interferometric recordings to show that the apical organ of Corti's micromechanics contains a strong nonlinearity with respect to length changes of the outer hair cells. This nonlinearity allows the organ of Corti to operate as an electromechanical transistor: the elongation of the outer hair cells can sensitively regulate how much sound stimulation is transmitted to the inner hair cells. These results could, for instance, explain how stimulation of the inner hair cells can be regulated by efferent nerve fibers. (COI: No)

International Scientific Program Committee Symposium

Symposium9

Metabolic syndrome and bone metabolism (TPS, Thailand)

(March 29, Fri., 10:00-12:00, Room I)

S9-1

Diabetic osteopathy and impaired intestinal calcium absorption in diabetes mellitus

Narattaphol Charoenphandhu[1,2,3,4] (*[1]Center of Calcium and Bone Research (COCAB), Faculty of Science, Mahidol University, Bangkok, Thailand; [2]Department of Physiology, Faculty of Science, Mahidol University, Thailand; [3]Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand; [4]The Academy of Science, The Royal Society of Thailand, Dusit, Thailand*)

Diabetic mellitus (DM) not only impairs glucose metabolism but also deteriorates several organs, including heart, kidney, retina, as well as intestine and bone. Previously, it was believed that only type 1 DM (T1DM) could lead to low bone mineral density (BMD) and increased fracture risk. Nevertheless, several recent studies have confirmed that type 2 DM (T2DM) and perhaps hyperglycemia in maturity onset diabetes of the young are able to damage bone cell function (cellular failure) and its extracellular collagenous scaffold (matrix failure), thereby impairing osteoblast-mediated bone formation and bone strength. Moreover, T1DM also downregulates the expression of calcium transporters (e.g. TRPV6) for intestinal calcium uptake, leading to a decreased calcium supply for bone formation and repair. Chronic inflammation in DM further negatively affects bone by inducing production of osteoclastogenic cytokines and bone resorption. Prolonged hyperglycemia and advanced glycation end-products may aggravate osteopathy independent of insulin resistance. Our recent study in Goto-Kakizaki T2DM rats showed that normalization of blood glucose was unable to rescue DM-associated bone loss. Therefore, early prevention of prediabetic condition is probably beneficial for mitigating diabetic osteopathy. Since several anti-diabetic drugs (e.g., thiazolidinediones) often aggravate bone loss, a search for novel therapeutic approaches for DM-associated osteopathy is worth exploring. Supported by TRF. (COI: No)

S9-2

Is Metabolic Syndrome a Concern for Osteoporosis?

Siriporn C Chattipakorn[1,2] (*[1]Neurophysiology Unit, Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Thailand; [2]Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Thailand*)

The osteoporosis and metabolic syndrome (MetS) have become important global health issues. MetS is a combination of abdominal obesity, impaired glucose tolerance or insulin resistance, dyslipidemia, and hypertension. Osteoporosis is characterized by bone fragility and susceptibility to fracture attributed to bone loss. Previous studies demonstrated that each component of MetS acts on bone tissue in various ways. Growing evidence of the relationship between MetS and osteoporosis are still controversial findings. Some studies have shown a positive correlation between body mass and bone density, suggesting that obesity prevents osteoporosis. However, the existing evidence has shown an inverse relationship between bone quality and obesity. Therefore, both positive and negative influences of MetS on bone exist. Evidence on the effects of each component of MetS on bone and its metabolism have been reported. In addition, the possible underlying mechanisms involved in osteoporosis via MetS have been proposed. The impairment of calcium homeostasis, inflammation and oxidative stress are common links between MetS and process of bone formation and resorption. We also demonstrated that obese-insulin resistance impaired osteoblastic insulin signaling, osteoblast proliferation, and osteoblast survival and resulted in osteoporosis in the jaw bone as well as the tibia. The existing evidence suggests that correction of MetS should be considered for the prevention of osteoporosis. (COI: No)

S9-3

The effect of high-fat diet on maternal bone microstructure and the metabolic parameters in rats

Panan Suntornsaratoon[1,2]; Narattaphol Charoenphandhu[1,2] (*[1]Department of Physiology, Faculty of Science, Mahidol University, Thailand; [2]Center of Calcium and Bone Research, Faculty of Science, Mahidol University, Thailand*)

Gestational diabetes mellitus (GDM) is a common pregnancy complication with hyperglycemia due to reduced insulin secretion from pancreatic ß cells. Approximately 10–25% of pregnant women developed an abnormal glucose tolerance in pregnancy which normally returned to normal postpartum. However, women with GDM are prone to develop type 2 diabetes mellitus (T2DM) later in life, and may have offspring with increased risk of obesity and also T2DM later in life. Interestingly, high fat feeding of dams during the perinatal period led to high serum total cholesterol, LDL, HDL, triglyceride, VLDL and hyperglycemia which resemble blood chemistry of T2DM in 100-day-old offspring even they were raised on normal diet. Our recent investigation showed that non-mated rats with T2DM also exhibited bone microstructural defects, premature growth plate chondrocyte apoptosis, suppressed osteoblast function and stimulated osteoclast activity. It is not known at present whether insulin resistance during perinatal period is associated with changes in gut microbiota or is a causative factor in bone microstructural defect in maternal and offspring similar to dyslipidemic T2DM in non-mated female rats. It was found that high-fed feeding during pregnant and lactation impaired maternal glucose tolerance by oral glucose tolerance test, and impaired maternal bone microstructure as investigated by micro-computed tomography. (COI: No)

S9-4

Proton-mediated regulation of physiological and pathological osteoclast functions

Miyuki Kuno (*Department of Anesthesiology, Osaka City University, Japan*)

Proton-mediated regulation of physiological and pathological osteoclast functions

Osteoclasts, bone-resorbing cells, secrete protons and undergo drastic H^+ metabolism. The plasma membrane expresses a vacuolar H^+-ATPase, a voltage-gated H^+ channel (Hv channel), H^+ leaks and molecules transporting H^+ in combination with Cl^- or Na^+. Thus proton is an important signaling molecule to regulate osteoclast functions. Extracellular Ca^{2+}, H^+ and inorganic phosphate (Pi), which accumulate in the resorption pit, will either stimulate or inhibit the H^+ fluxes and osteoclast activities. For example, Pi increases activities of Hv channels and upregulates NADPH oxidase-mediated production of reactive oxygen species (ROS). ROS are generated during RANK-RANKL-mediated signalling cascade, and are essential for differentiation, activation, and survival of osteoclasts. Also, ROS are considered to be a key element causing age-related disturbances in bone. ROS production is enhanced by PMA, an Hv channel activator, and is reduced by zinc, an Hv channel blocker, suggesting that Pi-induced enhancement of Hv channels contribute to support ROS production in osteoclasts. More or less of resorption, both do harm skeletal integrity. Thus Hv channels, as well as other H^+ fluxes, may have both beneficial and toxic effects on bone metabolism. Focusing on Hv channels, the roles of protons and H^+-mediated regulation mechanisms in osteoclasts will be discussed. (COI: No)

Symposium10

Neural circuit basis of behavioral physiology

(March 29, Fri., 10:00-12:00, Room J)

S10-1

Functional Dissection of the Central Glucoregulatory circuits

Shi-Bing Yang; Hsin-Ju Tsai (*Institute of Biomedical Sciences, Academia Sinica, Taiwan*)

Hypothalamus is the major commander to control somatic activities and innate behaviors such as hunger, thirst and body temperature. For example, the hypothalamus receives peripheral information such as nutrients (glucose, fatty acids and amino acids) and hormones (leptin, ghrelin and insulin) and modulates the energy metabolism of a variety of peripheral tissues such as the liver and skeletal muscle via the autonomic nervous system, by controlling insulin sensitivity as well as the hepatic gluconeogenesis. Earlier studies have found that acute infusion of glibenclamide, a K_{ATP} channel blocker, into the 3rd ventricle abolishes hormonally and nutritionally induced suppression of hepatic gluconeogenesis. In addition to that, our data indicated that central infusion of glibenclamide paradoxically impaired glucose tolerance in aged mice. Our genetic labeling have further revealed that the neurons in the dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH) and arcuate nucleus are involved in the central regulation of peripheral glucose homeostasis. Lastly, our results also showed the pharmacoactivation of neurons in the DMH induced the glucose intolerance in mice fed with high-fat diet. Our study has demonstrated that central nervous system may become a promising therapeutic for treating metabolic disorders. (COI: No)

S10-2

Modeling of group size dependent aggressive behavior in the cricket

Hitoshi Aonuma (*Research Institute for Electronic Science, Hokkaido University, Japan*)

Animals alter their behaviours on the demand of changing circumstances. It is one of the common interests between biologists and robotics engineers to understand the emergence of real time adaptive behaviours.

Here we focus on group size dependent aggression in the cricket. Individual interaction is one of the important factors for making decision animals. Cricket aggressive behaviour provide us good model system to understand how animals alter their behaviours on the demand of changing social circumstances.

It is widely observed in animals that dominant hierarchy is established by agonistic behaviour, through the complex interaction among physiological, motivational, and behavioural systems. Male cricket exhibits intensive aggressive behaviour when it encounters another male. The battle starts out slowly and escalates into a fierce struggleto establish dominant-subordinate relationship. Pharmacological behaviour experiments suggest that nitric oxide signalling mediates biogenic aminesystem in the brain, which in turn mediates aggressive motivation.Based on the results of behavioural experiments and physiological experiments, we built dynamical behaviour model (finite automaton model andoscillator model)and oscillator modeltounderstand the mechanisms of social adaptability.Our models demonstrate that important mechanism underlying social adaptability is a multiple feedback structure that is composed of feedback loop in the nervous systems and through the social interaction. (COI: No)

S10-3

Neural basis of infant attachment and separation anxiety

Kumi O Kuroda[1]; Sachine Yoshida[1,2] ([1]*Lab for Affiliative Social Behavior, RIKEN Center for Brain Science, Japan;* [2]*Department of Anatomy, Faculty of Medicine, Toho University, Japan*)

Mammalian infants develop selective attachment toward the mother, and contribute actively to maintain the maternal proximity through a wide range of attachment behaviors. One of primitive attachment behaviors, the Transport Response is a set of cooperative calming responses expressed during maternal carrying and has been identified in both human infants and rodent pups (Esposito et al, 2013; Yoshida et al, 2013). Infants also show prominent anxiety responses upon maternal separation, and express significant distress signs, including distress vocalizations and plasma corticosteroid elevation. Such "separation anxiety" has been widely acknowledged, and the long-term effects of maternal separation in neural and behavioral development in infants, such as stress response, have been extensively studied. The acute brain mechanisms that perceive maternal separation and elicit stress responses, however, remain largely unknown. Recently, we established the model of separation anxiety in mouse pups and found that the pups could recognize 30-minutes of maternal separation after the second postnatal week. Moreover, pups under maternal separation modulate their Transport Response, at least partly via the anterior cingulate cortex (Yoshida et al, 2018).

The role of the rodent and primate animal models to understand the development and abnormalities of infant attachment system will also be discussed. (COI: No)

S10-4

Set the threshold for surrender in social conflicts

Ming-Yi Chou[1]; Hitoshi Okamoto[2] ([1]*Department of Life Science, National Taiwan University, Taiwan;* [2]*RIKEN Center for Brain Science, Japan*)

Most of animal conflicts aim at establishing social hierarchy rather than causing lethal damage to opponents. The timing of stopping fights and escaping from the conflicts is important to obtain the best cost and benefit ratio during fighting. We identified two subnuclei of the dorsal habenula (dHb) antagonistically regulating the end point of conflicts: the lateral subnucleus (dHbL) raises the threshold for surrender and the medial subnucleus (dHbM) lowers it during zebrafish fighting. Losing experience caused a reduction in neural transmission in the dHbL-dorsal interpeduncular nucleus (dIPN) circuit. Silencing of the dHbL and the dHbM caused higher tendency to lose and win, respectively. Thus, both the dHbL and the dHbM affect the outcome of fight but play opposite roles on aggressive behaviors (COI: Properly Declared)

S10-5

Dissecting the neural circuits mediating female fertility in health and disease

Rebecca Campbell (*Centre for Neuroendocrinology, Department of Physiology, University of Otago, New Zealand*)

Pulsatile secretion of gonadotropin-releasing hormone (GnRH) is necessary for mammalian fertility and, as such, inappropriate pulse patterns result in infertility. Polycystic ovary syndrome (PCOS) is associated with a hyperactive GnRH pulse generator that results in high frequency luteinizing hormone (LH) secretion and downstream ovarian consequences. Elevated GnRH neuron activity is thought to result from impaired gonadal steroid hormone feedback and dysregulation of the afferent neuronal network important in GnRH neuron regulation. Our work, using a prenatally androgenized mouse model of PCOS, is identifying specific anatomical circuit abnormalities associated with the PCOS phenotype and employing opto- and chemo-genetic manipulations to assess their functional relevance. In this lecture, I will discuss newly identified roles of arcuate nucleus neuropeptide Y and GABAergic neuron populations in mediating GnRH/LH pulsatility. These findings are expanding our current understanding how GnRH/LH pulsatility is regulated and revealing potential PCOS treatment targets. (COI: No)

Symposium11

Advances in the mastication and swallowing physiology to prepare for an aging society

(March 29, Fri., 10:00-12:00, Room K)

S11-1

Properties of *Phox2b*-expressing premotor neurons targeting jaw-muscle motoneurons

Tomio Inoue (*Department of Oral Physiology, Showa University School of Dentistry, Japan*)

Last-order premotor neurons of trigeminal motoneurons innervating the jaw-muscles are located in various brainstem regions, including the supratrigeminal region (SupV). The SupV plays a critical role in controlling mastication, because that region receives abundant inputs from the orofacial structures, the cerebral cortex, amygdala, lateral hypothalamus and the central pattern generator for mastication. Using rat brainstem slice preparations, we investigated the physiological and morphological properties of SupV premotor neurons. Stimulation of SupV evoked glutamatergic, GABAergic or glycinergic postsynaptic responses in both jaw-closing and jaw-opening motoneurons. We then divided SupV premotor neurons into those firing at a frequency higher (HF neurons) or lower (LF neurons) than 33 Hz during a steady-state condition. Neurons expressing *Phox2b*, which encodes a transcription factor essential for autonomic nervous system development, are abundantly present in SupV and were designated as Phox2b^{+}. Nearly all Phox2b^{+} in SupV were glutamatergic, whereas most *Phox2b*-negative neurons (Phox2b^{-}) in SupV were GABAergic or glycinergic. Furthermore, the majority of Phox2b^{+} were LF neurons, while most Phox2b^{-} were HF neurons. Nearly half of the Phox2b^{+} and Phox2b^{-} in SupV sent their axons to the trigeminal motor nucleus. These results suggest that premotor Phox2b^{+} in SupV have properties distinct from premotor Phox2b^{-} and may play important roles in mastication. (COI: No)

S11-2

Development of masticatory performance as a novel biomarker of general health

Takahiro Ono (*Graduate School of Medical and Dental Sciences, Niigata Univ., Japan*)

It has been widely recognized that chewing ability in daily life is one of the keys for healthy long life. However scientific evidence was yet to be established due to the lack of objective and quantitative method for evaluating chewing ability. Nokubi et al. in Osaka University have developed the system for assessing chewing ability in which masticatory performance as the increased surface area of testing gummy jelly after 30 times chewing can be calculated by the fully automated machine (Nokubi et al., *J Oral rehabil*, 2013). We have joined the cohort study for investigating the risk factor of cardiovascular disease in urban general population (Suita study) since 2008 and conducted comprehensive evaluation of oral health including masticatory performance. In these ten years, cross sectional analysis for detecting the factors influencing masticatory performance (Kosaka et al., *J Clini periodont*, 2014), for investigating the relationship between metabolic syndrome and masticatory performance (Kikui et al., *J Dent*, 2017), and longitudinal analysis for detecting the factors influencing on the change in masticatory performance (Kosaka et al., *J Dent Res CTR*, 2018) have been done. And also, we have confirmed that the results of visual scoring method with 10 steps evaluation can be reliable as a biomarker of general health as well as oral health (Nokubi et al,*Gerodontology*, 2013). Summary of our cohort study and future prospect of our research will be presented in this symposium. (COI: No)

S11-3

Are respiratory-swallowing disturbances indicators of early dementia?

Mathias Dutschmann; Davor Stanic (*Florey Institute of Neuroscience and Mental Health, Australia*)

In the TauP301L mouse model of tauopathy we have detected irregular coordination between swallowing and breathing, which arise before cognitive and memory deficits.
Injections of water into the oral cavity triggered swallowing, but the tonic drive of vagus nerve activity (VNA – representative of epiglottal closure) was reduced by ~75%. Moreover, inspiratory PNA bursts occurred during the swallowing sequence, which shortened the swallowing-induced apnoea, and is a risk indicator for aspiration. These respiratory-swallowing parameters continued to deteriorate asTauP301L mice aged.
Tauopathy and neurofibrillary tangle-related neuropathological morphology was identified in TauP301L mice by immunohistochemistry. In brainstem areas critically involved in coordinating swallowing and breathing, cell bodies and fibers immunoreactive for phosphorylated and paired helical filament-tau were distributed within the Kölliker-Fuse (KF) nucleus, Nucleus ambiguus, and to a lesser degree, Nucleus of the solitary tract. This coincided with a progressive loss of Forkhead box protein P2 immunoreactive KF neurons as TauP301L mice aged.
Our results have identified respiratory-swallowing disturbances as a potential detection diagnostic for dementia. They also demonstrate that respiratory-related neurons in the brainstem are susceptible to neurodegeneration in TauP301 mice and lead to uncoordinated swallowing-breathing patterns and inefficient closure of the epiglottis during swallowing. (COI: No)

S11-4

Coordination between swallowing and breathing: pathophysiology and its clinical significance

Yoshitaka Oku (*Department of Physiology, Hyogo College of Medicine, Japan*)

Swallowing normally occurs during expiration to physiologically prevent aspiration. However, swallowing can occur immediately following inspiration (I-SW pattern) and breathing can resume with inspiration (SW-I pattern). Such abnormal breathing-swallowing (B-S) coordination tends to increase due to diseases and aging. Parkinson disease patients with decreased swallowing safety have a higher frequency of SW-I pattern.
We evaluated B-S coordination in elderly volunteers. Interestingly, 7.5 % subjects had a high (>40%) SW-I rate. Abnormal B-S coordination occurred when timing of swallows was inappropriate. Patients with dysphagia tended to swallow at late expiration due to a delay in eliciting swallows.
Recent large-scale cohort studies showed that gastroesophageal reflux disease (GERD) was related to frequent exacerbations of chronic obstructive pulmonary disease (COPD), however, proton pump inhibitors did not reduce the exacerbation rate. We hypothesize that impairment of swallowing function underlies the association between GERD and COPD exacerbation. We show that COPD patients with a higher frequency of I-SW and/or SW-I patterns have higher frequency of exacerbations. Further, we suggest that a low-pressure CPAP may improve the B-S coordination in COPD patients.
More studies are needed to elucidate whether high SW-I rates causes pulmonary aspiration and/or exacerbation, and whether an early intervention improves the outcome. (COI: No)

S11-5

Non-invasive methods to evaluate the swallowing function

Makito Iizuka[1]; Kazuhide Tomita[2]; Reiko Takeshima[3]; Masahiko Izumizaki[1] (*[1]Department of Physiology, Showa University School of Medicine, Japan; [2]Department of Physical Therapy, Ibaraki Prefectural University of Health Sciences, Japan; [3]Center for Medical Sciences, Ibaraki Prefectural University of Health Sciences, Japan*)

Elevation of the larynx is essential for airway protection during the pharyngeal phase of swallowing. The elevation causes apposition of the arytenoids to the base of the epiglottis and closes the laryngeal vestibule. During normal swallowing, the larynx elevates from 21.1 mm to 33.9 mm in healthy subjects. Pathologically reduced or delayed laryngeal elevation is the most common cause of aspiration in persons with dysphagia. Therefore, precise measurement of the laryngeal movement is useful in evaluating the swallowing function. In the clinical setting, however, laryngeal movement is not routinely measured in dysphagia patients, since there is no simple, easy and safe way to do this. Recently, we succeeded in developing a new piezo sensor array sheet for the noninvasive detection of larynx movement during swallowing, even in subjects with no apparent laryngeal prominence. Using this sensor sheet, four parameters, namely, the swallowing latency, the maximum rising velocity, the maximum lowering velocity and the upper part staying period, can be obtained easily and repeatedly. In this symposium, we are going to present this new method in detail and also summarize the recent advancement of other non-invasive methods to study the physiology and pathophysiology of swallowing, including the efficacy of swallowing rehabilitation. (COI: No)

Symposium12

New insights into baroreflex function for cerebral and cardiovascular regulation: Implications for human health and disease

(March 29, Fri., 10:00-12:00, Room L)

S12-1

The effect of baroreflex function on cerebral blood flow regulation during exercise

Shigehiko Ogoh (*Department of Biomedical Engineering, Toyo University, Japan*)

Although cerebral blood flow (CBF) was traditionally thought to remain unchanged during exercise to maintain cerebral homeostasis, it has been established recently that exercise causes an increase in CBF that is associated with cerebral neural activity. However, the CBF response to exercise is not simple; therefore, its physiological mechanism and significance remain unknown. The arterial baroreflex does not directly control vasculature at rest as well as during exercise because the sympathetic nervous system via baroreflex function is surmised to have a limited effect on cerebral vasculature in humans. On the other hands, several studies have indicated an interaction between CBF and autonomic function. We have demonstrated that cardiac baroreflex function contributes to dynamic cerebral autoregulation. Moreover, in a human study, during exercise, sympathetic blockade at the neck level eliminated the β1-blockade-induced attenuation in CBF, indicating that arterial baroreflex control of sympathetic nerve activity directly influences CBF during exercise as well as at rest. Cerebral vasomotion via baroreflex, however, is a paradoxical reaction with little physiologic benefit. Thus, a physiologically significant direct effect of baroreflex function on cerebral vasculature remains unclear. Given the present background, in this symposium, I would like to discuss about the role of baroreflex function on CBF regulation. (COI: No)

S12-2

Withdrawn

S12-3

Sex Differences in Baroreflex Function

Qi Fu[1,2] ([1]*Internal Medicine, University of Texas Southwestern Medical Center, USA, China;* [2]*Institute for Exercise and Environmental Medicine at Texas Health Presbyterian Hospital, USA*)

Women have been found to have blunted cardiovagal baroreflx sensitivity during a hypertensive stimulus compared with men, but baroreflex sensitivity is similar between sexes during a hypotensive stimulus. Sex differences in cardiovagal baroreflex sensitivity may be caused by a lower vagal response to baroreceptor activation in women. Conversely, recent research demonstrates that there is no sex difference in sympathetic baroreflx sensitivity in young individuals. With women of more advanced age, sympathetic baroreflx sensitivity decreases, which appears to be associated with greater arterial stiffness in older women than older men. The decreased sympathetic baroreflx sensitivity in older women may predispose them to an increased prevalence of hypertension or cardiovascular disease. The baroreflex is reset during dynamic exercise. Among healthy young individuals, neither sex nor menstrual cycle phase affects the magnitude of baroreflex resetting. However, augmented baroreflex control of blood pressure responses to acute hypotension were observed in young women in the mid-luteal phase compared with the early and late follicular phases at rest and during exercise, suggesting progesterone may be the underlying mechanism. Young women were also found to exhibit greater baroreflex control of heart rate during exercise, an effect that was present throughout the menstrual cycle. Whether there are sex differences in baroreflex function during exercise in older people remains unknown. (COI: No)

S12-4

Exercise pressor reflex and arterial baroreflex function in cardiovascular disease

Scott Alan Smith (*School of Health Professions, Department of Health Care Sciences, University of Texas Southwestern Medical Center, USA*)

Exercise programs that include both dynamic and static forms of physical activity have been shown to improve cardiovascular health in both hypertensive and heart failure patients. Unfortunately, in these patients, exercise elicits excessive increases in sympathetic nerve activity, heart rate, and blood pressure. These exaggerated elevations are potentially dangerous as they increase the risk for adverse cardiovascular events such as acute myocardial infarction, arrhythmia, cardiac arrest and stroke during exercise. As a result, the levels of exercise prescription considered safe in these patients is often constrained to low to moderate intensities of short duration. Determining the causes of autonomic cardiovascular dysfunction during acute bouts of exercise is, therefore, clinically important. To this end, emerging evidence suggests that the exercise pressor reflex, a feed-back neural reflex originating in skeletal muscle, contributes significantly to the abnormal circulatory response to physical activity in both hypertension and heart failure. Moreover, the arterial baroreflex, a feed-back neural reflex emanating from the carotid arteries and aortic arch, has also been implicated as an important contributor. As such, the focus of this symposium presentation will be on the independent actions of these two reflexes as well as their interactive behavior after the pathogenesis of cardiovascular disease. (COI: No)

S12-5

Modulation of cardiac baroreflex by central command in daily life

Kanji Matsukawa; Kei Ishii; Ryota Asahara (*Department of Integrative Physiology, Hiroshima University, Japan*)

We have reported that central command blunts the sensitivity of the aortic baroreceptor-heart rate (HR) reflex at onset of static exercise in conscious animals. This study aimed to examine which baroreflex control of cardiac sympathetic nerve activity (CSNA) or parasympathetic nerve activity is altered at onset of spontaneous motor behavior, which ocurred in paralyzed, decerebrate cats. CSNA exhibited an increase at exercise onset, followed by increases in HR and mean arterial pressure (MAP). With development of the pressor response, CSNA and HR decreased near baseline, although spontaneous motor activity was not terminated. Atropine methyl nitrate delayed the initial increase in HR but did not alter the response magnitudes of HR and CSNA, while atropine augmented the pressor response. The baroreflex-induced decreases in CSNA and HR elicited by brief occlusion of the abdominal aorta were challenged at onset of spontaneous motor activity. Spontaneous motor activity blunted the baroreflex reduction in HR by aortic occlusion but did not alter the baroreflex inhibition of CSNA. Atropine abolished the baroreflex reduction in HR but did not influence the baroreflex inhibition of CSNA. It is likely that central command increases CSNA and decreases cardiac vagal outflow at onset of spontaneous motor activity and that central command must attenuate cardiovagal baroreflex sensitivity against a rise in MAP. (COI: No)

Symposium13

The role of the sympathetic nerves in health and disease

(March 29, Fri., 10:00-12:00, Room M)

S13-1

Longterm effects of renal denervation in an ovine model of hypertensive chronic kidney disease

Kate M Denton; Reetu R Singh (*Department of Physiology, Monash University, Australia*)

Although the efficacy of radio frequency catheter based renal denervation (RDN) as a treatment for hypertension has been disputed, the majority of data suggest that there is a place for RDN in the clinic and trials are ongoing. Our studies in an ovine model of hypertensive chronic kidney disease (CKD) demonstrates that the reduction in blood pressure following RDN is sustained for 3 years, with marked improvements in glomerular filtration rate, albuminuria and left ventricular hypertrophy. Moreover, our data demonstrates that whilst significant nerve growth occurs, that this is not restored to control levels at 3 years post-RDN in the CKD group. This finding may explain in part the longterm benefits of RDN. These finding supports the use of catheter based RDN in CKD patients. (COI: No)

S13-2

Sympathetic regulation in anaphylactic shock or feeding suppression

Mamoru Tanida (*Department of Physiology II, Kanazawa Medical University, Japan*)

The sympathetic nervous system functions as defensive system during cardiovascular shock. Here, we examined effects of anaphylactic shock on efferent renal sympathetic nerve activity (RSNA) in anesthetized and ovalbumin-sensitized mice. Within 3 min after antigen injection, RSNA decreased along with a transient hypertension. Thereafter, RSNA showed a progressive increase during sustained hypotension. In contrast, heart rate continuously increased. Sinoaortic denervation, but not vagotomy, significantly attenuated the renal sympathoexcitation and tachycardia after antigen. Thus, the anaphylaxis-induced sympathoexcitation and tachycardia at the late stage are mediated by carotid sinus baroreceptors.

Nesfatin-1, feeding suppressor, generated in the hypothalamus, acts on the neurons by paracrine signaling. Hypothalamic injection of nesfatin-1 activated the extracellular signal-regulated kinase (ERK) in rats. Furthermore, the activity of sympathetic nerves to the kidneys, liver, and white adipose tissue and blood pressure was stimulated by the nesfatin-1 injection, and these effects were abolished by inhibition of ERK. Moreover, nesfatin-1 increased the number of activated ERK-positive neurons in the paraventricular nucleus and co-expression of the protein in neurons expressing corticotropin-releasing hormone (CRH). Thus, nesfatin-1 regulates the sympathetic nervous system through ERK signaling in the CRH neurons of paraventricular nucleus to maintain cardiovascular function. (COI: No)

S13-3

The importance of sympathetic nervous system influences in the coronary vasculature

James T. Pearson[1,2]; Daryl O. Schwenke[3]; Hirotsugu Tsuchimochi[1]; Takashi Sonobe[1]; Vijayakumar Sukumaran[1]; Mikiyasu Shirai[4] (*[1]Department of Cardiac Physiology, National Cerebral & Cardiovascular Center, Japan; [2]Department of Physiology, Monash University, Australia; [3]Department of Physiology, University of Otago, New Zealand; [4]Department of Advanced Medical Research for Pulmonary Hypertension, National Cerebral & Cardiovascular Center, Japan*)

Sympathetic overactivation is considered to play an important role in both ischemic and non-ischemic heart disease. It is well established that acute myocardial infarction evokes sustained increases in cardiac sympathetic nerve activity that can exacerbate left ventricle function and remodeling. However, there are two sides to every coin. The sympathetic nervous system (SNS) exerts tonic influences on coronary vasomotor tone through beta-adrenergic receptors (vasodilation) and alpha-adrenergic receptors (vasoconstriction). Here we show how acute ischemia induces coronary microvessel dilation that is prevented by pretreatment with non-selective beta-blockade. On the other hand, chronic overactivation of the renin-angiotensin-aldosterone system and the SNS in metabolic syndrome and hypertensive diabetes contribute to a progressive decline in coronary endothelial function and coronary flow reserve, leading to remodeling and congestive heart failure. Our recent studies show that sympathetic overactivation in prediabetic rats contributes to the loss of endothelium derived hyperpolarisation factor (EDHF)-mediated microvessel perfusion through increased oxidative/nitrosative stress and upregulation of endothelin-1. Herein we consider the importance of beta- and alpha-adrenergic receptors in the modulation of the coronary circulation by the SNS. (COI: No)

S13-4

Altered differential control of cardiac and renal sympathetic nerve activity in hypertension

Rohit Ramchandra[1]; Darvina Mahesh[1]; Jaap Joles[2]; Tycho Tromp[1,2] (*[1]Department of Physiology, The University of Auckland, New Zealand; [2]University Medical Centre, Utrecht University, The Netherlands*)

There is increasing evidence that hypertension is initiated and maintained by elevated sympathetic tone. Increased sympathetic drive to the heart is linked to cardiac hypertrophy in hypertension and worsens prognosis. However, cardiac sympathetic nerve activity has not previously been directly recorded in hypertension. We hypothesized that directly recorded cardiac sympathetic nerve activity levels would be elevated during hypertension. Adult ewes either underwent unilateral renal artery clipping (n=12) or sham surgery (n=15). Two weeks later, electrodes were placed in the contralateral renal and/or cardiac nerves to record sympathetic nerve activity. Baseline levels of sympathetic nerve activity as well as baroreflex control of heart rate and sympathetic drive were examined. Unilateral renal artery clipping induced hypertension (mean arterial pressure 109±2 vs 91±3 mmHg in shams, $p<0.001$). The heart rate baroreflex curve was shifted rightwards but remained intact. In the hypertensive group, cardiac sympathetic burst incidence (bursts/100 beats) was increased (39±14 vs 25±9 in normotensives, $p<0.05$), whereas renal sympathetic burst incidence was decreased (69±20 vs 93±8 in normotensives, $p<0.01$). Renovascular hypertension is associated with differential control of cardiac and renal sympathetic nerve activity; baseline cardiac sympathetic nerve activity is increased whereas renal sympathetic nerve activity is decreased. (COI: No)

S13-5

Cortical and subcortical structures involved in the generation of muscle sympathetic nerve activity

Vaughan G Macefield[1,2,3]; Luke A Henderson[4] (*[1]Human Autonomic Neurophysiology Lab, Baker Heart and Diabetes Institute, Melbourne, Australia; [2]School of Medicine, Western Sydney University, Australia; [3]Neuroscience Research Australia, Australia; [4]Discipline of Anatomy & Histology, Sydney Medical School, University of Sydney, Australa*)

Concurrent recording of muscle sympathetic nerve activity (MSNA) and functional magnetic resonance imaging (fMRI) of the brain allows one to functionally identify structures involved in the generation of MSNA and hence the beat-to-beat control of blood pressure. Using MSNA-coupled fMRI we have functionally identified the human homologue of the rostral ventrolateral medulla (RVLM) – the primary output nucleus for MSNA – and demonstrated a positive relationship between MSNA and BOLD signal intensity in the dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH), insula, dorsolateral prefrontal cortex (dlPFC), posterior cingulate cortex (PCC) and precuneus. Conversely, there is an inverse relationship between MSNA and BOLD signal intensity in the caudal ventrolateral medulla (CVLM), nucleus tractus solitarius (NTS) and the midbrain periaqueductal gray (PAG). During the pathophysiological increase in MSNA associated with obstructive sleep apnoea, and the resultant increase in blood pressure, signal intensity was higher in dlPFC, mPFC, ACC and precuneus than in controls. However, it was lower in RVLM in OSA than in controls; we interpret this as reflecting a withdrawal of active inhibition of the RVLM in OSA. These changes were reversed following 6 months of continuous positive airway pressure. We conclude that multiple cortical and subcortical areas are functionally coupled to the RVLM, which is functionally coupled to the generation of spontaneous bursts of MSNA. (COI: No)

Local Organizing Committee Symposium

Symposium14

Advances in understanding cerebellar LTD and motor learning: Masao Ito Symposium

(March 29, Fri., 15:10-17:10, Room A)

S14-1

Discovery and investigation of cerebellar long-term depression at Masao Ito's lab

Masanobu Kano[1,2] (*[1]Department of Neurophysiology, Graduate School of Medicine, The University of Tokyo, Japan; [2]International Research Center for Neurointelligence* (*WPI-IRCN*)*, The University of Tokyo Institutes for Advanced Study* (*UTIAS*)*, The University of Tokyo, Japan*)

The Marr-Albus-Ito theory is perhaps the most influential hypothesis for cerebellar motor learning. This theory is based on the assumption that the strength of a subset of parallel fiber (PF) to Purkinje cell (PC) synapses is modified when they are activated conjunctively with climbing fiber (CF) synaptic inputs. However, direct demonstration of this form of synaptic plasticity was not available until the discovery of long-term depression (LTD) by Masao Ito and his coworkers in 1982. In decerebrate rabbits, they demonstrated that responses of PCs to either mossy fiber or PF stimulation underwent LTD after the mossy fiber or PF stimulation was applied in conjunction with CF stimulation at 1-4 Hz for 100 to 300 times. In 1987, Sakurai in Ito's lab reported LTD in acute cerebellar slices, and presented the first evidence that CF-induced elevation of intracellular Ca^{2+} levels in PCs is crucial for LTD induction. Then, in 1990, Hirano at Gunma University independently demonstrated LTD in cultured PCs. The success of recording LTD in cerebellar slices and cultured PCs paved the way for the intense investigation of molecular/cellular mechanisms of LTD in the 1990s and 2000s. In this introductory talk, I will give an outline of early work on LTD at Masao Ito's lab in the1980s. I will also talk about how metabotropic glutamate receptor 1 (mGluR1) plays crucial roles in LTD and motor learning, the concept of which stemmed from my thesis work at Ito's lab. (COI: No)

S14-2

Temporal aspects of cerebellar long-term synaptic depression

Keiko Tanaka-Yamamoto; Taegon Kim; Yukio Yamamoto (*Center for Functional Connectomics* (*CFC*)*, Korea Institute of Science and Technology* (*KIST*)*, Korea*)

An important feature of long-term synaptic plasticity is that plastic changes in synaptic transmission lasts for long time after short-lasting stimulation, yet such time course of synaptic plasticity has not been systematically explained. Our previous studies of cerebellar long-term depression (LTD) demonstrated that a positive feedback kinase loop extends a time of kinases being active and consequently works for gradual LTD expression by enhancing the internalization of AMPA-type glutamate receptors (AMPARs). We have also found mechanisms that prevent sporadic activation of this loop at the basal state, yet boost the activation after the LTD induction. However, the subsequent events accomplishing the LTD maintenance after enhanced AMPAR internalization was not clarified. We recently addressed this issue by developing a genetically-encoded, photosensitive inhibitor of late endosome (LE) sorting, and then discovered that LTD relies on timely regulated LE sorting working at the end of enhanced AMPAR internalization. Due to such LE sorting, the number of AMPARs residing within endocytic recycling pathways was reduced, which leads to LTD maintenance.Further, experimental and computational analyses revealed a switch-like property of LE sorting. Thus, our results indicate that recycling AMPARs are reduced by transient LE sorting of internalized AMPARs, and that this process works as a switch accomplishing the transition from the expression to the maintenance of LTD. (COI: No)

S14-3

LTD and the search for the cerebellar memory trace

Christian Hansel (*Department of Neurobiology, University of Chicago, USA*)

Over the last years, Masao Ito's cerebellar learning theory with LTD at its core has faced multiple challenges as well as further experimental support. A widespread consensus view holds that LTD is important for cerebellar learning, but that it is one of several plasticity mechanisms that need to be activated. In this talk, I will present new data from our laboratory that show that LTD recorded under realistic, near-physiological conditions displays needs for its instructive climbing fiber signal that differ from those previously established. Based on two-photon calcium imaging as well as electrophysiological recordings from intact animals, I will demonstrate the relevance of these specific complex spike patterns in vivo. In addition, I will propose a new model of the cellular machinery underlying delay eye blink conditioning that includes synaptic and intrinsic plasticity components. (COI: No)

S14-4

New optogenetical tool clarified that the cerebellar LTD was essential for motor learning

Shinji Matsuda (*Department of Engineering Science, The University of Electro-Communications, Japan*)

Synaptic plasticity has been thought to be the underlying mechanism for learning and memory. Long-term depression (LTD) is one of the most well studied forms of synaptic plasticity. It has been clarified that the clathrin mediated endocytosis of AMPA type glutamate receptor (AMPA receptor) is the molecular basis for LTD induction. However, it is still unclear whether the endocytosis of AMPA receptor directly regulate memory and learning. Here we developed the new optogenetic tool, PhotonSABER, which can regulate the endocytosis of AMPA receptor in a spatially and temporally controlled manner. We clarified that the endocytosis of AMPA receptor during the LTD induction was inhibited by light stimulation both in hippocampal and cerebellar Purkinje neurons. Our electrophysiological experiments also indicated that the induction of LTD was almost completely blocked by light stimulation in the cerebellar slices from Purkinje cell specific PhotonSABER knock in mice. We found that the light stimulation to the bilateral flocculi of PhotonSABER knock in mice inhibited cerebellar motor learning, as well as the decrease of the synaptic AMPA receptor in the flocculus. Moreover, the other cerebellar motor learning, adaptation of vestibulo-ocular reflex, was also inhibited by the fiberoptic illumination to the bilateral flocculi. Our results indicated that the cerebellar LTD is directly linked to the motor learning. (COI: No)

S14-5

Specialization of the rules for cerebellar LTD at different parallel fiber-Purkinje cell synapses

Jennifer L Raymond (*Department of Neurobiology, Stanford University School of Medicine, USA*)

It was almost half a century ago that Professor Masao Ito first proposed that long-term depression (LTD) at the parallel fiber-to-Purkinje cell synapses supports cerebellum-dependent learning. Recently, my laboratory discovered that the properties of cerebellar LTD vary across parallel fiber synapses, and can be exquisitely specialized for a particular behavioral task. More specifically, the timing requirements for LTD can be tightly tuned for the feedback delay for errors to get reported on the cerebellar climbing fibers. This provides a synaptic solution for the temporal credit assignment problem, and a mechanism for temporally precise learning. (COI: No)

Local Organizing Committee Symposium

Symposium15

Thermal biology: A new world of life science (whole day symposium) part II

(Co-organized by Grant-in-Aid for Scientific Research on Innovative Areas 'Thermal Biology' of MEXT, Japan)

(March 29, Fri., 15:10-17:10, Room B)

S15-1

Effects of temperature on seasonal adaptation: Towards the understanding of human seasonality

Takashi Yoshimura[1,2,3] (*[1]Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Japan; [2]Graduate School of Bioagricultural Sciences, Nagoya University, Japan; [3]Division of Seasonal Biology, National Institute for Basic Biology*)

The appropriate timing of various seasonal processes, such as reproduction, migration and hibernation, is crucial to the survival of animals living in temperate regions. Although this phenomenon attracts great interest, its underlying mechanisms are not well understood. By using non-model organisms that have highly sophisticated seasonal responses, we have uncovered the universality and diversity in the signal transduction pathway regulating seasonal rhythms in vertebrates. Although humans are not typically considered seasonal animals, some evidence suggests that seasonal variation in physiology and behavior also exists in humans. For example, the wavelength settings for the unique yellow hue are shifted to shorter wavelengths in summer compared with those in winter. Seasonal affective disorder patients, experiencing recurrent winter episodes of depressed mood, overeating and hypersomnia, show electroretinogram changes in winter, with lower sensitivity compared with healthy subjects. These observations highlight the potential importance of the retina in seasonality, but the molecular basis of these seasonal changes remains unknown. We have recently discovered dynamic plasticity in phototransduction regulates seasonal changes in color perception in Japanese medaka fish (*Oryzias latipes*), an excellent model for studying seasonal adaptation. I will discuss how we can better understand human seasonal rhythms using unique animal models. (COI: No)

S15-2

Mechanisms of psychological impacts on thermoregulation and metabolism

Kazuhiro Nakamura (*Department of Integrative Physiology, Nagoya University Graduate School of Medicine, Japan*)

Psychological stress and emotion impact on the brain circuit systems that regulate body temperature and metabolism. Many psychological stressors stimulate sympathetic outflows to thermoregulatory effectors to increase body temperature. We have studied the central circuit mechanism by which psychological stress signals elicit sympathetic thermogenesis in brown adipose tissue and tachycardia leading to stress-induced hyperthermia. This central circuit involves a stress-activated excitatory (glutamatergic) pathway from the dorsomedial hypothalamus to the sympathetic premotor region in the medulla oblongata. We recently identified a cortical area that provides excitatory stress inputs to the hypothalamomedullary sympathetic pathway to drive the stress responses. Selective ablation of the corticohypothalamic pathway eliminates the stress-induced sympathetic responses, but does not affect basal thermoregulation, indicating the essential role of this stress pathway in driving stress-induced sympathetic responses.

Our study has also focused on the role of the oxytocinergic nervous system, which is activated by emotional stimuli, in central metabolic regulation, since ablation of oxytocin neurons causes obesity as seen in Prader-Willi syndrome. We have found that a hypothalamo-medullary oxytocinergic pathway stimulates sympathetic thermogenic outflow to brown adipose tissue. This oxytocinergic sympathoexcitation may explain the chronic impact of emotion and stress on metabolism. (COI: No)

S15-3

TRP ion channels – internal/deep-brain temperature sensors and guardians of homeostasis?

Jan Erik Siemens[1]; Gretel B. Kamm[1]; Juan C. Boffi[2]; Hong Wang[1,4]; Thomas Kuner[2]; Kun Song[1,3] (*[1]Department of Pharmacology, Heidelberg University, Germany; [2]Department of Functional Neuroanatomy, Heidelberg University, Germany; [3]Max Delbrück Center for Molecular Medicine (MDC), Germany ; [4]The Brain Cognition & Brain Disease Institute, University Town of Shenzhen, China*)

Transient receptor potential (TRP) ion channels have been identified as versatile, multimodal molecular sensors. Particularly, several members of the extended TRP ion channel family detect temperature changes in the somatosensory nervous system. Pharmacology and genetic deletion experiments have shown that TRP channels are necessary for mediating responses to painfully hot or cold temperatures and they become sensitized under inflammatory conditions leading to exacerbated nociceptive signals. TRPs have therefore emerged as targets for analgesic therapy.

Besides constituting a warning system alerting us about noxious thermal conditions, temperature detection in the innocuous range serves another important feat: Mammalian organisms possess the remarkable ability to maintain internal body temperature (T_{core}) within a narrow range close to 37°C despite wide environmental temperature variations. The brain's neural "thermostat" is made up by central circuits in the hypothalamic preoptic area (POA), which orchestrate peripheral thermoregulatory responses to maintain T_{core}. How the POA detects temperature and integrates temperature information to achieve thermal balance is largely unknown.

I will present our recent findings that implicate TRP channels in hypothalamic thermoregulation. I will conclude with an outlook on potential future experiments geared to address key questions concerning internal temperature detection and thermoregulation. (COI: No)

International Scientific Program Committee Symposium

Symposium16

Gastrointestinal Control of Energy Metabolism (CAPS, China)

(March 29, Fri., 15:10-17:10, Room C)

S16-1

Gastric mTORC1 as a fuel sensing mechanism and its role in lipid homeostasis

Weizhen Zhang (*Department of Physiology and Pathophysiology, Peking University Health Science Center, China*)

Gastric mechanistic target of rapamycin (mTOR) signaling is inversely associated with the expression and secretion of ghrelin, a 28-aa peptide hormone produced by X/A-like cells. We hypothesized that mTOR signaling in X/A like cells controls global lipid metabolism. To test this hypothesis, we established a *ghrl-cre* transgene in which the cre enzyme is expressed in X/A-like cells under the control of the ghrelin-promoter. $mTOR^{flox/flox}$ and $TSC1^{flox/flox}$ mice were bred with *ghrl-cre* mice to generate mG or TG mice, within which mTOR signaling was suppressed or activated respectively. Lipid metabolism in liver and adipose depots was analyzed. Under the control of the ghrelin-promoter, cre enzyme is exclusively expressed in stomach X/A-like cells in adult animals. Knockout of mTOR in X/A-like cells increased circulating acyl-ghrelin and promoted hepatic lipogenesis with effects on adipose depots. Activation of mTOR signaling by deletion of TSC1, its upstream inhibitor, decreased ghrelin expression and secretion, altering lipid metabolism as evidenced by resistance to HFD-induced obesity and hepatic steatosis. Both ghrelin administration and rapamycin, an inhibitor of mTOR, altered the phenotypes of TG mice. Our observations indicate that Gastric mTOR signaling in X/A-like cells contributes to organism lipid homeostasis by regulating hepatic and adipose lipid metabolism. Gastric mTOR signaling may provide an alternative strategy for intervention in lipid disorders. (COI: No)

S16-2

Gut-derived Dopamine and Its Regulation on Intestinal Barrier Function

Jinxia Zhu; Xiaoyan Feng; Chenzhe Liu; Xiaoli Zhang (*Department of Physiology and Pathophysiology, Capital Medical University, China*)

Dopamine (DA), an important catecholaminergic neurotransmitter, is generated in central nervous system and peripheral organs. About 50-70% of the body's DA comes from gut, including enteric neurons, gastrointestinal (GI) epithelia, enteric immune cells and pancreatic β cells. DA plays important roles in aging, brain-gut axis, GI motility, epithelial ion transport, inflammation and tumor. Recent studies have reported that a substantial level of DA existed in the gut lumen and it can be produced by intestinal bacteria, but the role of luminal DA in gut is unclear. In our study, the basolateral DA induced a short-circuit current (△Isc) in duodenum, while luminal DA increased the duodenal HCO3- secretion without change in △Isc. A substantial amount of DA was observed in the rat feces and its concentration was much higher than that in colonic tissue and serum. In rat colonic mucosa preparations, DA added to luminal side markedly elevated the levels of DA and its metabolite in colonic tissue, which was blocked by dopamine transporter inhibitor. Pretreatment with monoamine oxidase or catechol-O-methyl transferase inhibitors obviously decreased the decomposition of DA and elevated the DA content in colonic mucosa. Furthermore, gut-derived DA also mediated duodenal/colonic mucosa resistance and permeability, and mucus secretion. In conclusion, luminal DA may play important roles in the GI mucosal protection and gut functional regulation. (COI: No)

S16-3

Hormonal and neuronal regulatory mechanisms of gastrointestinal motility in the Suncus murinus

Ichiro Sakata; Takafumi Sakai (*Graduate school of Science and Engineering, Saitama University, Japan*)

Gastrointestinal motility is regulated by hormonal factors, as well as the autonomic and enteric nervous systems. Recently, we established *Suncus murinus* (suncus) as a small model animal to investigate gastrointestinal motility. Suncus produces the gastrointestinal hormones motilin and ghrelin, and the anatomy of the stomach and the patterns of gastric motility in suncus are similar to those in humans. We used this animal model to investigate the regulatory mechanisms of gastric contraction in suncus in the fed and fasting states. In the interdigestive state, ghrelin is involved in the regulation of phase II of the migrating motor complex (MMC) via the action of the vagal afferent. Additionally, coordinated activity of ghrelin and motilin stimulates strong gastric contractions corresponding to phase III of the MMC, and ghrelin-mediated GABAergic neurons play a key role in motilin-induced gastric contraction. In the postprandial state, we observed that hormonal regulation of ghrelin and motilin induces postprandial gastrointestinal contraction. In this discussion, we describe the usefulness of suncus as a unique animal model to investigate gastrointestinal motility and summarize the hormonal and neuronal regulation of gastrointestinal motility. (COI: No)

S16-4

The X/A-like cell as a regulator of food intake

Andreas Stengel[1,2] ([1]*Psychosomatic Medicine, University Tuebingen, Germany;* [2]*Psychosomatic Medicine, Charité University, Germany*)

Initially ghrelin has been identified in gastric X/A-like cells. Since ghrelin is the only known peripherally produced and centrally acting hormone to stimulate food intake this attracted a lot of attention. Subsequently, other peptide products of this cell type such as desacyl ghrelin and nesfatin-1 have been identified. Since these peptides were shown to inhibit food intake it has been hypothesized that the X/A-like cell could act as a dual regulator of food intake. The present talk will highlight these developments and discuss potential routes of future research. (COI: No)

S16-5

Regulation of GLP1 secretion and mitochondrial function by Berberine in colon enterocytes

Jianping Ye (*Central Lab, Shanghai Jiaotong University Affiliated 6th People's Hospital East, China*)

L-cell dysfunction is reported for GLP-1 reduction in type 2 diabetes. However, the mechanism of dysfunction remains unknown. We investigated the mechanism in diet-induced obese (DIO) mice, which were treated with berberine (100 mg/kg/day) for 8 weeks to regulate GLP-1 expression. Mitochondrial activities of the colon enterocytes were compared among three groups of mice (lean, DIO and DIO+berberine) at the end of treatment. A cellular model treated with palmitic acid (PA) was used in the mechanism study. A reduction in GLP-1 expression was observed in DIO mice with mitochondrial stress responses in the colon enterocytes. The mitochondria exhibited cristae loss, membrane rupture and mitochondrial swelling, which was observed with an increase in ATP abundance, complex I activity, and deficiency in the activities of complexes II and IV. Those changes were associated with dysbiosis and a reduction in SCFAs in the colon of DIO mice. In the cellular model, an increase in ATP abundance, loss of mitochondrial potential and elevation of apoptosis were induced by palmitic acid (PA). All of the alterations in DIO mice and the cellular model were attenuated by berberine. The mitochondrial stress responses were observed in the colon enterocytes of DIO mice for GLP-1 reduction. The stress was prevented by berberine in the restoration of GLP-1 expression, in which BBR may act through direct and indirect mechanisms. (COI: No)

Local Organizing Committee Symposium

Symposium17

Teaching physiology; International perspectives (whole day symposium) part II

(March 29, Fri., 15:10-17:10, Room D)

S17-1

Integration of social practice and medical knowledge in an outcome-based curriculum at NCKU Medical School

Mei-Ling Tsai (*Department of Physiology, National Cheng Kung University, Taiwan*)

An Epistemology for clinical medicine is derived from interpretative action and interaction. The former is based on medical research and biomedical data. The latter is based on the interpersonal communication through various means. However, medical education now highly addresses on scientific training and data interpretation. Very few courses are designed to build their knowledge through personal interaction. To empower their capacities of inductive reasoning skills and knowledge constuctivism, service learning will be a best practice. In the 21st century, impact of ageing on workforces of health care in the society becomes obvious but no course in medical programs is designed to enhance student's self-awareness on the elderly care. Therefore, conversion of service learning to social practices allowed the students to conduct health check-ups for the elderly and interact with the elderly with simple intrview, and then offer health education to the elderly. Our post-class evaulation showed that shadowing coupled with the approach of learning by teaching improves their skills of constructing knowledge through interpersonal communication. (COI: No)

S17-2

PHY-STORY : Students Discovering and Telling their Stories of Physiology

Cheng Hwee Ming (*Department Physiology, Faculty of Medicine, University Malaya, Malaysia*)

Physiological knowledge continues to grow and finer mechanistic details at the cellular level are elucidated to fit into the whole body homeostatic schemes. With the increasing volume of new information on cellular events, the big picture of integrated Physiology can be lost. In addition, it is also noted that students tend to become rootless and deficient in appreciating the historical milestones in the development of current Physiological knowledge. Besides an intentional inclusion of physiologist icons and key classical experiments in our lectures, students can be motivated to discover such PHY-stories for themselves. Short assignments to search out and present these narratives will enhance learning of Physiology. Several of my students in Universiti Malaya also initiated a Facebook group to initiative such a focus for their classmates. During small group tutorials, encouraging students to express, in their own words, their grasp of phenomenons like e.g. Haldane's effect, Starling's cardiac mechanism, renal autoregulation provides opportunity for self discovery of their own understanding and misconceptions. Directed, self learning using PHY-story can be an engaging activity to help our students think outside their power-point boxes. (COI: No)

S17-3

How to make students alert during lectures

Mangala Gunatilake (*Dept. of Physiology, Faculty of Medicine, University of Colombo, Sri Lanka*)

Within this ever-changing world, it is our duty as teachers to enhance student learning and we must make every effort to keep students alert specially during large group teaching. Lectures being a well-accepted teaching mode to impart knowledge to students in large classes, they should be more interactive to attract students' interest in the area of study. Inclusion of more patient based scenarios, asking questions from the related areas during the lecture, interactions with students in a friendly manner are some of the techniques that could maintain alertness of students. Aa per my experience, students prefer to interact with the lecturer when lecturer tries to be in the same level with the students while walking through the lecture hall without standing in the stage. In the feedback obtained, students indicated they prefer this method as they could concentrate more on the subject to grasp necessary information. (COI: No)

S17-4

Teaching Physiology - Students' Voice

Noriyuki Koibuchi (*Gunma University Graduate School of Medicine, Japan*)

Student- centered education is the most important key word that all teachers should keep in mind. To fulfill this issue, we should keep trying to improve our curriculum. To construct better curriculum, it is indeed important to hear students' opinion. During the final program for the whole day symposium on Physiology education, therefore, we will invite several undergraduate/postgraduate students who are taking Physiology course and ask them to present their experience on physiology education followed by the panel discussion on how to create the best student-centered curriculum to facilitate students' motivation to actively learn physiology. (COI: No)

Symposium18

Dynamics of membrane trafficking and intracellular signaling

(March 29, Fri., 15:10-17:10, Room E)

S18-1

Optogenetic control of diverse molecular and cellular processes in the mouse brain

Won Do Heo[1,2] ([1]*Department of Biological Sciences, KAIST, Korea;* [2]*Center for Cognition and Sociality, IBS, Korea*)

My group has been developing various bio-imaging and optogenetic tools for the study of cell signaling in live cells as well as neuronal functions *in vivo*. Novel optogenetic toolkit developed by my group is highly advantageous compared with conventional approaches in that it allows finely manipulated signaling pathways in a spatial and temporal resolution, thereby making it possible to dissect and analyze the transient dynamics of signaling processes within a defined period. These tools are very useful not only for imaging based researches in cell biology, but also for the studies in neuroscience. Recently developed optogenetic strategies have brought significant changes the way in which signaling in living cells is studied in neurobiology and other disciplines. Novel optogenetic toolkit my group has been developing are capable of providing what channelrhodopsins could not offer previously, contributing in a disparate perspective of neuroscience. We are applying the new technologies to the study of spatiotemporal roles of signaling proteins and second messengers in synaptic plasticity and learning and memory in normal and disease mouse models. (COI: No)

S18-2

Imaging secretory cells and molecular configurations of exocytic proteins

Noriko Takahashi[1]; Hiroyasu Hatakeyama[1]; Tomomi Oshima[1]; Yuichi Morimoto[2]; Haruo Kasai[2] ([1]*Department of Physiology, Kitasato University School of Medicine, Japan;* [2]*Structural Physiology, Graduate School of Medicine, The University of Tokyo, Japan*)

Insulin is secreted from pancreatic beta cells and regulates blood glucose levels. We have investigated the SNARE assembly at the plasma membrane of pancreatic beta cells and presynaptic terminals of cortical neurons, using two-photon fluorescence lifetime imaging (2p-FLIM) of Forster resonance energy transfer (FRET). We have revealed that the trans-SNARE assembly was detected in the presynaptic terminals in the resting state, and such assembly was correlated with the release probability. In additions, a domain-swapped conformation was detected, where a helix of SNAP25 (SN1) bound to the second helix (SN2) of the other SNAP25 molecule and formed oligomer of SNARE complex. Such a structure was suggested to underly the ultrafast exocytosis from the synapse. In contrast, such trans-SNARE complex involving vesicular SNARE molecule was undetectable in the resting state of beta cells, but was observed just before insulin exocytosis. Recently we also began to image the pancreatic islets *in vivo* using two-photon microscopy, and to image the insulin granule movement inside the cells using confocal microscopy. Combined with the data from these methods, we are investigating the regulatory system of insulin secretion. (COI: No)

S18-3

Fluorescence Imaging of membrane dynamics and intracellular signaling

Yusuke Ohba (*Department of Cell Physiology, Faculty of Medicine, Hokkaido University, Japan*)

Endocytosis mediates the internalization of a variety of endogenous or exogenous substances, and is accurately regulated by intracellular signaling. We have previously reported that the complex formed by the small GTPase Ras and phosphoinositide 3-kinase (PI3K) is translocated from the plasma membrane to endosomes, signaling from which thereby regulates clathrin-independent endocytosis (Tsutsumi *et al*., Cell Signal, 2009; Fujioka *et al*., PLoS One, 2011). However, the precise molecular mechanism through which the Ras-PI3K complex is recruited to the endosome has yet to be determined. Here, we have identified the amino acid sequence responsible for endosomal localization of the Ras-PI3K complex, which was named RAPEL after Ras-PI3K endosomal localization. PI3K lacking RAPEL failed to translocate to endosomes, and expression of RAPEL suppressed endocytosis, demonstrating that the sequence dictates the regulation of Ras-PI3K signaling in the endosome. To further elucidate the underlying molecular mechanism, we screened RAPEL binding factors and identified several mitochondrial membrane proteins as candidates. Expression levels of these proteins were either negatively or positively correlated with endosomal localization of Ras-PI3K complex and endocytic activities, suggesting the possibility of involvement of mitochondrial in the regulation of endocytosis. In fact, physical contact between endosomes and mitochondria was decreased under the suppression of the mitochondrial factor. (COI: No)

S18-4

Essential role of class II PI3K in endocytosis and endosomal signaling

Kazuaki Yoshioka[1]; Khin Thuzar Aung[1]; Azadul Kabir Sarker[1]; Sho Aki[1]; Kuntal Biswas[1]; Noriko Takuwa[1,2]; Yoh Takuwa[1] ([1]*Department of Physiology, Kanazawa University, Japan;* [2]*Department of Health Science, Ishikawa Prefectural Nursing University, Japan*)

PI3K family regulates diverse dynamic membrane events. While class I and class III of PI3K are well-characterized, functional roles of class II PI3K isoforms (PI3K-C2α, -C2β and -C2γ) were largely unknown. We demonstrated that global C2α-KO mice were embryonic lethal due to severe defects in angiogenesis. The inducible endothelial cell (EC)-specific C2α deletion resulted in a similar phenotype. Mechanistically, C2α was required for ligand-induced endocytosis of the angiogenic receptors including VEGFR2, S1P1 and TGFβ receptor, and thereby receptor-mediated endosomal activation of Rho, Rac, and Smad2/3. C2α and also C2β were required for clathrin-mediated fluid-phase endocytosis (pinocytosis) in EC. Both C2α and C2β were co-localized with clathrin-coated pits and vesicles. However, C2β but not C2α was highly co-localized with actin filament-associated clathrin-coated structures (CCS), and required for actin filament formation at the CCS. Among several different cell type-specific class II PI3K KO mice, smooth muscle (SM)-specific KO of both C2α and C2β in female mice resulted in impaired parturition due to defective uterine SM contraction. In contrast, the single KO of either C2α or C2β did not impair parturition. The double KO mice showed defective endosomal Rho activation and diminished myosin phosphorylation. These results indicate that C2α and C2β are involved in clathrin-mediated endocytosis with cell type-specific functional compensations. (COI: Properly Declared)

S18-5

Morphological changes of plasma membrane and protein assembly during clathrin-mediated endocytosis

Shige H. Yoshimura[1]; Aiko Yoshida[1,2]; Yoshitsuna Itagaki[1]; Yuki Suzuki[3] ([1]*Graduate School of Biostudies, Kyoto University, Japan;* [2]*Graduate School of Medicine, Hokkaido University, Japan;* [3]*Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, Japan*)

Clathrin-mediated endocytosis (CME) proceeds through a series of morphological changes of the plasma membrane induced by a number of protein components. Although the spatiotemporal assembly of these proteins has been elucidated by fluorescence-based techniques, the protein-induced morphological changes of the plasma membrane have not been fully clarified in living cells. Here, we visualize membrane morphology together with protein localizations during CME by utilizing high-speed atomic force microscopy (HS-AFM) combined with a confocal laser scanning unit. The plasma membrane starts to invaginate approximately 30 s after clathrin starts to assemble, and the aperture diameter increases as clathrin accumulates. Actin rapidly accumulates around the pit and induces a small membrane swelling, which, within 30 s, rapidly covers the pit irreversibly. Inhibition of actin turnover abolishes the swelling and induces a reversible open±close motion of the pit, indicating that actin dynamics are necessary for efficient and irreversible pit closure at the end of CME. (COI: No)

International Scientific Program Committee Symposium

Symposium19

Mitochondrial Physiology and Pathophysiology (KPS, Korea)

(March 29, Fri., 15:10-17:10, Room F)

S19-1

Mitochondrial quality control and its metabolic regulation by reactive persulfide species

Motohiro Nishida[1,2] (*[1]Division of Cradiocirculatory Signaling, National Institute for Physiological Sciences, National Institutes of Natural Sciences; [2]Department of Translational Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kyushu University*)

Mitochondria are dynamic organelles that continuously undergo fission and fusion, which are necessary for maintaining bioenergetic homeostasis in cells. Mitochondrial fission and fusion cycle is precisely regulated by three GTP-binding proteins, dynamin-related protein 1 (Drp1), mitofusins (Mfn1 and mfn2) and optic atrophy 1 (Opa1), and these three G proteins have redox-sensitive cysteine (Cys) residues. Especially, mitochondria predominantly show tubular form in adult cardiomyocytes and are reported to be fragmented by exposure to electrophilic chemical substances. We found that electrophilic modification of Cys-624 on Drp1, caused by endogenous or exogenous electrophiles, increased Drp1 GTPase activity as well as cardiac vulnerability to mechanical stress in rodent hearts. In contrast, reactive sulfide species such as Cys persulfides that are produced in cells are likely involved in electrophile metabolism. Protein Cys persulfide detection assay revealed that endogenous Drp1 abundantly formed Cys persulfide in rat cardiomyocytes, and exposure to environmental electrophiles such as methylmercury (MeHg) reduced Drp1 persulfide level. Supplementation of sulfur to Cys-624 by exogenous treatment with NaHS as a sulfur substrate for 24 hours completely abolished electrophile-mediated sulfur deprivation of Drp1 protein as well as exacerbation of cardiac cell injury induced by mechanical stretch. These results strongly suggest that formation of Cys persulfide on Drp1 proteins play a key role in mitochondrial quality control and bioenergetics by negatively regulating Drp1 activity. (COI: No)

S19-2

Regulation of systemic energy metabolism in altered mitochondrial proteostasis

Minho Shong (*Department of Internal Medicine, Chungnam National University, Korea*)

Recent *in vivo* studies in *C. elegans* and *Drosophila* revealed that UPRmt activation by inhibition of mitochondrial electron transport chain (ETC) functions increases lifespan. This effect is modulated by cell-autonomous and cell-non-autonomous factors, known as mitokines. However, extrapolation of these studies into mammalian systems is extremely difficult because generalized impairment of ETC function in mice uniformly results in progressive deterioration of organ functions and premature death. Recently, we successfully generated and demonstrated relevant mouse models of tissue-specific mitochondrial unfolded protein response (UPRmt) activation and ETC deficiency that are reminiscent of complex human disorders, e.g., neurodegeneration, Parkinson's disease, insulin resistance, and type 1 and 2 diabetes. These models are based on tissue-specific knockout of *Crif1*, which encodes a factor required for biogenesis of ETC subunits. Preliminary observations on phenotypes of tissue-specific *Crif1*-deficient mice revealed unique UPRmt activation e.g., adaptive transcriptomic changes and secretome responses (mitokines), which can be considered to be part of the phenomenon of "mitohormesis". Based on these observations, we postulated that tissue-specific UPRmt and mitokine responses are critical cell-non-autonomous modifiers in disease progression, and that individual mitokines may act as disease markers and potential therapeutic targets in complex human disorders. (COI: No)

S19-3

Roles of mitochondrial dynamics in cellular function, development, and differentiation

Naotada Ishihara[1,2]; Takaya Ishihara[1,2]; Emi Ogasawara[1,2]; Tadato Ban[2] (*[1]Graduate School of Science, Osaka University, Japan; [2]Institute of Life Science, Kurume University, Japan*)

Mitochondria are highly dynamic organelles that change their morphology in response to cellular signaling and differentiation. Dynamin-related GTPase Drp1 plays a key role in mitochondrial fission. Autosomal dominant mutation of human Drp1 leads to neonatal lethality, suggesting that mitochondrial dynamics is important for development and differentiation in mammals. To assess the physiological role of mitochondrial fission, we generated Drp1 KO mice by using Cre-loxP system. Drp1 knockout (KO) mice have developmental abnormalities, and die after embryonic day 12.5. Neural cell-specific Drp1 KO mice die shortly after birth due to brain hypoplasia with apoptosis, due to failed proper distribution of mitochondria. Recent studies have revealed that mitochondrial dynamics also plays an important role in the formation and maintenance of cardiomyocytes. We found that muscle-specific Drp1 KO mice showed neonatal lethality due to dilated cardiomyopathy. The Drp1 ablation in heart and primary cultured cardiomyocytes resulted in severe mtDNA nucleoid clustering and led to mosaic deficiency of mitochondrial respiration. The functional and structural alteration of mitochondria also led to immature myofibril assembly and defective cardiomyocyte hypertrophy. Thus, the dynamics of mtDNA nucleoids regulated by mitochondrial fission is required for neonatal cardiomyocyte development by promoting homogeneous distribution of active mitochondria throughout the cardiomyocytes. (COI: No)

S19-4

Mitochondrial oxidative stress associated with calcium and phosphate

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Intracellular Ca^{2+} homeostasis is particularly important for signal transduction and energy metabolism. Oxidative stress by glucolipotoxicity triggers aberrant ER Ca^{2+} release and thereby depletion of the ER Ca^{2+} store leading to serious ER stress. ER Ca^{2+} depletion also induces perilysosomal Ca^{2+} dysregulation and defective autophagic flux. Secondary to ER Ca^{2+} release, further increases in cytosolic and mitochondrial matrix Ca^{2+} aggravate mitochondrial oxidative stress and cytotoxicity. All these pathogenic changes were prevented by mitochondrial ROS scavengers. eliciting a vicious cycle. Inorganic phosphate (P_i) also plays an essential role in cell signaling and metabolism. Elevated P_i have serious cardiovascular complications, the underlying mechanisms of which remains unclear. Our group observed that cellular P_i uptake elicited cytosolic alkalinization which facilitates P_i transport into mitochondrial matrix. Increased mitochondrial P_i uptake accelerated superoxide generation, upregulation of osteogenic genes and calcific changes in aortic smooth muscle cells. Vascular calcification by high P_i was prevented by inhibition of mitochondrial ROS as well as Ca^{2+} and P_i transports. We suggest that there could be a close connection between intracellular Ca^{2+} and P_i regulation, which might be a novel therapeutic target for various metabolic diseases. (COI: No)

Symposium20

Adaptation mechanisms to external or internal environmental changes of respiratory system

(March 29, Fri., 15:10-17:10, Room G)

S20-1

Vaginal delivery is a strong adaptation signal to start spontaneous breathing

Keiko Ikeda[1,2]; Hiroshi Onimaru[3]; Kiyoshi Kawakami[2] (*[1]Department of Physiology, International University of Health and Welfare, Japan; [2]Division of Biology, Center for Molecular Medicine, Jichi Medical University, Japan; [3]Department of Physiology, Showa University School of Medicine, Japan*)

Na pump is the membrane protein responsible for maintaining $Na^+$and K^+ gradient across the cell membrane and consists of α and ß subunits. A glial-specific human α2 subunit isoform of the pump, *ATP1A2*, plays an important role in neuronal excitability during pregnancy. Point mutations in the *ATP1A2* cause familial hemiplegic migraine. To get insight into the pathophysiology of migraine and functional roles of the α2 in the brain, we examined the phenotype of *Atp1a2* knockout homozygous mouse fetuses (*Atp1a2*$^{-/-}$). All the *Atp1a2*$^{-/-}$ were alive in the wom but died after birth due to lack of spontaneous breathing. Interestingly, they showed different phenotype depending on the mode of delivery. About half of the *Atp1a2*$^{-/-}$ born through the birth canal, i.e., by vaginal delivery, started spontaneous breathing and survived several hours at most. On the contrary, all *Atp1a2*$^{-/-}$ born by caesarian section showed complete loss of breathing activity, followed by immediate death. We confirmed that vaginal delivery boosted the neural activities in both *Atp1a2*$^{-/-}$ and wild type by electrophysiological analyses using brainstem spinal cord preparation. Moreover, some of channel proteins showed different levels of expression between vaginal and caesarean deliveries. The results indicate that the stimulus of vaginal delivery is a strong signal for newborn to adapt environment and to start spontaneous respiration through maturation of respiratory neural network in both *Atp1a2*$^{-/-}$ and the wild-type. (COI: No)

S20-2

Pontine modulation of medullary respiratory circuit activity

Rishi R Dhingra; Mathias Dutschmann (*Division of Systems Neurophysiology, The Florey Institute of Neuroscience & Mental Health, Australia*)

The breathing pattern is generated by a columnar central pattern generator network extending rostrally from the ventrolateral medulla thru the dorsolateral pons. The eupneic respiratory pattern consists of three distinct respiratory phases: inspiration, post-inspiration and late-expiration. It has been known for more than a century that dorsolateral (dl) pontine brain areas are of major importance for respiratory pattern formation. For instance, inhibition of the Kölliker-Fuse (KFn) or medial parabrachial nuclei can severely disrupt the respiratory motor pattern by significantly delaying the inspiratory off-switch, which triggers a pathological two-phase breathing pattern called apneusis. These compelling data implicate that the formation of the eupneic breathing pattern depends on a presently undefined pontine modulation of medullary respiratory circuit activity. Here, we utilize high-density planar silicon multi-electrode arrays to assess the impact of dl pontine lesion (local microinjection of the GABA(A)R agonist isoguvacine) on medullary respiratory neuron activities in the pre-Bötzinger and Bötzinger complexes in the perfused preparation of rats. Using this approach, we characterize the dl pontine-dependent modulation of tonic- and phasic-respiratory neurons within the medullary core of the respiratory network. (COI: No)

S20-3

Hypoxic responses of the respiratory system

Yasumasa Okada[1]; Itaru Yazawa[2]; Kotaro Takeda[3]; Shuntaro Okazaki[4]; Makoto Uchiyama[5]; Yuki Kurita[5]; Isato Fukushi[1]; Shigefumi Yokota[6]; Yasuo Mori[5]; Hiroshi Onimaru[7] (*[1]Clin. Res. Ctr., Murayama Med. Ctr., Japan; [2]Global Res. Ctr. for Innovative Life Sci., Hoshi Univ. Sch. of Pharm. & Pharmaceut. Sc, Shinagawa, Japan; [3]Sch. of Hlth. Sci., Fujita Hlth. Univ., Japan; [4]Waseda Univ., Japan; [5]Dept. of Synthetic Chem. and Biol. Chem., Grad. Sch. of Engineering, Kyoto Univ., Japan; [6]Dept. of Anat. and Neurosci., Shimane Univ., Japan; [7]Showa Univ. Sch. of Med., Japan*)

Adequate maintenance of oxygen homeostasis is essential for the life. It has been described that hypoxia is sensed solely by peripheral chemoreceptors in respiratory control. However, hypoxia augments ventilation in unanesthetized awake animals even after peripheral chemodenervation, indicating the existence of the central hypoxia-sensitive mechanism. We hypothesized that astrocytes are a key player in hypoxic sensing, and tested the effects of pharmacological suppression of astrocytic activation using arundic acid in in vivo mice. Arundic acid suppressed hypoxic ventilatory augmentation and post-hypoxic persistence of ventilatory increase. Further, to investigate the purely central mechanism of hypoxic respiratory regulation, we used in vitro brainstem-spinal cord preparations isolated from newborn rats or mice. The preparation generates spontaneous respiratory output but lacks peripheral tissue, and is ideal in the study of purely central mechanism of hypoxic respiratory responses. When the superfusate gas composition is switched from control (95% O2, 5% CO2) to hypoxic (95% N2, 5% CO2), respiratory frequency increases transiently followed by a decrease in respiratory frequency. By calcium imaging we confirmed hypoxia activated astrocytes in the respiratory regions in the ventrolateral medulla. We propose that not only a subset of neurons but astrocytes in the medulla play roles in respiratory hypoxic sensing. (COI: No)

S20-4

How hypoxia blunts respiratory arousal from sleep

Peter George Burke (*Neuroscience Research Australia, Australia*)

Obstructive sleep apnoea causes significant intermittent arterial hypoxemia and hypercapnia, and leads to cardiorespiratory stimulation and arousal from sleep. These adjustments in breathing, circulation and vigilance are initiated by feedback by O_2/CO_2 chemoreceptors such as the carotid body, and by airway mechano-, thermal and pressure sensors. Brainstem neuronal networks receive and integrate this respiratory afferent information, and activate physiological and behavioural effectors via a sequence of central neuronal connections that remain poorly defined. Identifying these key intermediate neuronal circuits, such as the circuitry responsible for the arousal by carotid body activation, is vitally important. So too is defining the endogenous factors and mechanisms that modulate the information flow along these circuits that shape neural gain (ie ventilatory response) and adaptive behaviour (ie arousal threshold). My talk will focus on recent efforts using optogenetic methodologies to test the contribution of specific brainstem pathways in rodents for the hypoxic or hypercapnic-induced cardiorespiratory stimulation and arousal from sleep. I will also present evidence that increased endogenous adenosine tone, via sleep deprivation or mild CNS hypoxia, suppresses brainstem circuits triggering arousal. This metabolic neuromodulator may underlie the increased arousal threshold seen in obstructive sleep apnoea. (COI: No)

S20-5

Impact of cervical spinal cord injury on respiratory motor control

Kun-Ze Lee (*Department of Biological Sciences, National Sun Yat-sen University, Taiwan*)

Inspiratory flow is trigger by contraction of the diaphragm and intercostal muscles, which are innervated by the cervical phrenic and thoracic intercostal motoneurons, respectively. These spinal respiratory motoneurons were driven by the brainstem premotor neurons and spinal interneurons, therefore, cervical spinal cord injury is usually associated with respiratory compromises due to interruption of bulbospinal respiratory pathways and/or damage of phrenic motoneurons. The present study was aimed to investigate how cervical spinal cord injury influences inspiratory pump muscles at different injured stage in clinically-relevant spinal cord contusion rodent model. Our results demonstrated that both compensatory plasticity and spontaneous recovery could be evoked in the phrenic and intercostal motor system after cervical spinal cord injury. In addition, we noticed that the upper airway response evoked by vagal bronchopulmonary C-fiber activation was attenuated in cervical spinal cord contused rats. These results indicated that cervical spinal cord injury not only affects spinal respiratory activity but also has a significant impact on the vagal-mediated respiratory reflex and upper airway motor control. (COI: No)

Symposium21

New Paradigm in Physiology and Pathophysiology of Coagulation-fibrinolysis System

(March 29, Fri., 15:10-17:10, Room H)

S21-1

Overview of the cross-talk between the coagulation-fibrinolysis System and cellular functions

Tetsumei Urano; Yuko Suzuki (*Department of Medical Physiology, Hamamatsu University School of Medicine, Japan*)

The coagulation-fibrinolysis system plays a primary role in the hemostasis. The activity of coagulation and fibrinolysis are precisely regulated in a spatiotemporal manner, to which surfaces of vascular endothelial cells and platelets greatly contribute, thereby preventing the intravascular clot formation and contributing to maintaining vascular integrity. The coagulation-fibrinolysis system cross-talks with a variety of physiological and/or pathological phenomena other than thrombus formation and lysis including inflammation and immunity. The proteinase-activated receptors, a unique family of G protein-coupled receptor, plays a critical role as a key molecule at the interface between coagulation-fibrinolysis and these phenomena, by mediating various cellular effects of the serine proteinase in the coagulation-fibrinolysis system. In this symposium, the recent advancements in the field of physiology and pathophysiology of the coagulation-fibrinolysis system will be discussed from two points of view, i.e., from a point of the coagulation-fibrinolysis factors and from a point of their receptor. (COI: Properly Declared)

S21-2

Cell surface-modified fibrinolysis; contribution of vascular endothelial cells and platelets

Yuko Suzuki; Hideto Sano; Naoki Honkura; Tetsumei Urano (*Department of Medical Physiology, Hamamatsu University School of Medicine, Japan*)

Vascular endothelial cells (VECs) play essential role in keeping the fluidity of circulating blood by expressing dynamic antithrombotic effects. Once vascular wall is injured, haemostatic thrombus is promptly formed via an initiation of coagulation cascade following to the recruitment and activation of platelets. The lysis of the thrombi is also finely regulated, and those formed in excess or at unnecessary place are quickly dissolved. To achieve these missions with accuracy, spatiotemporal regulation of platelets activation, coagulation, and fibrinolysis are essential. Recent advances in genetic engineering and optical instrumentation have allowed us to demonstrate where, when, and how these processes take place. Our recent results obtained by real-time imaging analysis of cell surface-modified fibrinolysis using fluorescence microscopies will be discussed focusing on VEC surface and activated platelets. (COI: No)

S21-3

Novel role of coagulation factor XI as a regulator of vascular smooth muscle function

Katsuya Hirano; Wenhua Liu (*Department of Cardiovascular Physiology, Faculty of Medicine, Kagawa University, Japan*)

Coagulation factor XI (FXI) is involved in the intrinsic coagulation pathway. The studies with FXI-knockout mice suggest the involvement of FXI in pathogenesis of atherosclerotic lesions and inflammation. However, the underlying mechanism remains unclear. Serine proteinases exert cellular effects by acting as agonist for proteinase-activated receptor (PAR). PAR has been demonstrated to contribute to the pathogenesis of vascular diseases, including atherosclerosis. Here, I present the first evidence that FXI exerts direct effect on vascular smooth muscle via PAR_1, one of four subtypes of PAR. Fura-2 fluorometry revealed that FXI induced Ca^{2+} signal in a manner mainly dependent on Ca^{2+} influx from the extracellular space in rat embryo aorta smooth muscle A7r5 cells. The Ca^{2+} release from the intracellular store sites was observed only at high concentrations. The Ca^{2+} signaling effect of FXI was abolished by PAR_1 antagonist and proteinase inhibitor. FXI cleaved the recombinant protein containing the extracellular domain of PAR_1. The experiments with pharmacological inhibitors and siRNA indicated the involvement of voltage-dependent Ca^{2+} channel, $Ca_V1.2$, in the FXI-induced Ca^{2+} influx. Wound healing assay demonstrated that FXI accelerated the rate of migration by 2.5-fold of the control. $Ca_V1.2$ inhibitor and PAR_1 antagonist partly inhibited the FXI-induced cell migration. Our new discovery propose a novel role for FXI as a regulator of smooth muscle function. (COI: No)

S21-4

Endosomal Platforms for Protease Signaling

Nigel W. Bunnet (*Columbia University, USA*)

G protein-coupled receptors (GPCRs) regulate most physiological processes and are the target of >30% of drugs. GPCRs at the cell surface detect extracellular ligands and couple to heterotrimeric G proteins, and drug discovery focuses on the targeting cell surface GPCRs. GPCR signaling at the plasma membrane is terminated by b-arrestins, which mediate desensitization and endocytosis. Many GPCRs can continue to signal from endosomes by b-arrestin- and G protein-dependent mechanisms. We investigated the contribution of endocytosis of GPCRs for proteases and neuropeptides for signaling pain, including protease-activated receptor-2 (PAR_2) the neurokinin 1 receptor (NK_1R), and calcitonin-like receptor (CLR). We observed that receptor endocytosis is necessary for the activation of signals in subcellular compartments that mediate sustained excitation of nociceptors. Dynamin, clathrin and b-arrestin inhibitors attenuate neuronal excitation and nociception. GPCR antagonists that are conjugated to the lipid cholestanol or encapsulated into nanoparticles target GPCRs in endosomes. These antagonists selectively inhibit endosomal GPCR signaling, prevent sustained excitation of pain-transmitting neurons, and demonstrate superior anti-nociception in preclinical models of nociceptive, inflammatory and neuropathic pain. Thus, endosomal GPCRs can generate compartmentalized signals that underlie complex pathophysiological events *in vivo*, and GPCRs in endosomes are a viable therapeutic target. (COI: Properly Declared)

S21-5

Fibrinolysis and immunity: a new paradigm

Robert Lindsay Medcalf (*Australian Centre for Blood Diseases, Monash University, Australia*)

The fibrinolytic system is well known for its role in removing blood clots and fibrin deposits via the generation of the potent protease, plasmin that is generated from its inactive precursor plasminogen after activation by either tissue-type plasminogen activator (tPA) or urokinase. However, studies over recent years have begun to shed light on a broader function for this enzyme cascade. Indeed, tPA-mediated plasmin formation occurs on numerous other non-fibrin substrates, many of these include misfolded proteins occurring as a consequence of cell death or injury. Moreover, plasmin has been shown to promote phagocytosis of human dendritic cells in a manner that avoids dendritic cell activation, suggestive of an immunosuppressive effect of plasmin. It has been suggested that manipulation of the fibrinolytic system, either via t-PA mediated thrombolysis, where plasmin levels transiently increase many hundred fold, or by blocking plasmin generation using antifibrinolytic agents, would impact either positively or negatively on the host immune response and subsequent infection risk. This presentation will overview current data linking the fibrinolytic system with immune function and the potential cllnical significance of this new paradigm. (COI: No)

Symposium22

Proton signalings and proton-related functions

(March 29, Fri., 15:10-17:10, Room I)

S22-1

Hv1/VSOP voltage-gated proton channel inhibits migration in response to fMLF in neutrophils

Yoshifumi Okochi; Yasushi Okamura (*Integrative Physiology, Graduate School of Medicine, Osaka University, Japan*)

Neutrophils are immune cells that kill pathogens by secreting degradative enzymes and reactive oxygen species (ROS). We have shown that voltage-gated proton channel Hv1/VSOP regulates intracellular pH and membrane potential in neutrophils and is necessary for keeping NADPH oxidase activity high, leading to enough ROS production. On the other hand, we recently found that Hv1/VSOP negatively regulates ROS production in neutrophils. When Hv1/VSOP-deficient neutrophils are stimulated with fMLF at low concentrations, these cells exhibited increased ROS production before and after fMLF stimulation. Because fMLF is chemoattractant, we examined the migration in Hv1/VSOP-deficient neutrophils. Hv1/VSOP-deficient neutrophils exhibited enhanced migration in low fMLF concentrations. Receptor internalization and $[Ca]_{in}$ increase were normal under the fMLF stimulation, suggesting that the receptor sensitivity for fMLF in Hv1/VSOP-deficient neutrophils is not altered. Instead, increased and prolonged ERK activity was observed in Hv1/VSOP-deficient neutrophils. Inhibition of NADPH oxidase activity blunted the enhanced migration and ERK activity. These results indicate that Hv1/VSOP negatively regulates ERK signal by inhibiting ROS production in neutrophils, preventing the excess migration in response to fMLF. (COI: No)

S22-2

Controlling the innate immune signaling by the proton-coupled peptide transporters

Toshihiko Kobayashi; Noriko Toyama-Sorimachi (*Department of Molecular Immunology and Inflammation, Research Institute, National Center for Global Health and Medicine, Japan*)

The endosome/lysosome compartments play important roles in many aspects of cellular events including immune responses, controlling the receptor activation and the quality of downstream signaling pathways. In the phagocytic cells such as dendritic cells or macrophages, endosome/lysosome system is used for engulfment and digestion of the pathogens or self-antigens and following activation of the innate sensors, including Toll-like receptors (TLRs) or NOD-like receptors (NLRs). The activation of TLRs or NLRs is closely coupled with environment of endo/lysosomal compartment, such as pH, ion, and amino acid concentrations, which is regulated by various transporters.

Recent studies have revealed that SLC15A3 and SLC15A4, lysosome-resident proton-coupled oligopeptide transporters that transport amino acids and oligopeptides from the lysosomal lumen to the cytosol, facilitate the innate immune responses. SLC15A3 mediates inflammatory responses depending on cytoplasmic sensors including NOD2 or STING, whereas SLC15A4 plays a critical role for regulating TLR7- or TLR9-mediated type I interferon (IFN-I) production that is dependent on the V-ATPase activity and mTOR pathway. We review how these transporters optimize the inflammatory signaling at endosome/lysosome and discuss their potential as therapeutic targets for inflammatory diseases. (COI: No)

S22-3

Withdrawn

S22-4

Proton imaging in the brain using CCD-type ion image sensor

Hiroshi Horiuchi[1,2,4]; Junko Ishida[1,4]; Masakazu Agetsuma[1,2,4]; Kazuaki Sawada[3,4]; Junichi Nabekura[1,2,4] ([1]*Division for Homeostatic Development, National Institute for Physiological Sciences, Japan;* [2]*Department of Physiological Sciences, The Graduate School for Advanced Study, Hayama, Japan;* [3]*Department of Electronic and Information Engineering, Toyohashi University of Technology, Japan;* [4]*Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Japan*)

The regulation of pH is essential as a homeostatic function in all tissues. Especially, neural activity induces rapid pH changes in the intracellular and extracellular fluid in the brain. Recent study has reported that protons are released from nerve ending as a neurotransmitter, and decrease of extracellular pH can directly induce neural activity (Du et al, 2014). Classically, it has been known that pH in the brain during cerebral ischemia is decrease. Alkalization of astrocytes using optogenetics inhibits neural death during cerebral ischemia (Beppu et al, 2014). Furthermore, pH decrease occurs in psychological diseases including schizophrenia and bipolar disorder (Hagihara et al, 2017). Previously, pH imaging in the brain has been studied using magnetic resonance imaging (Magnotta et al, 2012). However, this technique have a disadvantage in terms of spatio-temporal resolution. ISFET has been applied for neurophysiological measurements (Bergveld et al, 1970), and a charge transfer type pH image sensors were realized to visualize chemical phenomena (Hizawa et al, 2005; Martinoia et al, 2001). However, miniaturization has been required to apply these techniques to pH imaging in vivo. In this study, CCD ion imaging sensor was inserted into the visual cortex to visualize pH condition, and visual stimulation was applied to stimulate neurons in the visual cortex. Interestingly, we found that different topical pH changes for each direction of the stimulation. (COI: No)

S22-5

Withdrawn

Symposium23

Glia and Neurological Diseases: from Physiological to Pathological Roles of Astrocytes and Microglia

(March 29, Fri., 15:10-17:10, Room J)

S23-1

Physiological function of microglia and their effect on neuronal circuits

Hiroaki Wake (*Division of System Neuroscience, Kobe University Graduate School of Medicine, Japan*)

Microglia are highly motile immune-reactive cells that play integral roles in the response to brain infection and damage and in the progression of various neurological diseases. During development, microglia also help sculpt neural circuits, via both promoting synapse formation and by targeting specific synapses for elimination and phagocytosis. Microglia are also active surveyors of neural circuits in the mature, healthy brain detecting synapse activity. Using *in vivo* imaging of neurons and microglia in awake, behaving mice, we will show the functional consequences of microglia-synapse contacts in mature mice and their effect on the neuronal circuit activity. In addition, we will discuss how microglia sense the systemic immune condition to modify the neuronal circuit activity. Our finding provides a plausible physical basis for understanding how alterations in immune status can impact on neural circuit plasticity and on cognitive performances such as learning. (COI: No)

S23-2

The roles of astrocytes and microglia in glutamate release after brain injury

Wen-Biao Gan; Sally Levinson; Joseph Cichon; Mirko Santello (*Skirball Institute, New York University School of Medicine, USA*)

Abnormally high levels of extracellular glutamate that occur after traumatic brain injury (TBI) and stroke are thought to be excitotoxic but the source of such heightened levels of glutamate is not well understood. We used the genetically-encoded glutamate sensor, iGluSnFr, and two-photon microscopy to study the pattern of glutamate released after traumatic injury in the living mouse cortex and explored the mechanisms underlying its release and regulation. We observed glutamate release after laser or stab-induced injury in two phases: the first phase occurred within minutes and was largely due to physical damage of neuronal processes. The second phase of glutamate elevations occurred over many hours and was dependent on astrocytic activity and microglial signaling. Our results highlight glial cells as a key player in the release and regulation of glutamate in brain injury. (COI: No)

S23-3

Bidirectional regulation of synapse remodeling by reactive astrocytes

Schuichi Koizumi (*Department of Neuropharmacology, Interdisciplinary Graduate School of Medicine, University of Yamanashi, Japan*)

When pathological condition, astrocytes become "reactive astrocytes" and contribute to both beneficial and hazardous brain functions. Here, I showreactive astrocyte-mediated synapse remodeling in the somatosensory cortex (S1) and the striatum. (1) Mechanical allodynia: We previously showed that when partial sciatic nerves are ligated (PSL), S1 astrocytes became synaptogenic and re-wired S1 neuronal networks, thereby leading to cross-talk between nocuous and innocuous circuits and mechanical allodynia. For this, upregulation of mGluR5 in S1 astrocytes has a pivotal role. When mGluR5 is selectively deleted in astrocytes, both uncontrolled synapse formation and mechanical allodynia were abolished. Thus, mGluR5 could be a key molecule that control astrocyte-mediated synapse formation and mechanical allodynia. (2) Brain ischemia: After transient brain ischemia, phagocytic astrocytes were observed within ischemic penumbra region in the later stage of ischemia.Phagocytic astrocytes upregulated ABCA1 and its pathway molecules, MEGF10 and GULP1, which were required for their phagocytosis. In addition, upregulation of ABCA1 was sufficient for the phagocytosis. Together, these findings suggest that astrocytes should be transformed into phagocytic phenotype via increasing ABCA1 and its related molecules. Judging from the spatiotemporal pattern of the phagocytic astrocytes, they have distinct roles from microglia, and would contribute to remodeling of the penumbra networks. (COI: No)

S23-4

The role of cortical astrocytes in establishing peripheral neuropathic pain

Sun Kwang Kim (*Department of Physiology, College of Korean Medicine, Kyung Hee University, Korea*)

Neuropathic pain following peripheral nerve injury is characterized by mechanical allodynia, a painful response to innocuous tactile stimulation. Although this chronic pain has been known to be induced by glial activation and altered nociceptive transmission within the spinal cord, an effective treatment is still insufficient, suggesting that novel therapeutic targets are critically needed. One such target may be the remodeling of synaptic connections in the primary somatosensory (S1) cortex that is highly associated with the severity of neuropathic mechanical allodynia. However, the causal relationship of S1 synapse remodeling to mechanical allodynia and its underlying cellular/molecular mechanisms remain unknown. Here we show that partial sciatic nerve ligation (PSL) injury induces an early re-emergence of immature metabotropic glutamate receptor 5 signaling in S1 astrocytes, which elicits spontaneous somatic Ca^{2+} transients, thrombospondin-1 release and synapse formation. Such activation of S1 astrocytes was evident only during a critical period (~1w post-injury), correlating with the temporal changes in S1 extracellular glutamate levels and dendritic spine turnover following PSL injury. Blocking this astrocytic signaling pathway suppressed mechanical allodynia, while activating this pathway in the absence of injury induced long-lasting allodynia. Thus, these synaptogenic astrocytes are a key trigger for S1 synaptic rewiring that mediates neuropathic pain. (COI: No)

Symposium24

Complexity and Diversity of Motility Regulation in Smooth Muscle

(March 29, Fri., 15:10-17:10, Room K)

S24-1

Morphological Study of Motility Regulation Mechanisms in Gastrointestinal Tract

Hiromi Tamada[1,2] (*[1]Graduate School of Medicine, Nagoya University, Japan; [2]Japan Society for the Promotion of Science, Japan*)

Gastrointestinal motilities are regulated not only by the enteric nervous system (ENS) but also by the interstitial cells of Cajal (ICC). ICC are the mesenchymal cells distributed along the intestine and are known as pacemaker cells and intermediators between nerves and smooth muscle cells. There are several subtypes which have different morphological features and functions depending on where they are distributed. In our study, new subtypes of ICC have been found in the subserosal layer (ICC-SS) and around the submucosal plexus (ICC-SMP). According to their morphological features, the new ICC subtypes are assumed to possess new functions, which are completely different from the typical ICC functions. This talk will present a review of histological findings with the electron microscopy for ENS, the smooth muscle cells, ICC, and discuss a novel concept of ICC study. Furthermore, the neurogenesis mechanism in ENS with *c-kit* mutant mice, which are known to lose ICC expression, will be also discussed. It has been known that neurogenesis in ENS is not observed *in vivo*, although the precursor cells which have the potential to differentiate into neurons *in vivo* remain in the adult gut. In our study with the ablation of enteric neurons, prominent neurogenesis was observed in *c-kit* mutant mice. Finally, new electron microscopic techniques to reveal three-dimensional ultrastructures will be introduced. They could be a powerful strategy to understand complicated interactions among cells. (COI: No)

S24-2

Differnece of pacamaking activity of interstitial cells of Cajal between small and large intestine

Jae Yeoul Jun (*Department of Physiology, University of Chosun, Korea*)

Interstitial cells of Cajal (ICC) are pacemaker cells that generate slow waves by producing pacemaker potentials. However, the configuration and frequency of pacemaker potentials are different between small intestine and large intestine, suggesting that pacemaker mechanisms of ICC may different between small intestine and large intestine. This study provides evidence that mechanisms of pacemaker activity in ICC are different between small intestine and large intestine. The pacemaker potentials and $[Ca^{2+}]_i$ oscillations were suppressed by selective ANO1 channel inhibitors in large intestinal ICC but not in small intestinal ICC. In knockdown of ANO1 with siRNA reduced the frequency of pacemaker potential only in colonic ICC. T-type Ca^{2+} channel inhibitors as well as specific blockers for HCN channels also suppressed the pacemaker activity of large intestinal ICC only. In immunohistochemistry, HCN1 and 3 are detected in large intestinal ICC. These results suggested the functional mechanism of pacemaker activity between small and large intestinal ICC is different. ANO1 and T-type Ca^{2+} channels have important functional role for generating of pacemaker activity in large intestinal ICC but not in small intestinal ICC, although ANO1 and T-type Ca^{2+} channels are exit in ICC. In addition, HCN channel is involved in regulating of pacemaker activity in large intestinal ICC but not in small intestinal ICC. (COI: No)

S24-3

Characteristic motility regulation of smooth muscle in lower urinary tract

Shunichi Kajioka[1]; Tomoko Maki[2]; Maya Hayashi[2]; Nouval Shahab[1]; Shinsuke Nakayama[3]; Toshiyuki Sasaguri[1]
(*[1]Department of Clinical Pharmacology, Kyushu University, Japan; [2]Department of Urology, Kyushu University, Japan; [3]Department of Cell Physiology, Nagoya University, Japan*)

It is generally accepted that physiological synchronous motilities of detrusor and urethral smooth muscle to keep continence and periodic micturition are under neural control. However, we have found some interesting and characteristic myogenic regulation of detrusor and urethral smooth muscle leading to the systematic control of continence by themselves. I would like to introduce these unique relaxation-contraction mechanisms especially focused on detrusor smooth muscle different from other smooth muscle organs from macro- to micro-scopic point of view.

Firstly, we found that the Ca^{2+}-sensitization caused by the activation of PKC and especially ROK pathway leading to MLCK activation is well-developed in detrusor. Thus MLCK-related contraction involving Ca^{2+}-sensitization is possible through thick-filament (myosin) -linked mechanism even at low $[Ca^{2+}]_i$.

On the other hand, thin-filament (actin) -linked mechanism are considered to be supportive to thick one. However, we have observed that cardiac TnT (cTnT) is expressed in various smooth muscles and is most abundant in detrusor smooth muscle. We confirmed cTnT plays a crucial role in detrusor smooth muscle in a physiological range of $[Ca^{2+}]_i$, especially below 1 μM.

These cooperations of thick filament-liked (Ca^{2+}-sensitiasation) and thin filament-linked (cTnT expression) causes well efficiency at voiding urine. At the same time, mal-cooperation causes pathophysiological condition, such as OAB. (COI: No)

S24-4

Regulation of thick and thin filaments organization during smooth muscle contraction

Masaru Watanabe[1]; Naoya Nakahara[2]; Yukisato Ishida[1,3]
(*[1]Laboratory of Physiology, Graduate School of Human Health Sciences, Tokyo Metropolitan University, Japan; [2]The Jikei University, Japan; [3]Bunkyo Gakuin University, Japan*)

Structure of contractile (thick and thin) filament is known to be unstable in smooth muscle than those in striated muscle. On the other hand, it is still unclear whether the structural organization and/or the amount of the contractile filaments in the smooth muscle cells changeduring the relaxation-contraction cycles. Several groups including us have presented that the amount of the thick and/or thin filaments in the several types of smooth muscle was increased during muscle contraction, and these findings indicate that structural changes in the thick and thin filaments regulate smooth muscle contraction. Recently, we found that a myosin II inhibitor disrupted the structural organization of the thick filaments, resulting in suppression of contractile force in the skinned smooth muscle of the guinea-pig taenia cecum. X-ray diffraction studies on the skinned taenia cecum in the resting state, showed that the myosin II inhibitor induced decreases in the intensity of both the 14.4 nm merdional reflection arising from the arrangement of myosin molecules in the thick filaments, and the diffuse equatorial peak at 1/11.4 nm^{-1} originated from lattice-like arrangement of the thin filaments. These results indicated that functional changes in smooth muscle myosin molecules regulate the organization of thick-filaments, resulting in thin-filaments structure and/or organization even in the absence of cross-bridges (irrespective of phosphorylation-dephosphorylation steps of MLC_{20}). (COI: No)

Symposium25

Calcium signaling in heart disease

(March 29, Fri., 15:10-17:10, Room L)

S25-1

Alterations of shear-Ca^{2+} signaling in atrial myocytes under chronic pressure and volume overload

Sun-Hee Woo; Min-Jeong Son; Qui A Le; Joon-Chul Kim (*College of Pharmacy, Chungnam National University, Korea*)

Hemodynamic disturbance by heart failure and hypertension increases cardiac shear stress. Using micro fluid-jet in single atrial cells, we have reported that shear stress induces longitudinal Ca^{2+} wave (L-wave) and transverse wave (T-wave). Although both waves are caused by autocrine action of ATP, released via connexins, $P2Y_1$ and P2X receptors mediate L-wave and T-wave, respectively. Here, we present a basis for the observations of two types of waves and its role in atrial remodeling under chronic pressure and volume overload. Most of left atrial cells showed L-waves, while right atrial myocytes mainly produced T-waves under shear. $P2Y_1$ receptor proteins were higher in left than in right atrial cells. $P2X_4$ protein level was higher in right than left atrial myocytes. Treatment of $P2X_4$ antibodies suppressed P2X component of shear-induced cation currents. Chronic myocardiac infarction (MI) and transverse aortic constriction (TAC) increased L-wave frequency and $P2Y_1$ receptor expression. Chronic TAC, not MI, resulted in increases in the $P2X_4$ currents and $P2X_4$ proteins. These results indicate that left and right atrial myocytes have different context of P2 receptor subtypes, thereby generating two different Ca^{2+} waves. Our data further suggest differential role of atrial shear-P2 receptor signaling in chronic MI and afterload increase. (COI: No)

S25-2

Mechanisms for sex differences in drug-induced arrhythmia

Junko Kurokawa (*School of Pharmaceutical Sciences, University of Shizuoka, Japan*)

Sex hormonal regulation in cardiac ion channels accounts for functional responses to sympathetic nervous system stimulation, which show sex differences in susceptibility of arrhythmias associated with QT prolongation (TdP: torsade de pointes). Although it has been known that women have a greater TdP risk than men in both congenital and acquired long QT syndrome, the sex difference becomes evident only when compared with adult men and adult women at the follicular phase, implying androgen and progesterone have protective effects on TdP. Accumulating clinical evidence suggests a protective role for progesterone (P_4). We have demonstrated that a nitric oxide (NO) production induced by stimulation of cardiac progesterone receptors through a non-genomic pathway suppresses L-type Ca^{2+} currents ($I_{Ca,L}$) under cAMP-stimulated condition, suggesting a cross-talk between NO and cAMP/PKA signaling. In this study, our pharmacological analysis revealed that the cross-talk is established by phosphodiesterase 2 (PDE2) at lipid raft of T-tubules of cardiac myocytes. In order to visualize cAMP/PKA activities in living cell, FRET-based cAMP/PKA biosensors, which are anchored to membrane rafts or non-raft regions, respectively, were employed. The FRET analysis suggested a subcellular localization of the cross-talk. These results suggest that a compartmentalized PKA activity may involve a cross-talk between the non-genomic PRs pathway and beta-ARs pathway to regulate $I_{Ca,L}$. (COI: No)

S25-3

Sarcoplasmic reticulum calcium leak promotes atrial fibrillation

Wenjun Xie; Ying Qi; Jingjing Li; Wenjin He (*School of Life Science and Technology, Xi'an Jiaotong University, China*)

Abnormal intracellular calcium signals have been recently proposed to play important roles in the pathogenesis of AF. In atrial myocytes, the main intracellular calcium release channel on the sarcoplasmic reticulum (SR) is type 2 ryanodine receptor (RyR2). We found several mice model harboring leaky RyR2, including RyR2 human catecholaminergic polymorphic ventricular tachycardia (CPVT) mutation and RyR2-S2808D that mimic chronic PKA phosphorylation of RyR2, can be stimulated into AF. Hypertension is the leading risk factor of AF. We also examined SR calcium release in hypertension-related AF using transverse aortic constriction (TAC) mice model. 4 week after TAC surgery, the mice displayed increased AF susceptibility accompanied by increased diastolic SR calcium leak in myocytes from left atria. The increased pressure induced pathological stretch in left atria (the left atria size increased by 38%). Applying stretch (12%) to atrial myocytes can induce a burst increase of SR calcium release, which can be inhibited by dithiothreitol, dantrolene and S107. The AF in TAC mice can also be prevented by dantrolene or S107 treatment. Our results indicated that SR calcium leak promotos AF, which can be the target in developing effective interventions to prevent and treat AF. (COI: No)

S25-4

Mechanism and therapeutic strategies for arrhythmogenic diseases caused by RyR2 mutations

Nagomi Kurebayashi (*Department of Pharmacology, Faculty of Medicine, Juntendo University, Japan*)

The type 2 ryanodine receptor (RyR2) is a Ca^{2+} release channel on the endoplasmic reticulum (ER) and plays a central role in E-C coupling in the heart. Abnormal activity of RyR2 is known to cause arrhythmia via spontaneous Ca^{2+} release from ER in diseased hearts such as chronic heart failure. In addition, mutations in RyR2 have been linked to various types of cardiac arrhythmias including catecholaminergic polymorphic ventricular tachycardia (CPVT), idiopathic ventricular fibrillation (IVF) and long QT syndrome (LQTS). At present over 300 arrhythmogenic mutations have been reported. We have developed an efficient and quantitative approach for functional evaluation of mutant RyR2s by measuring $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{ER}$, and Ca^{2+}-dependent RyR2 activity by $[^3H]$ryanodine binding assay using HEK293 expression system (Murayama et al, Human Mut., 2016, Uehara et al. J. Gen. Physiol., 2017, Fujii et al. Heart Rhythm, 2017). We found that all CPVT-linked mutations exhibit gain-of-function (GOF) phenotypes whereas other IVF and LQTS-related mutations include both GOF and loss-of-function (LOF) phenotypes. Furthermore, as an attempt to develop therapies for abnormal RyR2-related arrhythmias, we recently discovered several RyR2 inhibitors by high-throughput screening approach. Several compounds successfully suppressed Ca^{2+} sparks and waves in mouse cardiomyocytes. These compounds are promising candidates for novel anti-arrhythmic drugs. (COI: No)

S25-5

Conjunct JPH2-CAV3 Transcription Enhanced Ca Signaling Efficiency in Hibernating Ground Squirrels

Shi-Qiang Wang; Lei Yang; Rong-Chang Li; Bin Xiang; Yi-Chen Li; Li-Peng Wang; Xiao-Ting Wang (*College of LIfe Sciences, Peking University, China*)

Cardiac excitation-contraction (E-C) coupling is controlled by the signaling between L-type Ca^{2+} channels (LCCs) in the cell membrane/T-tubules (TTs) and ryanodine receptors (RyR) in the sarcoplasmic reticulum (SR). In heart failure, decreased expression of junctophilin-2 (JPH2) decreased the efficiency of LCC-RyR signaling and compromised the gain of E-C coupling. We found that the LCC-RyR signaling efficiency were increased significantly during hibernation in ground squirrels. The structural consolidation of LCC-RyR signaling apparatus was in parallel with increased expression of JPH2 and caveolin-3 (CAV3), the interaction between which anchors the SR to TTs. Interestingly, the promoters of both *JPH2* and *CAV3* exhibited binding sites for the serum response factor (SRF), which initiated *JPH2* and *CAV3* transcription via its interaction with myocardin. During hibernation, the expression of myocardin was increased. By adenoviral infection, overexpression of myocardin, but not that of SRF, increased *JPH2* and *CAV3* expression, improved LCC-RyR signaling efficiency and enhanced E-C coupling gain. The myocardin-mediated conjunct regulation of *JPH2/CAV3* transcription explained the cardiac specificity of JPH2 expression, and revealed a stoichiometry-optimized mechanism for E-C coupling regulation. This finding elucidated the mechanism for maintained cardiac contractility in hibernation, and provides new ideas for studying the "loss-of-function" remodeling in heart diseases. (COI: No)

Symposium26

Synaptic remodeling and beyond in health and disease

(March 29, Fri., 18:30-20:00, Room A)

S26-1

Neuronal activity-dependent synaptic pruning by microglia

Ryuta Koyama (*Graduate School of Pharmaceutical Science, The University of Tokyo, Japan*)

Microglia are recognized as main executors of synaptic pruning. Inconsistent findings are, however, that microglia engulf less active synapses during development, whereas neuronal activity enhances microglia to touch spines more frequently, resulting in the growth of touched spines in adulthood. Thus, it remains unclear whether and how neuronal activity modulates microglia-neuron interactions and synaptic engulfment. Here we established a live imaging system of microglia-neuron interactions *in vitro* in which neuronal activity can be modulated using the DREADD system. We found that increased neuronal activity enhances the interaction between the processes of ramified microglia with axons, suggesting that increased neuronal activity serves as a "find-me" signal. To further examine the activity-induced interactions *in vivo*, we used a mouse model of febrile seizures which are accompanied by the development of spontaneous seizures. We found that the dentate inhibitory synapses were extensively labeled with complements and microglia engulf the inhibitory synapses after febrile seizures, resulting in the increased activity of dentate neural circuits. Additionally, the engulfment of inhibitory synapses were blocked when the activity of dentate inhibitory neurons were suppressed using DREADD. Thus, our findings suggest that the increased neuronal activity serves as a "find-me" signal whereas complements serve as "eat-me" signals to orchestrate synaptic pruning by microglia. (COI: No)

S26-2

Interleukin-1ß-mediated effects of inflammation on visual circuit development in the zebrafish

Edward S Ruthazer; Cynthia M Solek; Nasr Al Farooqi; Niklas S Brake (*Montreal Neurological Institute, McGill University, Canada*)

Neuroinflammation caused by maternal infection during fetal development is implicated in the etiology of neurodevelopmental disorders. We have used the transparent larvae of *Danio rerio* (zebrafish) to observe structural and functional changes in the central nervous system tha result from immune activation during development. Just 2 h treatment of larvae with bacterial lipopolysaccharide (LPS) to mimic infection on day 3 post-fertilization causes rapid and long-lasting arborization defects in retinal ganglion cell axons *in vivo,* with LPS treatment increasing growth and complexity of axonal projections. Expression of the pro-inflammatory cytokine interleukin 1ß (IL-1ß) is necessary and sufficient to manifest these effects. Morpholino knockdown of PU.1 to prevent differentiation of myeloid cells, including microglia, abrogates the effects of LPS on acute axon dynamics, but only partially prevents IL-1ß upregulation, indicating a parallel role for microglia in this process. To assess the effects of early immune activation on circuit function, we have begun to perform rapid 3D 2-photon imaging of visually evoked Ca^{2+}responses from hundreds of neurons simultaneously in *Tg(Elav3:H2B-GCaMP6s)* transgenic fish. Understanding the mechanisms by which inflammation causes structural-functional dysregulation of developing neuronal circuits may provide insights to help mitigate or prevent neurodevelopmental and neuroinflammatory disorders. (COI: Properly Declared)

S26-3

Photooxygenation reduces the Aβ level in the brains of Alzheimer disease model mice

Yukiko Hori[1]; Shuta Ozawa[1]; Youhei Sohma[2]; Motomu Kanai[2]; Taisuke Tomita[1] ([1]*Laboratory of Neuropathology and Neuroscience, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan;* [2]*Laboratory of Synthetic Organic Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan*)

Several lines of evidence suggest that the aggregates of amyloid-β peptide (Aβ), including oligomers and fibrils, have toxicity for synapses and cells, resulting in relating with pathogenesis in Alzheimer disease (AD). We previously found that the photooxygenation of synthetic Aβ by small compound catalysts, which are activated by irradiation of light, reduced the aggregation potency and neurotoxicity of Aβ (Taniguchi et al., Nat Chem 2016). To verify the effects of oxygenation on deposited Aβ *in vivo*, we carried out the photooxygenation experiment using brains of APP knockin (NL-G-F; Saito et al., Nat Neurosci 2014) mice. One to four oxygen adducts in Aβ were detected by MALDI-TOF-MS. Notably, the mobility of Aβ was shifted to 10 kDa on SDS-PAGE/immunoblot analysis, suggesting that the photooxygenation changed the conformation and/or biochemical character of Aβ. We then injected the catalyst every day into hemi-hippocampus of living mice followed by irradiation by LED fiber. After 7 times of reactions, we found that 10 kDa Aβ was also detected under the reaction. In addition, this photooxygenation reaction decreased the total amount of Aβ in the brain. These results suggest that the catalyst photooxygenated the deposited Aβ in the AD model mice brain, and the photooxygenated Aβ was metabolized faster than naive Aβ. Thus, artificial photooxygenation by the catalyst would be a novel strategy for AD prevention and treatment. (COI: No)

S26-4

Regulation of aberrant synaptic remodeling in the thalamus triggered by peripheral nerve injury

Yoshifumi Ueta; Mariko Miyata (*Dept Physiol, Div Neurophysiol, Grad Sch Med, Tokyo Women's Med Univ, Japan*)

Peripheral nerve injury rapidly reorganizes somatotopic maps in the adult brain. In mice, whisker deafferentation induces synaptic remodeling of ascending fibers on neurons in the barreloids of the ventral posteromedial (VPM) nucleus. VPM neurons receive multiple fibers derived not only from whisker but also from non-whisker regions of the brainstem, resulting in reorganized whisker map. However, it is unclear what mechanisms regulate this aberrant plasticity. We found that tonic inhibition via extrasynaptic $GABA_A$ receptors containing α4 subunits was increased in VPM neurons immediately after the deafferentation. Continuous infusion of $GABA_A$ receptor agonist induced the synaptic remodeling in intact mice, whereas α4 subunits-deficient neurons were devoid of this remodeling. We next examined the involvement of microglia which are associated with formation and refinement of synapses. Microglial activity was increased in the whisker sensory pathway after the nerve injury. We found that the depletion of microglia suppressed nerve injury-induced synaptic remodeling in VPM. Whisker deafferentation induces mechanical hypersensitivity on the lower jaw. This ectopic hypersensitivity was disappeared by conditional deletion of α4 subunits from VPM or microglia depletion from the brain. Thus, our results demonstrate key mechanisms regulating aberrant plasticity changes in synaptic structure and function triggered by peripheral nerve injury. (COI: No)

S26-5

Activity-dependent synapse elimination in the developing cerebellum

Naofumi Uesaka; Tzu-Huei Kao; Masanobu Kano (*Graduate School Medicine, University of Tokyo, Japan*)

Developing neural circuits are refined by an activity-dependent process known as synapse elimination. Synaptic connections are initially weak and excessive, but necessary connections are selectively strengthened while unnecessary ones are eliminated during postnatal development. Postnatal refinement of climbing fiber (CF) to Purkinje cell (PC) synapses in the cerebellum has been a representative model of synapse elimination in the developing brain. CF synapse elimination consist of at least four distinct phases: (1) Selective strengthening of a single CF out of multiple CFs innervating each PC from postnatal day 3 (P3) to around P7, (2) translocation and expansion of innervation territory of the strongest CF (`winner' CF) to PC dendrites from P9, (3) elimination of somatic synapses of the `winner' CF and those of weaker CFs (`loser' CFs) from P7 to around P11, (4) elimination of somatic synapses of both the `winner' CF and `loser' CFs from around P12 to P17. We and other groups have demonstrated that neural activity in postsynaptic Purkinje cells is crucial for the functional differentiation and the elimination of loser CFs. However, whether activity of other players including presynaptic CFs and glia plays roles in CF synapse elimination has not been tested. In this symposium, we will present results of our on-going research about activity-dependent synapse elimination and discuss how activity regulates developmental synapse elimination. (COI: No)

Symposium27

Regulation of cell functions by phosphoinositides

(March 29, Fri., 18:30-20:00, Room B)

S27-1

A new mechanism of Ca2+-independent voltage-dependent secretion in dorsal root ganglion neurons

Zhuan Zhou[1]; Yuan Wang[1]; Hiroki Arima[2]; Rong Huang[1]; Yuqi Hang[1]; Xingyu Du[1]; Feipeng Zhu[1]; Zuying Chai[1]; Changhe Wang[1]; Yasushi Okamura[2] (*[1]Peking University, China; [2]Osaka University, Japan*)

The somata and axons of primary sensory neurons including dorsal root ganglion (DRG) neurons, release neurotransmitters and neuropeptides. Physiological action potentials trigger the Ca^{2+}-independent but voltage-dependent secretion (CiVDS) in somata of freshly isolated DRG neurons from both rats and mice (Chai et al, *Neuron*, 2017). We find that plasma membrane PIP2 does not regulate CiVDS by using the voltage-sensitive phosphatase (VSP) (Okamura et al, *Physiol Rev*, 2018) in dorsal root ganglion neurons. This provides a new mechanism of neurotransmitter release via CiVDS in neurons.

References:

Chai, Z. *et al.* CaV2.2 Gates Calcium-Independent but Voltage-Dependent Secretion in Mammalian Sensory Neurons. *Neuron* **96**, 1317-1326 (2017).
Okamura, Y., Kawanabe, A. & Kawai, T. Voltage-Sensing Phosphatases: Biophysics, Physiology, and Molecular Engineering. *Physiological reviews* **98**, 2097-2131 (2018). (COI: No)

S27-2

Functional analysis of voltage-sensing phosphatase in mouse sperm

Takafumi Kawai[1]; Haruhiko Miyata[2]; Hiroki Nakanishi[3]; Souhei Sakata[1,4]; Yoshifumi Okochi[1]; Masahiko Watanabe[5]; Kenji Sakimura[6]; Takehiko Sasaki[7,8]; Masahito Ikawa[2]; Yasushi Okamura[1] (*[1]Graduate School of Medicine, Osaka University, Japan; [2]RIMD, Osaka University, Japan; [3]Research Center for Biosignal, Akita University, Japan; [4]Dept. of Physiolgy, Osaka Medical College, Japan; [5]Graduate School of Medicine, Hokkaido University, Japan; [6]Brain Research Institute, Niigata University, Japan; [7]Graduate School of Medicine, Akita University, Japan; [8]Medical research institute, Tokyo Medical and Dental University, Japan*)

Voltage-sensing phosphatase (VSP) shows phosphoinositides phosphatase activity that is coupled to membrane potential. In the present study, we confirmed that VSP protein is expressed in matured sperm, suggesting that VSP functions in sperm of mice. Indeed, we found that the concentration of PIP_2 in VSP-/- sperm is significantly higher than that of hetero and WT sperm of mice, suggesting that endogenous VSP regulates PIP_2 level in sperm. Further experiments revealed that VSP-/- sperm showed severe defect in their motility after capacitation, but not before capacitation, resulting in significant reduction in success rate of fertilization in in vitro fertilization experiment. Ca^{2+} imaging experiments revealed that Ca^{2+} influx induced by capacitation-inducing medium is significantly facilitated in tail of VSP-KO sperm. Furthermore, lowering extracellular Ca^{2+} rescued the defect in motility of VSP-KO sperm after capacitation. We further found that the difference in Ca^{2+} influx between $VSP^{+/-}$ and $VSP^{-/-}$ was diminished by treating sperm with valinomycin, K^+ ionophore, suggesting that VSP regulates K^+ channel activities. We confirmed that K^+ current that is caused by Slo3, sperm specific K^+ channel is enhanced in $VSP^{-/-}$ sperm by electrophysiology. These lines of evidence suggest that VSP degrades PIP_2 in sperm, and the concentration of PIP_2 indirectly control Ca^{2+} influx during capacitation of sperm by regulating Slo3 channel. (COI: No)

S27-3

Regulation of ion channel functions by phosphoinositides

Byung C. Suh (*Department of Brain and Cognitive Sciences, DGIST, Korea*)

A growing body of data supports a view of the phospholipids of cell membrane as a key regulator of many ion channels and cellular excitability of neurons. The decrease in the proportion of polyphosphoinositides that occurs in response to the stimulation of G protein-coupled receptors (GPCRs) mostly reduces the open probability of ion channels and diminishes whole current size passing through the channel pores. Our research has been documented the functional mechanism of stimulus-induced phospholipid dynamics in ion channel regulation using a wide variety of experimental approaches. Most of the evidence suggests that normal ion channel activity requires membrane polyphosphoinositides, especially phosphatidylinositol 4,5-bisphosphate (PIP_2). The molecular basis for the PIP_2–dependent activation of ion channels and its physiological roles vary in diverse types of ion channels and cells. Here, I will introduce the molecular properties of phospholipid regulation in voltage-dependent Ca^{2+} and Cl^- channels. (COI: No)

S27-4

Molecular mechanisms of phosphoinositide signaling

Junko Sasaki[1]; Satoshi Eguchi[2]; Hiroki Nakanishi[3]; Takehiko Sasaki[1] (*[1]Medical Research Institute, Tokyo Medical and Dental University, Japan, Japan; [2]Department of Medical Biology, Graduate School of Medicine, Akita University, Japan; [3]Research Center for Biosignal, Akita University, Japan*)

Phosphoinositides are molecules that contain phosphatidylinositol, which has a glycerol backbone, two fatty acids linked to the glycerol through ester bonds, and an inositol phosphate head group that is phosphorylated at the 3, 4, or 5 position of the hydroxyl residue. Phosphoinositides are membrane-signaling lipids that regulate numerous biological functions, including proliferation, survival, adhesion, migration, and vesicular trafficking. By virtue of LC/MS/MS and gene targeted mice lacking a phosphoinositide metabolizing enzyme, we revealed that an aberrant metabolism of phosphoinositide leads to a wide variety of conditions mimicking human diseases. We will discuss the potential importance of the fatty acid moiety of PtdIns(3,4,5)P3 for the proinflammatory role. (COI: No)

Symposium28

Molecular evidences Link Physical Exercise to Cardiovascular Improvement

(March 29, Fri., 18:30-20:00, Room C)

S28-1

Non-coding RNA basis of exercise induced physiological hypertrophy

Junjie Xiao (*Institute of Cardiovascular Sciences, School of Life Science, Shanghai University, China*)

Exercise can induce physiological hypertrophy and protects against pathological cardiac remodeling. Non-coding RNA controls essential biological progresses, however, their roles in exercise induced physiological hypertrophy are not completely clear. We found that miR-222, which was elevated in exercised heart, was necessary for exercise induced physiological hypertrophy. miR-222 could increase cardiomyocytes cell size and proliferation. It could also protect cardiomyocytes apoptosis. HIPK1, HIPK2, HMBOX1 and P27 were identified as target genes of miR-222. Besides that, we also found miR-17-3p was increased in exercised heart. miR-17-3p promoted cardiomyocyte hypertrophy, proliferation, and survival. TIMP-3 was identified as a direct target gene of miR-17-3p whereas PTEN was indirectly inhibited by miR-17-3p. Increasing miR-222 or miR-17-3p could protect against adverse remodeling after cardiac ischemia/reperfusion injury. Recently, we also found lncRNA and circular RNA contributed to exercise induced physiological hypertrophy. (COI: No)

S28-2

Exercise Training Prevents Cardiac Injury Induced by Sympathetic Stress

Han Xiao; Youyi Zhang (*Institute of vascular medicine, Peking University Third Hospital, China*)

Aims: Stress-induced cardiac inflammation often triggers and/or accelerates heart injury. Rapid over-activation of β-adrenergic receptor (β-AR) upon stress plays pivotal roles in mediating cardiac inflammatory responses. Here we set out to determine if exercise training prevents cardiac injuries induced by sympathetic stress and investigate the underlying mechanisms. **Methods:** 10 weeks old C57BL6 male mice were treated with/without running 6 weeks and then treated with ISO (β-AR agonist, isoproterenol, 5 mg/kg body weight) or vehicle. **Results:** ISO induced cardiac macrophage infiltration (Mac3 staining) and fibrosis (sirus red staining) were significantly attenuated in the running group. Cytokine array profiling demonstrated that chemokines dominated the initial cytokines upregulation upon β-AR insult, which promoted macrophage infiltration. Further study showed that the rapid inflammasom/IL-18 activation was the critical up-stream regulator for elevated cardiac chemokine expression upon ISO treatment. ISO induced inflammasome activation and increased chemokines were blocked in the running group. These protective effects of running were diminished in AMPKa2-/- mice. **Conclusions:** Running exercise prevents cardiac inflammation and fibrosis induced by β-AR sympathetic overactivation. The underlying mechanism is that running targets inflammasome activation and subsequently decreases chemokines secretion. These protective effects of running were dependent on AMPK. (COI: No)

S28-3

Targeting a critical regulator of exercise-induced cardiac hypertrophy, PI3K, in the failing heart

Julie Rae Mcmullen (*Baker Heart and Diabetes Institute, Australia*)

Phosphoinositide 3-kinase (PI3K, p110α) is downstream of the insulin-like growth factor 1 receptor (IGF1R) and is a key regulator of exercise-induced heart growth (physiological cardiac hypertrophy) and protection. In contrast, other pathways such as G-protein coupled receptor pathways have been shown to be critical for pathological cardiac hypertrophy and heart disease. Utilizing a gene therapy approach we have demonstrated that increasing PI3K activity in mouse models with established cardiac dysfunction and pathology is beneficial. My laboratory has subsequently been exploring the regulation of PI3K-regulated mRNAs, microRNAs and lipid species in the heart as a potential treatment strategy for heart failure and complications including atrial fibrillation. Profiling models of physiological and pathological cardiac hypertrophy is being used to identify drug targets and biomarkers. (COI: No)

Symposium29

New insights into central mechanisms underlying hypertension

(March 29, Fri., 18:30-20:00, Room D)

S29-1

Central mechanisms of hypertension: brain-heart-kidney connection

Yoshitaka Hirooka (*Department of Medical Technology and Sciences, International University of Health and Welfare, Japan*)

Augmented sympathetic activity and renin-angiotensin system are responsible for the development of hypertension and hypertension-related cardiovascular complications. We suggest that the brain AT1 receptor activation and inflammatory changes induce increased central sympathetic outflow thereby causing the development of hypertension. These changes are affected by the peripheral immune and inflammatory abnormality. Increased oxidative stress occur not only the peripheral vasculature, but also within the brain. I show some data of Toll like receptor 4 and regulatory T cells as immune abnormality related to the sympathetic activation in hypertension and heart failure. Second, I introduce some of recent clinical studies related renal denervation in hypertension. Experimental studies suggest that renal denervation prevents immune cell activation and renal inflammation in hypertension. We also found that renal denervation reduced central sympathetic outflow in mice model of chronic kidney disease. Interestingly, an antihypertensive therapy with an angiotensin receptor blocker reduced sympathetic activity associated with decreased brain oxidative stress. It is conceivable that the brain mechanisms play an important role in the beneficial effect of renal denervation. (COI: No)

S29-2

Visceral afferent modulation for regulating sympathetic activity in cardiorespiratory disease

Julian FR Paton (*Department of Physiology, University of Auckland, New Zealand*)

For decades, the treatment of cardiovascular disease has been to target end organs. However, autonomic imbalance, particularly sympathetic discharge, is poorly controlled by frontline medications. This is important as reduced parasympathetic drive to the heart and excessive levels of sympathetic activity contribute to both the development and maintenance of cardiovascular disease. Recently, we proposed an *afferent activation hypothesis of autonomic imbalance* by which visceral afferents that produce reflex increases in sympathetic activity become sensitised and generate aberrant tone. My presentation will describe some mechanisms and functional consequences of visceral sensory neurone hyper-excitability as it contributes to hypertension, heart failure and disordered breathing in animals and humans. I will discuss putative clinical therapeutic strategies to alleviate autonomic imbalance by modulating peripheral chemoreceptor and baroreceptor reflexes with novel pharmacological and device based strategies. Our findings invoke a paradigm shift away from end organs to targeting visceral sensory neurones for the management of diseases in which autonomic imbalance prevails. (COI: No)

S29-3

Role of hypothalamus on the cardiovascular regulation during repeated acute psychological stress

Jouji Horiuchi; Ena Yamamoto; Takatoshi Horiuchi; Misaki Ichikawa (*Department of Biomedical Engineering, Toyo University, Japan*)

Psychological stressors evoke autonomic and neuroendocrine responses. Although such responses increase the probability of survival in the face of threatening stimuli, they can also lead to acute cardiovascular disorders. In addition, in human with essential hypertension the increase in blood pressure and heart rate evoked by psychological stress is exaggerated compared to normotensive subjects, consistent with the view that repeated stress-evoked cardiovascular responses lead to sustained increases in blood pressure. Thus, finding ways to reduce the cardiovascular effects of stress is critical for health.

Neurons in the hypothalamus play an important role on the cardiovascular response evoked by psychological stress. Changes regarding interpersonal issues are thought to be a kind of psychological stress, but it is unclear how the hypothalamus neurons participate on the autonomic cardiovascular response during the stress. In addition, orexin-containing neurons are localized within a restricted region in the hypothalamus and may be involved in the autonomic response during various types of stress. In this presentation, we reveal the cardiovascular response and the role of orexin-containing neurons during a change in an interpersonal issue, such as a social defeat situation. We also discuss change in resting blood pressure and distributions of c-Fos and/or orexin containing neurons in the hypothalamus during temporal and repeated social defeat stresses in conscious rats. (COI: No)

S29-4

NTS gene expression profiles underlying basal blood pressure levels: Focus on disease and gender

Sabine S. S. Gouraud[1,2]; Makiko Onishi[3]; Linh Thuy Pham[2,3]; Ko Yamanaka[4]; Hidefumi Waki[4] ([1]*Dept. Biology, Ochanomizu University, Tokyo, Japan;* [2]*Grad Sch General Educational Research, Ochanomizu University, Japan;* [3]*Grad Sch Humanities and Sciences, Ochanomizu University, Japan;* [4]*Dept. Physiology, Grad Sch Health and Sports Science, Juntendo University, Japan*)

Essential hypertension (EH), an important public health challenge worldwide, develops from complex mechanisms involving genetic pre-dispositions and lifestyles. Pre-menopausal women exhibit a lower arterial pressure (AP), a lower sympathetic outflow and a greater baroreceptor reflex than age-matched men, however, the molecular mechanisms remain unknown. Because the nucleus tractus solitarius (NTS) is a pivotal region for regulating the set point of AP, we believe that this central site is involved in the mechanisms underlying the variation of basal AP levels related to disease and gender. Therefore, unmasking NTS genes associated with AP level differences is crucial to understand these mechanisms. Transcriptomics experiments were performed on a model of human EH, the spontaneously hypertensive rat (SHR), to characterize gene expression profiles of NTS. In this model, females exhibit a lower AP than their male counterparts. When compared with normotensive male Wistar Kyoto (WKY) rats, the NTS of male SHRs exhibited a specific inflammatory state with altered gene expression levels of some cytokines/chemokines, apoptosis-related and neurotrophic factors. However, gender appeared to affect the expression profiles of different groups of genes in the NTS of SHRs, suggesting that central mechanisms associated with AP level differences are various. Genome-wide transcriptome analysis is a powerful tool to understand the molecular basis of AP regulation by the NTS. (COI: No)

S29-5

Brain molecular mechanisms underlying anti-hypertensive effect of daily exercise

Hidefumi Waki[1]; Ko Yamanaka[1]; Kei Tsukioka[1]; Keisuke Tomita[1]; Miwa Takagishi[2]; Sabine S. S. Gouraud[3] ([1]*Department of Physiology, Graduate School of Health and Sports Science, Juntendo University, Japan;* [2]*Department of Therapeutic Health Promotion, Kansai University of Health Sciences, Japan;* [3]*Department of Biology, Faculty of Science, Ochanomizu University, Japan*)

Daily exercise is recommended to prevent primary hypertension. However, the mechanisms underlying the anti-hypertensive effects of exercise remain unknown. Here, we introduce potential mechanisms based on our findings. In particular, we discuss the nucleus tractus solitarii (NTS), which is a pivotal region for regulating the set-point of blood pressure (BP). We characterized the gene expression profiles of the NTS after long-term daily wheel running in SHRs, a genetic animal model of human essential hypertension, and by the following functional examinations, we found the molecular basis of the anti-hypertensive effects of exercise. In addition to the genetic components, we have also been investigating brain mechanisms underlying stress-induced hypertension and the protective effects of exercise. Chronic stress is known to be one of environmental factors for hypertension manifestation. Since the amygdala (AMY) is involved in emotional responses and BP control, we examined gene expression profiles in the AMY after chronic restraint stress in normotensive rats and the interference effects by daily exercise. We found that expression patterns in most of abnormally expressed genes by chronic stress returned to normal by daily exercise, suggesting that the AMY may be involved in mechanisms of both stress-induced hypertension and preventive effects of exercise. We hope our presentation will provide new insights into the molecular basis supporting the concept of Exercise is Medicine. (COI: No)

Symposium30

Substance abuse and addiction ~ From basic science to regulatory science

(March 29, Fri., 18:30-20:00, Room E)

S30-1

An overview of recent emergence of new psychoactive substances (NPS)

Ruri Kikura-Hanajiri (*Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of Health Sciences, Japan*)

A wide variety of new psychoactive substances (NPS) has emerged around the world over the past fifteen years. In Japan, to prevent the distribution of NPS, a new regulatory category titled "Designated Substances" was introduced into the Pharmaceutical Affairs Law and enforced in 2007. After the introduction of this new category, NPS such as tryptamines and piperazines disappeared rapidly from the illegal drug market. In their place, synthetic cathinones and herbal products containing synthetic cannabinoids have appeared. As quickly as these NPS were controlled, other analogs of the controlled substances began to appear on the illegal drug market, and the identification and control of these substances rapidly turned into a "cat and mouse game". Many fatalities related to these products have occurred, and car accidents caused by impaired consciousness after smoking the herbal products have been a serious problem in Japan. To fight their distribution, the Government has further strengthened the regulation and controls of these substances since the latter half of 2014. As a result of this drug control reinforcement, the number of distributors of these products and the emergence of novel substances dramatically decreased by 2015. Products containing newly emerged NPS are, however, still available through the Internet although they are in decline. To avoid health problems and abuse caused by new designer drugs, we have to continuously monitor the distribution of these products. (COI: No)

S30-2

High-throughput imaging analysis using cultured neurons for detecting phencyclidine-like substances

Kenji Hanamura[1]; Toshinari Mitsuoka[1]; Ruri Kikura-Hanajiri[2]; Yuko Sekino[3]; Tomoaki Shirao[1] (*[1]Department of Neurobiology and Behavior, Gunma University Graduate School of Medicine, Japan; [2]Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of Health Sciences, Japan; [3]Endowed Laboratory of Human Cell-Based Drug Discovery, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan*)

Substance abuse is a big issue in the world. In Japan, since synthetic cathinones and cannabinoids have been under the control of generic legislation, phencyclidine (PCP)-like substances have emerged. Therefore, it will be important to establish high-throughput system for efficiently detecting new psychoactive substances that can inhibit the activity of NMDA-type glutamate receptors (NMDAR). We have shown that NMDAR activation reduces the amount of an actin-binding protein drebrin in postsynaptic sites. We utilized the characteristics of drebrin to detect the inhibitory effects of PCP-like substances on NMDAR activity. After 3 weeks in vitro, cultured neurons were preincubated with PCP or PCP-like substances, such as 3-MeO-PCP and 3-MeO-PCMo, and then treated with 100 μM glutamate for 10 min. After fixation, cultured neurons were immunostained with anti-drebrin and anti-MAP2 antibodies. Drebrin cluster density along dendrites were automatically quantified by originally-developed protocol. Preincubation with PCP or PCP-like substances significantly reduced the glutamate effect. Maximal inhibitory rate of 3-MeO-PCMo was lower than that of PCP or 3-MeO-PCP. Thus, it is indicated that our high-throughput imaging system using cultured neurons is useful for detecting the effects of PCP-like substances on NMDA receptor activity. (COI: No)

S30-3

GIRK channels and NMDA receptor GluN2D subunit in signal pathways from addictive substances

Kazutaka Ikeda (*Department of Psychiatry and Behavioral Sciences, Tokyo Metropolitan Institute of Medical Science, Japan*)

G-protein activated inwardly rectifying potassium (GIRK, Kir3) channel is one of the effectors in signal pathways from addictive substances. We found associations between genetic polymorphisms in the GIRK subunit genes and sensitivity to addictive substances in mice and humans. We also found that several drugs inhibited GIRK channels *in vitro* and reduced preference for methamphetamine in mice. Furthermore, ifenprodil, a widely used drug for dizziness, also inhibited GIRK channels *in vitro* and inhibited alcohol use in patients with alcohol dependence in a prospective, randomized, controlled, rater-blinded study. On the other hand, ketamine and phencyclidine (PCP), noncompetitive NMDA-type glutamate (NMDA) receptor antagonists, increase locomotor activity in rodents and cause dependence and schizophrenia-like symptoms in humans. We found that acute and repeated administration of ketamine and PCP did not increase locomotor activity in mice lacking GluN2D, a subunit of NMDA receptor. GluN2D knockout mice did not show impairment of prepulse inhibition by PCP, either. PCP significantly increased extracellular levels of dopamine in the striatum and prefrontal cortex in wildtype but not in GluN2D knockout mice. Furthermore, DNA array experiments revealed that PCP-induced *fos* expression was abolished in GluN2D knockout mice. These results suggest that GIRK channels and GluN2D subunit are candidate targets for pharmacotherapy of drug and alcohol dependence. (COI: Properly Declared)

S30-4

The serotonin transporter (SERT) as a genetic risk factor for drug addiction

Bart A Ellenbroek (*School of Psychology, Victoria University of Wellington, Newzealand*)

Drug addiction is a world-wide problem with significant medical, judicial and social repercussions. While some pharmacological and non-pharmacological therapies are currently in use, relapse rates are very high and there is clearly a need for a better understanding of the neurobiological basis and the aetiology of drug addiction.

We are investigating to what extent a genetic reduction in the SERT affects the reinforcing properties of drugs of abuse. Using rat self-administration procedures, we have been able to show SERT knock-out ($SERT^{-/-}$) rats are substantially more sensitive to the rewarding properties of cocaine and MDMA (the active ingredient of "ecstasy") than wildtype ($SERT^{+/+}$) rats. On the other hand, these rats do not differ in their sensitivity to the rewarding effects of heroin. With respect to ethanol, female but not male $SERT^{-/-}$ showed enhanced drinking. In a recent RNA sequencing study, we have started to identify the genes that are dysregulated in $SERT^{-/-}$ and $SERT^{+/+}$ rats both before and after MDMA self-administration. Focusing on the frontal cortex, both mitochondrial and neuroplasticity-related genes were disproportionally affected by both the genetic reduction of the SERT and the effects of MDMA.

Together with the fact that a reduction in the SERT is a common genetic variation in humans, our data contributes to identifying potential risk factor for drug addiction and novel target for its treatment. (COI: No)

Symposium31

Genomics of Sports and Exercise

(March 29, Fri., 18:30-20:00, Room F)

S31-1

Implication of genetic polymorphisms on sports performance

Eri Miyamoto-Mikami (*Graduate School of Health and Sports Science, Juntendo University, Japan*)

Human physical performance is a complex multifactorial trait. Our meta-analyses indicate that almost half of the variations in muscle strength and endurance capacity are determined by genetic factors (Zempo et al., 2017; Miyamoto-Mikami et al., 2018). Athlete status is also significantly affected by genetic factors (De Moor et al., 2007). In the past 20 years, at least 155 genetic variants were reported to be associated with elite athlete status (Ahmetov et al., 2016). Although most of these variants were identified by candidate gene approach, within the last 5 years genome-wide approaches were introduced in sports science field. Sequencing of entire mitochondrial DNA in 185 elite Japanese athletes revealed several polymorphisms to be potentially associated with elite Japanese athlete status (Mikami et al., 2013). Furthermore, genome-wide association studies (GWAS) identified multiple loci for muscle strength and elite endurance athlete status. The incidence of sports injuries is negatively associated with athletic success. Therefore, we are performing GWAS for sports injuries to clarify the genetic component of sports injury risk. In this symposium, I will introduce genomics studies on sports performance and injuries. (COI: No)

S31-2

Genomic investigations of skeletal muscle function

Ola Hansson[1,2] ([1]*Department of Clinical Sciences, Lund University, Sweden;* [2]*Institute for Molecular Medicine Finland (FIMM), Helsinki University, Finland*)

The proportions of fiber types vary widely across a person's body and from one person to the next. Like many other complex traits, the phenotypes of skeletal muscle composition segregate within families, indicating that genetic variation partly determines variation in fiber characteristics. The oxidative and glycolytic potential and the contractile properties of skeletal muscle also vary considerably by fiber type, with the mitochondria-rich slow twitch fibers (Type I) having higher oxidative capacity, and fast twitch fibers (*e.g.*Type IIx) having higher glycolytic capacity. Elite athletes in physically demanding endurance sports are generally enriched for slow-twitch type I muscle fibers, whilst fast-twitch type II fibers are more common in elite power sports athletes. Despite a clear heritable component to many skeletal muscle phenotypes, very little is known of the specific loci that influence these traits. Heritability estimates indicate that ~45% of fiber type variance is due to genetic factors and this warrants an effort to identify genetic variants responsible for prevalence of a certain type of fibers. We have performed genome-wide association meta-analysis on ~650 Swedish males from 3 independent cohorts with histological phenotypes from skeletal muscle biopsies, identifying 4 independent genetic variants associated with the proportion of type IIx fibers and capillary density. (COI: No)

S31-3

A Kinesio-Genomic Effect of mtDNA Polymorphism in the MOTS-c on Diabetes

Hirofumi Zempo[1,2] ([1]*Department of Administrative Nutrition, Faculty of Health and Nutrition, Tokyo Seiei College, Japan;* [2]*Graduate School of Health and Sports Science, Juntendo University, Japan*)

Mitochondria play key roles in both insulin secretions from the pancreatic beta cell and insulin sensitivity of skeletal muscles. Mitochondrial DNA (mtDNA) is a double-stranded circular molecule of 16,569 bp and encodes a total of 37 classically known genes, including 2 rRNAs, 22 tRNAs and 13 polypeptide subunits for the oxidative phosphorylation system. Current study has revealed that 12S rRNA region contains small open-reading-frame called MOTS-c and that enhances muscular insulin sensitivity in mice (Lee et al. 2015). MOTS-c comprising 16 amino acid residues also circulates in human blood. We have reported that East Asian specific mtDNA (m.)1382 A>C polymorphism accompanies amino acid replacement from Lys (K) to Gln (Q) at the 14th amino-acid residue of the MOTS-c (Fuku et al. 2015). 5-8% of East Asians carry a variation in this region.

We have currently found that this polymorphism affects the prevalence of type 2 diabetes in Japanese men, but not in women. Additionally, the K14Q-MOTS-c variant leads to diminished insulin-sensitization in vitro. In male mice fed a high-fat diet, WT-MOTS-c reduced weight and improved insulin-sensitivity whereas K14Q-MOTS-c variant did not. This was not seen in female-mice, recapitulating the sex-specific effect seen in humans.

In this session, we will discuss the effect of m.1382 A>C polymorphism on diabetes, and the combination with the physical activity (i.e., kinesio-genomic effect). (COI: No)

Local Organizing Committee Symposium

Symposium32

Membrane transporters related to diseases and drug development

(March 29, Fri., 18:30-20:00, Room G)

S32-1

The amino acid transporter SLC6A19 as a target to improve metabolic diseases

Stefan Broer (*Research School of Biology, Australian National University, Australia*)

Dietary protein restriction and restriction of specific amino acids, such as branched-chain amino acids, methionine or tryptophan have been shown to induce metabolic benefits, such as improved glucose tolerance, increased use of fatty acids as energy metabolites and increased life span. The overall aim of our studies is to develop a pharmacological induced amino acid restriction phenotype. Dietary restriction of specific groups of amino acids is difficult, but absorption of methionine, branched-chain amino acids and tryptophan is mediated by a single transporter in the intestine, namely SLC6A19.

A mouse model lacking SLC6A19 was employed and a variety of metabolic tests performed. Biomarkers were developed to evaluate inhibition of SLC6A19 in vivo and a variety of assays were developed for high through-put screening of chemical compound libraries to identify novel inhibitors of SLC6A19.

SLC6A19 ko mice have improved glucose tolerance, browning of white adipose tissue, reduced activation of mTORC1 and increased production of FGF21 and GLP-1, improved insulin sensitivity, reduced hepatic glucose output and reduced liver triglycerides. GC-MS based metabolomic approaches were used to identify biomarkers of amino acid restriction in urine, faeces and serum samples. Cell lines and bacteria overexpressing SLC6A19 were used to generate high-throughput screening assays. These tools were used to identify novel compounds that inhibit SLC6A19 with high potency and specificity. (COI: Properly Declared)

S32-2

L-type Amino Acid Transporters and Cancer

Arthit Chairoungdua[1,2] ([1]*Department of Physiology, Faculty of Science, Mahidol university, Thailand;* [2]*Excellent Center for Drug Discovery (ECDD), Mahidol University, Thailand*)

L-type amino acid transporters (LATs) are a family of Na^+-independent transporters, which promote the transport of neutral amino acids into cells. Four members including LAT1 (SLC7A5), LAT2 (SLC7A8), LAT3 (SLC43A1) and LAT4 (SLC43A2) have been identified. LAT1 and LAT2 associate with 4F2hc (SLC3A2), also known as CD98, becoming the heterodimeric obligatory exchanger. In contrast, 4F2hc is not required for LAT3 and LAT4 functions. LAT1 and 4F2hc have been reported to be upregulated and play critical roles in the progression of many types of cancers. In addition, we also found the upregulation of LAT1 and 4F2hc in pluripotent embryonal carcimoma (EC) cells, NTERA2, the cancer stem cells of testicular germ cell tumors (TGCTs). The malignant phenotypes including cell viability, cell proliferation, and clonal ability are suppressed following the inhibition of LAT1 function by BCH, a well characterized LAT family inhibitor. These results highlight the potential roles of LAT1/4F2hc also in cancer stem cells. Although, roles of LAT3 and LAT4 in cancer are limited. However, anti-tumor activity of LAT3 have been reported recently in prostate cancer. These information suggesting the potential therapeutic applications L-type amino acid transporters especially LAT1 and LAT3 in cancer. In this presentation, the development of specific inhibitors for LAT1 and LAT3 will also be discussed. (COI: No)

S32-3

Phosphate balance in the body and epithelial phosphate transporters

Hiroko Segawa; Yuji Shiozaki; Ichiro Kaneko; Ken-Ichi Minamoto (*Department of Molecular Nutrition Institute of Biomedical Sciences, Tokushima University Graduate School, Japan*)

Inorganic phosphate (Pi) is a highlighted by the syndromes causes by hypo- or hyper phosphatemic states. The serum Pi concentration is determined by the balance between the intestinal absorption of Pi from the diet, storage, and of Pi in the bone, and the excretion of Pi through the urine.

SLC34A1 and SCL34A3 are predominantly expressed in the kidney. In human, SLC34A1 mutations cause idiopathic infantile hypercalcemia, and SCL34A3 mutation causes hereditary hypophosphatemic rickets with hypercalciuria.

Hyperphosphatemia is recognized as a contributor to vascular calcification in patients with chronic kidney disease (CKD) and hemodialysis patients and is independently associated with cardiac mortality. Normalization of blood phosphate levels has been a clinical target in patients with CKD. It is now known that intestinal phosphate absorption occurs via two distinct mechanisms: passive paracellular transport, and active transcellular transport. Intestinal Pi absorption by the paracellular route is a nonhormonally dependent process that occurs mainly through the tight junctions by passive diffusion. The cellular transport pathway in the small intestine requires SLC34A2, SLC20A1, and SC20A2. Recent study reported that inhibition of intestinal sodium absorption via Na^+/H^+exchanger 3 (SLC9A3) is accompanied by increased fecal Pi excretion.

This symposium will give a brief overview for controlling of phosphate in the body. (COI: Properly Declared)

S32-4

Genomic analysis of Japanese Cystinuria patients through a next-generation sequence

Shinichi Sakamoto[1]; Yukio Naya[2]; Yasuhiro Shigeta[3]; Masaaki Fujimura[4]; Chiaki Inada[1,8]; Yuzuru Ikehara[6]; Yoshikatsu Kanai[7]; Naohiko Anzai[5]; Tomohiko Ichikawa[1,8] ([1]*Department of Urology, Chiba University Graduate School of Medicine, Japan;* [2]*Department of Urology, Teikyo University Chiba Medical Center, Japan;* [3]*Nishifunabashi Urology Clinic, Japan;* [4]*Department of Urology, Saiseikai Narashino Hospital, Japan;* [5]*Department of Pharmacology, Chiba University Graduate School of Medicine, Japan;* [6]*Department of Tumor Pathology, Chiba University Graduate School of Medicine, Japan;* [7]*Department of Bio-system Pharmacology, Osaka University Graduate School of Medicine, Japan;* [8]*Division of Clinical Genetics, Chiba University Graduate School of Medicine, Japan*)

INTRODUCTION AND OBJECTIVES: Cystinuria is an autosomal recessive disease, caused by the two mutations in BAT1(SLC7A9)/rBAT(SLC3A1). Around 70% of Japanese Cystinuria patients possessed a characteristics mutation: P482L, which has not been found in European countries. Here we studied the genomic profile of the fifty-one Japanese Cystinuria patients.

METHODS: A next-generation sequence was performed among fifty-one patients who previously performed a direct sequence of SLC3A1/SCL7A9. Nextseq 500 was used for the sequencing.

RESULTS: Overall, 8 novel mutations were identified, includes 2 frameshift(fs) and 5 point mutations and one exon-intron boundary mutation. In SCL7A9, P482L homozygote and heterozygote were found in 16(31.4%) and 17(33.3%) of patients, respectively. In terms of genotype classification, the number of type A, B and AB patients was 5, 34 and 2, respectively. Compare to direct sequence, 9 patients were reclassified into the novel genotype. However, overall 19.6% of patients still not fit into an autosomal recessive inheritance, with 2 patients possessed no mutation.

CONCLUSIONS: Among 51 patients, 8 novel mutations were identified and 9 patients were reclassified into a novel genotype. However, 20% of patients did not fit into autosomal recessive genotype. Current data may suggest the potential contribution of another factor in the pathogenesis of Cystinuria. (COI: No)

Symposium33

New insights into Endocrinology and Metabolism

(March 29, Fri., 18:30-20:00, Room H)

S33-1

Effects of perinatal hypothyroidism on brain development

Izuki Amano; Yusuke Takatsuru; Ayane Kate Ninomiya; Hiroyuki Yajima; Miski Aghnia Khairinisa; Michifumi Kokubo; Machiko Suda; Asahi Haijima; Noriyuki Koibuchi (*Department of Integrative Physiology, Gunma University Graduate School of Medicine, Japan*)

Thyroid hormone plays critical roles in differentiation, growth, metabolism, and physiological functions. Thyroid hormone deficiency during the fetal and early neonatal period impairs brain development. Cretinism in humans, which results from severe untreated congenital hypothyroidism, manifests as intellectual disability, ataxia, deafness and mutism, and spasticity with stunted physical growth. After the initiation of newborn screening programs for congenital hypothyroidism in the mid 1970s, most infants with congenital hypothyroidism were identified during the early postnatal period. Although early initiation of thyroid hormone replacement therapy may be able to prevent severe neurologic sequela, several risks of perinatal hypothyroidism remain. These include 1) The effects of mild and/or moderate hypothyroidism that cannot be detected by newborn screening 2) The effects of nutritional iodine excess on brain development and 3) Unknown genetic mutations linked to hypothyroidism. To clarify them, we generated mice models using anti-thyroid drug induction or genetic models. We will report on novel thyroid hormone action in the developing brain and the mechanisms by which it acts. (COI: No)

S33-2

Revealing role of thyroid hormone on autophagy regulation in skeletal muscle

Ronny Lesmana[1,2] ([1]*Departement of basic science, Physiology Division, Faculty of Medicine, Universitas Padjadjaran, Indonesia;* [2]*Central Laboratory, Universitas Padjadjaran, Indonesia*)

Revealing TH regulation on autophagy function in skeletal muscle using both invitro and invivo models, we demonstrated that TH induces autophagy in a dose-and time-dependent manner in skeletal muscle. TH induction of autophagy involved reactive oxygen species (ROS)stimulation of 5 adenosine monophosphate-activated protein kinase (AMPK)-Mammalian target of rapamycin (mTOR)-Unc-51-likekinase1(Ulk1) signaling. TH increased mitochondrial protein synthesis and number as well as basal mitochondrial O2 consumption, ATP turnover, and maximal respiratory capacity. Surprisingly, mitochondrial activity and biogenesis were blunted when autophagy was blocked in muscle cells by Autophagy related gene (Atg) 5 short hairpin RNA (shRNA). We also observed that gene regulated fission and fussion were upregulated. In addition, micrograph result from electron microscope showed a altere shaped of mitochondria looks like hyperfussion. In summary, our findings showed that TH-mediated autophagy was essential for stimulation of mitochondrial biogenesis and activity in skeletal muscle. In case of Aging process, reducing levels of thyroid hormone level may play role in muscle performance and fiber distribution. (COI: No)

S33-3

The role of nuclear receptor corepressors NCoR1 and SMRT on physiologic function in the mouse

Megan Jean Ritter; Izuki Amano; Kristen Vella; Anthony N Hollenberg (*Weill Cornell Medicine, Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, USA*)

Thyroid hormone (TH) plays an essential role in physiologic process starting with development and this continues throughout life. Thyroid hormone receptor (TR) is a nuclear receptor that is activated upon TH binding to express thyroid hormone responsive genes. Two critical corepressors that bind to thyroid hormone response elements (TREs) in the absence of TH to inhibit gene transcription are the nuclear receptor corepressor 1 (NCoR1) and the silencing mediator of retinoid and thyroid hormone receptors (SMRT). Repression is mediated through histone deacetylation by complexing with histone deacetylase 3. In order to study the role of these two corepressors, we used a tamoxifen-inducible Cre recombinase (UBC-Cre-ERT2) to delete NCoR1, SMRT, or NCoR1 and SMRT in adult mice because global deletion of either NCoR1 or SMRT during embryogenesis is lethal. Interestingly, while postnatal deletion of either NCoR1 or SMRT did not impact mortality, knock-out (KO) of both NCoR1 and SMRT resulted in a rapidly lethal phenotype heralded by weight loss, hypoglycemia and hypothermia. Mice with NCoR1/SMRT KO had several histologic abnormalities including crypt hyperplasia and villi atrophy throughout the intestines in addition to hepatosteatosis. We will report on the role of NCoR1 and SMRT in physiologic functions. (COI: No)

S33-4

The Protective Roles of Cardiac Macrophages in Heart Failure

Munehiko Shibata (*Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center, USA*)

Chronic inflammation is involved in various cardiac diseases. However, it is still unclear how inflammation contributes to disease development. We analyzed cardiac immune cells by flow cytometry and found that the myocardium contained a large number of macrophages even under physiological conditions. Pressure overload induced by thoracic aortic constriction (TAC) further increased the number of macrophages. To assess the function of macrophages, we injured them prior to TAC. Surprisingly, macrophage dysfunction led to severe cardiac dysfunction, suggesting that macrophages are important for the adaptive response to pressure-overload. Metabolomic analysis showed that macrophage dysfunction had significant impact on myocardial metabolism. Macrophages appeared to control glycolytic metabolism, TCA cycle and oxidative phosphorylation. The cardiac macrophages exhibited M2-type macrophage phenotypes. Since STAT6 is essential to M2 differentiation, we generated hematopoietic Stat6 knockout mice. These mice, lacking STAT6 in myeloid cells, also had disturbances in cardiac glycolytic metabolism, confirming our data that macrophages regulate the cardiac metabolism. We then identified a mediator that is expressed only by cardiac macrophages. Administrating this factor to the macrophage-dysfunctional mice offset the metabolic dysfunction and rescued the heart failure after TAC. Our results demonstrate cardiac macrophages are essential to the adaptive response of the heart. (COI: No)

Symposium34

Life Style Related Diseases in Asia: Underlying Mechanisms, Functions and Behavioural Transitions

(March 29, Fri., 18:30-20:00, Room I)

S34-1

Obesity : a matter of fat taste

Naim A Khan (*Universite de Bourgogne, France*)

Obesity is a pathology that is arithmetically increasing worldwide. It is responsible for several pathologies, such as cancer, metabolic syndrome and cardiovascular diseases. The excessive consumption of lipids products is considered as a key factor, involved in this pathology. It has been well propounded that there exists *five basic taste* modalities, *e.g.,* sweet, sour, bitter, salty and umami. Recent compelling evidence from rodent and human studies raise the possibility for an additional *sixth taste* modality devoted to the perception of lipids. A number of studies have recently suggested that lingual CD36, a glycoprotein, mainly expressed by circumvallate papillae of the tongue, might be implicated in the perception of dietary fat taste. Our recent studies have not only supported the existence of the 6th taste modality, destined for the perception of fat, but also explored the intracellular signalling mechanisms, involved in this phenomenon. We have shown that lingual CD36, after activation by free fatty acids, induces increases in free intracellular calcium concentrations, ([Ca^{2+}]i). This signalling cascade is likely responsible for physiologic responses, induced by the detection of lipids in the oral cavity. Our studies show that fat taste signaling is altered in obese animals and there is a polymorphism of CD36 in obese subjects (COI: No)

S34-2

The autonomic modulation for alleviating life style diseases

Kishore Kumar Deepak (*Department of Physiology, All India Institute of Medical Sciences, India*)

Several traditional and newer health improvement programs work on the principle of autonomic modulation. Both of them may involve varying degrees of somato-visceral Stimulation. The somatic-autonomic link is very strong and purposeful. Autonomic Nervous system (ANS) can be modulated by almost all sensory inputs namely visceral sensory, somato-sensory and special sensory. For example, for mobilizing ANS, the physical exercise may rely on three things; firstly, demand imposed on ANS for increasing circulation; second, somato-sensory stimulation; third, motivational/emotional component. The autonomic responses get modulated after exercise. Similar evidences are available for other non-exercise stimuli and they cause the changes in the levels of autonomic functional status. The practice of rhythmic slow breathing (*pranayama*) also results in useful autonomic responses. To explain this, the author proposed a hypothesis that the repeated episodes sympathoexcitation would result in blunted sympathetic response. Most of autonomic responses are regulated by a well laid down feedback loops. When the feedback loops are repeatedly stimulated, they learn to operate on a lower level for a given autonomic load. It makes the system more efficient. Thus, the ANS has capacity for performance based adaptability. This makes the ANS as self sustaining, adapting, optimizing and self healing system. This concept of ANS may be used for improving health and alleviating diseases. (COI: No)

S34-3

Cognitive Neurophysiological Imaging and Neuromodulation in Obesity

Kanwal Preet Kochhar (*Department of physiology,All India Institute of Medical Sciences, India*)

Over the last half century, there has been a rapid nutrition transition for Asian population due to rapid socio-economic development and Lifestyle changes.The thrifty gene hypothesis plays an important part in rise of incidence of Obesity, Metabolic Syndrome, Coronary Disease, gut motility disorders as well as psychosomatic,immune and neuro degenerative disorders.Major transitions are in the increasing use of salt,sugar,fat, beverages and processed fast foods.

Role of traditional Indian Diet, coarse cereals, Spices and Probiotics can help in managing Obesity,Diabetes, Hypertension, Autonomic Dysfunction seen in Metabolic Syndrome in Asian people changing their food habits, geographical location for work, sleep patterns andbody rhythms Today the era of biomarkers has become saturated and prone to many confounding variables but cyclic regulations and physiological waveforms are a more consistent and robust marker of an individual neurohormonal and psychoneuroimmune profile.cognitive correlates of attention to food and food craving, activation of taste areas and neuromapping of brain activity in response to food cues can serve as personalized and patient specific diagnostic and therapeutic, tools .Cognitive training and behaviour therapy may reduce hyper-responsivity of reward regions to food cues and increase inhibitory control thus participants learn to show reduced reward and attention region responsivity leading to wiser food choices and reduced caloric intake. (COI: No)

S34-4

Role of Yoga-based intervention in managing obesity and inflammation

Raj Kumar Yadav (*Department of Physiology, All India Institute of Medical Sciences, India*)

Yoga is our Indian legacy. In recent years, we have done several yoga-based lifestyle intervention studies at Integral Health Clinic (IHC), dept of Physiology, All India Institute of
Medical Sciences, New Delhi, and published in peer-reviewed journals. In this symposium, we shall present the evidence-based scientific aspects of mind, medicine and meditation, the effects of yoga as medicine in obesity. The primary objective of this symposium is to discuss the effect of short-term yoga-based lifestyle intervention on different aspects of obesity.
In an interesting study (2012), the efficacy of the short-term yoga-based lifestyle intervention program in reducing stress and inflammation was assessed. The results demonstrated that the mean level of cortisol decreased while β-endorphins increased from baseline to day10. We also reviewed (2014) the available scientific data on efficacy of yoga-based lifestyle interventions in reducing obesity-related inflammation and, suggested the possible mechanism of action. We have observed that yoga-based intervention modifies levels of vitamin D and risk factors for diabetes type 2 in obesity as well as gene expression of stress, inflammation and ageing in obesity. (COI: No)

S34-5

Food addiction and its link to obesity

Siddharth Sarkar (*Department of Psychiatry and NDDTC, AIIMS, India*)

In the last few years, food addiction has been debated as an entity based upon phenomenological parameters. Excessive uncontrolled consumption of fatty and hyperpalatable foods has been likened to substance use disorders. Craving for starchy and fatty foods have been described, which has been reported to be difficult to control. Even animal models have been described for the phenotype of food addiction. This presentation attempts to highlight the link between such purported food addiction and obesity. The present literature on food addiction and current controversies regarding assessment of this condition will be discussed. The link of excessive consumption of hyperpalatable fatty food and occurrence of obesity, as per the recent studies would be presented. The assessment measures for defining the food addiction in the individuals would be alluded to. Subsequently, potential remedial measures for tackling such food addiction, both in terms of pharmacological and non-pharmacological options would be examined in the presentation. The challenge of such food addiction in the context of increasing prevalence of diabetes and metabolic disorders in Asia would be touched upon. (COI: No)

Symposium35

Frontiers in Ca^{2+} release research in skeletal muscle: 50th anniversary from discovery of Ca^{2+}-induced Ca^{2+} release

(March 29, Fri., 18:30-20:00, Room J)

S35-1

Identification of novel inhibitors of Ca^{2+}-induced Ca^{2+} release for RyR1-related muscle diseases

Takashi Murayama (*Department of Pharmacology, Juntendo University School of Medicine, Japan*)

Type 1 ryanodine receptor (RyR1) is a Ca^{2+}release channel in the sarcoplasmic reticulum and plays an important role in excitation-contraction coupling. Genetic mutations in RyR1cause various skeletal muscle diseases including malignant hyperthermia (MH). Because the main underlying mechanism of the pathogenesis is overactive Ca^{2+}-induced Ca^{2+}release (CICR) by gain-of-function of the RyR1 channel, inhibition of CICR is expected to be a promising treatment for these diseases. We here developed a novel high-throughput screening (HTS) platform using time-lapse fluorescence measurement of Ca^{2+}in the endoplasmic reticulum (ER) to identify RyR1 inhibitors. RyR1 carrying an MH mutation and a genetically-encoded ER Ca^{2+}indicator were stably expressed in HEK293 cells and time-lapse fluorescence was measured using a fluorometer. Since MH mutant RyR1 reduces ER Ca^{2+}in HEK293 cells through Ca^{2+}leakage, specific drugs that inhibit RyR1 increase ER $Ca^{2+}$by preventing Ca^{2+}leakage. By screening 1,535 compounds in a library of well-characterized drugs, we successfully identified oxolinic acid (OA), a quinolone antibiotic as a novel RyR1-specific inhibitor. The highly quantitative nature and good correlation with the channel activity validate this HTS platform by ER Ca^{2+}measurement to explore novel drugs for RyR1-related muscle diseases. (COI: No)

S35-2

Interaction of junctophilins and the $Ca_V1.1$ is essential for the skeletal muscle contraction

Tsutomu Nakada; Toshihide Kashihara; Masatoshi Komatsu; Mitsuhiko Yamada (*Department of Molecular pharmacology, Shinshu University School of Medicine, Japan*)

Close physical association of $Ca_V1.1$ L-type calcium channels (LTCCs) at the sarcolemmal junctional membrane (JM) with ryanodine receptors (RyRs) of the sarcoplasmic reticulum (SR) is crucial for excitation–contraction coupling (ECC) in skeletal muscle. However, the molecular mechanism underlying the JM targeting of LTCCs is unexplored. Junctophilins (JPs) stabilize the JM by bridging the sarcolemmal and SR membranes. We examined the roles of JPs in localization and function of LTCCs. Knockdown of JP1 or 2 in cultured myotubes inhibited LTCC clustering at the JM and suppressed evoked Ca^{2+} transients without disrupting JM structure. Coimmunoprecipitation and glutathione S-transferase pull-down assays demonstrated that JPs physically interacted with 12 amino acid residues in the proximal C-terminus of the $Ca_V1.1$. A JP1 mutant lacking the C-terminus including the transmembrane domain (JP1ΔCT) interacted with the sarcolemmal/T-tubule membrane but not the SR membrane. Expression of this mutant in adult mouse muscles *in vivo* exerted a dominant-negative effect on endogenous JPs, impairing LTCC–RyR coupling at triads without disrupting JM morphology, and substantially reducing Ca^{2+} transients without affecting SR Ca^{2+} content. Moreover, the contractile force of the JP1ΔCT-expressed muscle was dramatically reduced compared with the control. Taken together, JPs recruit LTCCs to the JM through physical interaction and ensure robust ECC at triads in skeletal muscle. (COI: No)

S35-3

Analysis of disease mutants of type 1 ryanodine receptor using molecular dynamics and Ca^{2+} imaging

Toshiko Yamazawa (*Department of Molecular Physiology, The Jikei University School of Medicine, Japan*)

Malignant hyperthermia (MH) is a disorder of Ca^{2+}-induced Ca^{2+} release (CICR) via the type 1 ryanodine receptor (RyR1) in skeletal muscles. More than 300 mutations have been reported in the RyR1 gene of patients with MH. There are three main mutation clusters (hotspots), which are found in the N-terminal (RyR1 amino acid residues 35–614) and central (2129–2458) regions in the cytoplasm and carboxyl (C)-terminal region (4637–4973) near or within channel-forming segments of the RyR1. However, few comprehensive study on Ca^{2+} homeostasis associated with structural properties in these mutants to give insight into the mechanism of the pathogenesis have been reported. Here, we combined functional studies and molecular dynamics (MD) simulation of RyR1 carrying disease-associated mutations at the N-terminal region. When expressed in HEK293 cells, the mutant RyR1 caused abnormalities in Ca^{2+} homeostasis, i.e., caffeine sensitivity, ER Ca^{2+} contents, resting cytoplasmic Ca^{2+} concentration. MD simulation of the mutant RyR1 revealed that alterations of hydrogen bonds/salt bridges between N-terminal subdomains strongly correlate with the channel function of RyR1.These results suggest these interactions are primary determinant for MH phenotype. (COI: No)

S35-4

Structural basis for the gating, insecticide binding and resistance of insect ryanodine receptor

Zhiguang Yuchi; Lianyun Lin; Zhiyuan Hao (*School of Pharmaceutical Science and Technology, Tianjin University, China*)

Diamide insecticides target insect ryanodine receptors (RyRs) and cause misregulation of calcium signaling in insect muscles and neurons, generating worldwide sales over 2 billion U.S. dollars annually. Several resistance mutations have been reported to reduce the efficacy of the diamides, but the exact binding sites and mechanism of resistance mutations are not clear. The recent breakthrough in the structural studies of mammalian RyRs has deepened our understanding, but the structural information about insect RyRs is still scarce. Here we present the crystal structure of RyR N-terminal domain (NTD) at 2.84 Å resolution from the diamondback moth (DBM). DBM RyR NTD consists of a beta-trefoil folding motif and a flanking alpha helix. Interestingly, two regions in NTD interacting with neighboring domains showed distinguished conformations in DBM relative to mammalian RyRs. We also generate several homology models of full-length DBM RyR representing different functional states and dock the diamide insecticides into the structural models. These models reveal the specific structural features, gating mechanism, ligand-binding sites, and insecticide-binding sites of DBM RyR. By comparing the structures of the wild type and insecticide-resistant mutants, we propose a model depicting how the mutations affect the insecticide binding. We also identify the key difference between mammalian and insect RyRs that may explain the species-specific binding properties of diamides. (COI: No)

Local Organizing Committee Symposium

Symposium36

Inter-tissue communications underlying metabolic and feeding control in living body (whole day symposium) part I

(March 30, Sat., 10:00-12:00, Room A)

S36-1

Dietary nutrients and genes that regulate growth in *C. elegans*

Masamitsu Fukuyama[1]; Toshiaki Katada[1,2] (*[1]Laboratory of Physiological Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan; [2]Molecular Cell Biology Laboratory, Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, Musashino University, Japan*)

Dietary restriction has been known to delay growth and aging in many species of animals. However, the mechanism underlying the phenomenon remains to be fully delineated. Under the starved condition, newly hatched *C. elegans* larvae suspend growth at a specific developmental stage by maintaining quiescence of stem and progenitor cells and survive over a week. Such arrested larvae can resume growth by reactivating these quiescent cells when supplied with *E. coli* as food.

We found that feeding fatty acids (or its precursor, ethanol) and essential amino acids (EAAs) together can release several types of progenitor cells from quiescence, as *E. coli*. When either the insulin/IGF signaling (IIS) pathway or mTORC1 is hyper-activated, feeding ethanol alone can reactivate quiescent neural and mesodermal progenitor cells in newly hatched larvae. These observations suggest that amino acids not only serve as building blocks and energy sources, but also act as cues to trigger the reactivation. Based on this hypothesis, and taking advantage of the fact that combination of EAAs and fatty acids/ethanol is sufficient to initiate growth, we have conducted genetic screenings for mutants that can grow even when fatty acids/ethanol or a single amino acids such as leucine and methionine are omitted. Successful isolation and phenotypic analysis of such mutants suggest that, in addition to the IIS and mTORC1 pathways, there seems to be additional nutrient-responsive pathways that regulate growth. (COI: No)

S36-2

Nutri-developmental biology: nutritional adaptability and adipose tissue remodeling

Tadashi Uemura[1,5]; Yukako Hattori[1]; Kaori Watanabe[1]; Taiichi Tsuyama[1]; Yasutetsu Kanaoka[1]; Shoko Mizutani[1]; Kohei Shimono[1]; Hironobu Uchiyama[2]; Shunsuke Yajima[2,3]; Masayoshi Watada[4] (*[1]Grad. Sch. of Biostudies, Kyoto Univ., Japan; [2]NGRC, Tokyo Univ. of Agri., Japan; [3]Dept. of Bioscience, Tokyo Univ. of Agri., Japan; [4]Grad. Sch. of Sci. and Eng., Ehime Univ., Japan; [5]AMED-CREST, Japan*)

Animal growth is influenced profoundly by the quality and quantity of nutrients consumed by juveniles. To unravel regulatory systems that govern nutritional adaptability to ensure growth, we have focused on divergent feeding habits among species. Some species, including humans, are nutritional generalists that can eat a wide variety of foods, while others are specialists, feeding on a narrow range of food resources. Molecular mechanisms underlying adaptabilities of generalist species for development are still poorly described. To address this question, we used five *Drosophila* species with distinct habits and compared responses of growing larvae to various diets using multi-omics. We discovered that robust metabolic regulation by systemic TGF-beta/Activin signaling governs strong adaptabilities of the generalist species to sugar-rich diets, and that some specialist species are defective in this signaling and unable to adapt to such diets.

We are also studying development of a central organ for the systemic control of growth and metabolism, the fat body (adipose tissue), in particular development of the adult fat body which replaces the larval fat body. Our *in vivo* imaging suggests that dynamic behaviors of adult fat body precursor cells contribute to the characteristic distribution and size of the adult fat body. We are aiming to explore the cellular origin of adult fat cells in larvae, and to identify genes participating in the migration and/or differentiation of these cells. (COI: Properly Declared)

S36-3

The neural circuit for prey capture in zebrafish: from vision to the hypothalamic feeding center

Koichi Kawakami; Akira Muto; Deepak Ailani (*Division of Molecular and Developmental Biology, National Institute of Genetics, Japan*)

The visual system plays a major role in prey recognition in diurnal animals. The initial step of the visual recognition of a possible prey or other small objects starts in the retina in zebrafish. The optic tectum of the midbrain, the largest retinorecipient structure, is involved in the computation of the object size. The optic tectum also serves as a visuotopic map on which the perceived prey is located. Previously we successfully visualized activation of the tectum upon visual recognition of the prey by calcium imaging of immobilized and freely-swimming larvae. In this study, we address a question how the visual information is conveyed to the deep brain areas, especially to the feeding center that mediates appetite. we performed real-time imaging of neuronal activity in freely-behaving and constrained zebrafish larvae and found that prey or prey-like visual stimuli activate the hypothalamic feeding center. Furthermore, we identified prey detector neurons in the pretectal area that project to the hypothalamic feeding center. Ablation of the pretecto-hypothalamic circuit abolished the prey capture behavior. Taken together, we demonstrated that the pretecto-hypothalamic pathway plays a crucial role in conveying visual information to the feeding center. Thus, this pathway possibly converts visual food detection into feeding motivation in zebrafish. These findings may contribute to pathology of human disorders related to appetite, such as bulimia, and anorexia. (COI: No)

S36-4

Hypothalamic control of glucose metabolism in skeletal muscle

Yasuhiko Minokoshi[1,2] (*[1]Division of Endocrinology and Metabolism, National Institute for Physiological Sciences, Japan; [2]Department of Physiological Sciences, School of Life Science, SOKENDAI The Graduate University for Advanced Studies, Japan*)

The hypothalamus plays a key role in the control of energy homeostasis. We have shown that the ventromedial hypothalamus (VMH) is an important hypothalamic nucleus to regulate glucose metabolism in some peripheral tissues. Hyperinsulinemic-euglycemic (HE) clamp studies showed that leptin increases whole-body glucose utilization and glucose uptake in red-type skeletal muscle, heart muscle and brown adipose tissue (BAT) through melanocortin receptor (MCR) in the VMH. Leptin did not change plasma insulin levels or locomotor activity in mice. Injection of leptin or MCR agonist into the VMH increased sympathetic nerve activity in red- but not white-skeletal muscle.The leptin-induced glucose uptake in skeletal muscle was blunted in mice lacking beta-adrenergic receptor (AR)s, whereas it was restored by forced expression of the beta2-AR in skeletal muscle. Activation of SF1 neurons by DREADD (designer receptors exclusively activated by designer drugs) technology, reduced food intake and increased energy expenditure in mice. It also increased glucose uptake in red-type skeletal muscle, heart muscle and BAT. During HE clamp, such activation of SF1 neurons enhanced insulin-induced whole-body glucose utilization, glucose uptake in the above tissues, and insulin signaling in red-type skeletal muscle. Thus, VMH-sympathetic nervous system plays a key role in glucose utilization and insulin sensitivity in certain peripheral tissues including red-type skeletal muscle. (COI: No)

S36-5

Fibroblast Growth Factor 21 mediates the inter-talk between major metabolic regulators

Karen SL Lam (*Department of Medicine, The University of Hong Kong*)

FGF21, fibroblastic growth factor 21, a metabolic hormone predominantly produced from the liver, mediates the inter-talk between various tissues regulating glucose and lipid metabolism. In a liver-adipocyte inter-talk, FGF21 stimulates the secretion of adiponectin which mediates some of the actions of FGF21 on hepatic and peripheral insulin sensitivity, and improves hepatic lipid metabolism. Conversely, FGF21 resistance in the adipose tissue of obese individuals would lead to hypoadiponectinemia and exacerbation of insulin resistance. In humans, serum FGF21 level exhibits a circadian rhythm, with a peak in the early morning, reminiscent of changes in growth hormone and cortisol levels. Indeed, growth hormone induces hepatic production of FGF21 through a mechanism dependent on lipolysis in the adipocytes. Through suppressing lipolysis, the rise in FGF21 will serve to complete this pituitary-adipocyte-liver inter-talk for the fine-tuning of endogenous lipolysis. In the mice, there is also evidence suggesting an important role of FGF21 in preventing fasting hypoglycemia, through the mediation of a liver-brain-adrenal inter-talk, activating the hypothalamic-pituitary-adrenal axis to increase cortisol production and hence gluconeogenesis. The impact of the above inter-talks amongst major tissues regulating glucose and lipid metabolism on the clinical application of FGF21-targeting approaches, using FGF21 mimetics or enhancers, will be reviewed. (COI: No)

Local Organizing Committee Symposium

Symposium37

Primate researches in Asian regions

(March 30, Sat., 10:00-12:00, Room B)

S37-1

Advantages of using Thai cynomolgus macaques for infectious disease and cognitive research

Suchinda Malaivijitnond[1,2]; Srichan Bunlungsup[1]; Taratorn Kemthong[1]; Suthirote Meesawat[1]; Mallika Imwong[3]; Yuzuru Hamada[4] (*[1]National Primate Research Center of Thailand-Chulalongkorn University, Thailand; [2]Department of Biology, Faculty of Science, Chulalongkorn University, Thailand; [3]Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Thailand; [4]Evolutionary and Morphology Section, Primate Research Institute of Kyoto University, Japan*)

Cynomolgus macaque (*Macaca fascicularis*) is one of the commonly used non-human primate (NHP) models for infectious disease and cognitive research. Among 10 subspecies, common (*M. f. fascicularis*; *Mff*) and Burmese (*M. f. aurea*; *Mfa*) cynomolgus macaques are found in Thailand. Though primatologists denote that genetics of Thai *Mff* are messy because they carry the genetic admixture of rhesus macaques (*M. mulatta*; *Mm*). Using 40 autosomal SNPs, the genetic admixture between *Mff* and *Mm* was maximized at the hybrid zone (15-20 °N) and a northern Thai *Mff* population at 16° 51′N carried 50% of *Mm* ancestry while a southern one living at 7° 12′N carried 15% of *Mm* ancestry. However, when we looked at another angle, these Thai *Mff* could be an excellent animal model for infectious disease research. For example, *Mm* are susceptible to *Plasmodium cynomolgi* (a sister taxon of human *P. vivax*) and tuberculosis while *Mff* are tolerance to those diseases. Thus, Thai *Mff* who carry different degrees of *Mm*'s genetics should be varied in *P. cynomolgi* or tuberculosis infection. *Mfa* are the only Asian NHP who use stone tools to forage for encased foods. Exploring their genetics using partial mtDNA, Y-chromosome gene, whole mtDNA and whole genome sequences, they are distinctive from their neighboring *Mff* species. Regarding these unique characteristics, we propose that their genetics have major effects on learning behavior and memory capacity and they should be beneficial for neuroscience research. (COI: Properly Declared)

S37-2

Tool-Use Behavior in Burmese Long-Tailed Macaques and Possible Adaptation for Learning

Michael D Gumert (*Nanyang Technological University, Singapore*)

Burmese long-tailed macaques (M*acaca fascicularis aurea*) use stone tools along the Andaman Sea Coast of Thailand and Myanmar. In this region, these macaques use stones as hammers to crack open mollusks, crustaceans, and plant matter. This behavior is found only in groups living along coasts and islands in the region, and occurs mostly on rock shores and in stony mangroves. The stone-hammering behavior of these macaques is not found in any other Old World monkey and is potentially unique to Burmese longtails. Very few, if any, other primates use stone tools regularly in coastal habitats. Where Burmese longtails hybridize with common longtails (*M. f. fascicularis*), we also find stone hammering behavior. There, however, are no known cases of common longtails using stone tools regularly on coasts outside of this hybridization zone. Surveys of the biogeography of macaques expressing tool behavior and field studies on groups of hybrids, point towards a strong association between stone hammering behavior and the Burmese subspecies. These results suggest Burmese longtails and their hybrids will be a useful model to understand how biological, social, and ecological conditions interact in the evolution and development of technological behavior in a primate. Here, natural selection could be operating on Burmese longtails in such a way that they have evolved special capacities that bias their learning and development of stone hammering behavior. (COI: No)

S37-3

Ruminant-Like Primate, Proboscis Monkey in Borneo

Ikki Matsuda[1,2,3,4] (*[1]Chubu University Academy of Emerging Sciences, Japan; [2]Wildlife Research Center, Kyoto University, Japan; [3]Japan Monkey Centre, Japan; [4]Institute for Tropical Biology and Conservation, University Malaysia, Malaysia*)

Proboscis monkeys (*Nasalis larvatus*), endangered and endemic to Borneo, are the largest foregut fermenting colobines. Despite of their unique morphological features such as "big nose and stomach", for decades, knowledge about the proboscis monkey had been gleaned from limited information of their behavior from boat surveys along riverbanks where they rest in the early mornings and late evenings. This was due to the inhospitable swampy habitats they live in, which made them near impossible to track once they move into inland forests. This resulted in patchy knowledge about the species' ecology. However, by the 2000s, the attention raised for these unique monkeys had gradually attracted more researchers devoting their academic pursuits to the further understanding of the species, with studies still predominantly focused on their socio-ecology, but extending to genetic analyses, and other novel topics about their digestive capabilities and the sexual selection of enlarged noses. I summarized the findings from our proboscis monkey long-term project in Sabah, Borneo, Malaysia, focusing on their incredible digestive strategy of rumination, the first documented among primate species with their foregut microbiome composition, has not been investigated so far. (COI: No)

S37-4

Neurobiology of Primate Brain-Body-Environment Interactions under Evolutionary Perspectives

Atsushi Iriki (*Lab. for Symbolic Cognitive Development, Center for Biosystems Dynamics Research, RIKEN, Japan*)

Human evolution has involved a continuous process of acquiring new cognitive capacity to form novel culture. The dramatic expansion of the primate brain that accompanied additions of new functional areas would have supported such continuous evolution. Extended brain functions would have driven rapid and drastic changes in their ecological niche, which in turn demanded further brain resources to adapt to it. In this way, primate ancestors have constructed a novel niche in each of the ecological, cognitive and neural domain, whose interactions accelerated their individual evolution through a process of the "Triadic Niche Construction". Human higher cognitive activity can therefore be viewed holistically as one component of the earth's ecosystem, eventually comprising today's terrestrial environment, the "Anthropocene". The primate brain's functional characteristics seem to play a key role in this triadic interaction. Subspecies of macaque monkeys in the islands along the coast of Indian Ocean are regular tool-users, whereas continental subspecies and their hybrids rarely are. Comparison of these two subspecies would imply epi-genetic factors to elucidate neurobiological evo-devo mechanisms that give rise to human intelligence under the viewpoint of mind-body interactions through gut microbiome and dietary patterns at natural habitat, that is, interactions among mind, body and social/ecological environment, in the evolutionary context. (COI: No)

Local Organizing Committee Symposium

Symposium38

Cutting-edge research topics on skeletal muscle plasticity in health and diseases

(Co-organized by Japanese Society of Physical Fitness and Sports Medicine)

(March 30, Sat., 10:00-12:00, Room C)

S38-1

Evidence for acute contraction-induced myokine secretion by cultured myotubes

Nobuharu L Fujii (*Department of Health Promotion Sciences, Graduate School of Human Health Sciences, Tokyo Metropolitan University, Japan*)

Skeletal muscle has been recognized as a secretory tissue producing bioactive molecules, termed as myokines, which are released in response to various stimuli. However, there was no evidence that acute muscle contraction regulates myokine secretion. Here, we present evidence that acute contractions induced myokine secretion from C2C12 myotubes. We found that change in cell culture medium unexpectedly stimulates release of abundant proteins from C2C12 myotubes and those proteins masks the contraction-regulated myokine secretion. Once the released proteins were eliminated, stimulated secretion of interrokine-6 (IL-6), which is the best-known myokine, increased in response to a 1-hour contraction evoked by electrical stimulation. Using this experimental condition, we also found that calcium chelating, rather than inhibition of muscle fiber movement, blocked contraction-induced IL-6 secretion from the myotubes. This is the first report to show an evidence for acute contraction-induced myokine secretion by skeletal muscle cells. In summary, we showed that acute muscle contraction evidently stimulates myokine secretion, and our experimental condition can be used for investigating the secretory mechanism of skeletal muscle cell and for novel myokine discovery in further studies. (COI: No)

S38-2

Sex difference in sarcopenia: mechanisms and interventions

Shuichi Machida (*Graduate School of Health and Sports Science, Juntendo University Graduate School of Health and Sports Science, Juntendo University, Japan*)

Aging is associated with sarcopenia, which is defined by reductions in skeletal muscle mass, strength, and physical function. Sarcopenia decreases the ability to perform activities of daily living, reduces quality of life, and increases fall-risk and associated injuries. Older women exhibit greater frailty and skeletal muscle mass and strength losses compared to that in older men. It is likely that the underlying causes of sarcopenia are different in women and men, which will require the identification of sex-specific therapeutic targets.

Skeletal muscles are generally considered to have high regenerative capacity. However, muscle regeneration capacity progressively declines with aging. Satellite cells are a group of adult muscle stem cells that play a key role in mediating muscle regeneration. Recent studies have shown that satellite cells progressively undergo sex-specific changes in cell-intrinsic characteristics and functions.

Skeletal muscles are comprised of aggregate of muscle fibers, and are classified as slow twitch and fast twitch muscle fibers based on their contractile characteristics. The characteristic of sarcopenia is the selective atrophy in the fast twitch muscle fibers. Furthermore, it has been reported that the size of muscle fibers in women is smaller than that in men, and this difference is significant in fast muscle fibers. On the other hand, the hypertrophy of muscle fibers due to resistance training is dominant in fast muscle fibers. (COI: No)

S38-3

Therapeutic potential of slow muscle programming for muscle wasting and muscular dystrophy

Gordon S Lynch[1]; Justin P Hardee[1]; Karen J Martins[1]; Timur Naim[1]; Stefan M Gehrig[1]; Gregory R Steinberg[2]; Rene Koopman[1]; James G Ryall[1] (*[1]Centre for Muscle Research, Department of Physiology, The University of Melbourne, Australia; [2]Division of Endocrinology and Metabolism, Department of Medicine, McMaster University, Australia*)

Preferential wasting of fast, glycolytic myofibres is common in many muscle wasting conditions, including Duchenne muscular dystrophy (DMD). Promoting a slow, oxidative phenotype could protect muscles from damage to improve patient quality of life, but its therapeutic potential for DMD is unclear. To address this issue, electrodes were implanted in wild type and dystrophic (*mdx* and *dko*) mice and hindlimb muscles wirelessly stimulated at low-frequency to characterise acute (single bout, 10 Hz, 12 h) and 'training' adaptations (10 Hz, 12h/d, 7 d/wk, 4 wk). In wild type mice, whole genome sequencing revealed 1764 transcripts that were differentially regulated in EDL muscles after a single bout of LFS. Repeated LFS bouts induced a fast-to-slow muscle phenotype in TA muscles after 4 weeks, with increased SDH activity and decreased myofibre diameter. Genes related to oxidative phosphorylation, fatty acid degradation and PPAR signalling were all highly enriched after training. Repeated bouts of LFS for 4 wk improved myofibre SDH activity independent of histopathology alterations in both dystrophy models. These findings highlight the utility of LFS to enhance mechanistic understanding of contraction-induced muscle plasticity, and its therapeutic potential to ameliorate the dystrophic pathology, improve muscle repair, and enhance patient quality of life.

Supported by the National Health & Medical Research Council of Australia (GNT1124474) (COI: No)

S38-4

Adiponectin and skeletal muscle – new insights and potential implications

Katsumasa Goto (*Department of Physiology, Graduate School of Health Sciences, Toyohashi SOZO University, Japan*)

Skeletal muscle exhibits a large plasticity in response to various intracellular and extracellular stimuli. Aging also has an impact on skeletal muscle mass and function. Sarcopenia, aging-associated skeletal muscle atrophy, is considered as a risk factor for falls in older adults. Recent cohort studies show high level of plasma adiponectin is associated with atrophy and weakness of skeletal muscle in older adults. Although adiponectin is an adipokine, its expression is confirmed skeletal muscle cells. In general, adiponectin binds to adiponectin receptors (AdRs), exhibits insulin-sensitizing effects in skeletal muscle cells. We hypothesize that high adiponectin levels may have atrophic effects on skeletal muscle cells. Aging-associated up-regulation of adiponectin in fast skeletal muscle was observed. Agonist for AdRs, AdipoRon, suppressed myogenic differentiation of C2C12 cells in a dose-dependent manner. Knockdown of AdR in C2C12 myoblasts partially attenuated AdipoRon-induced suppression of C2C12 differentiation. Evidences suggest that aging-associated up-regulation of adiponectin in skeletal muscle may induced muscle atrophy in especially fast skeletal muscle. This study was supported, in part, by KAKENHI (Grant Numbers JP16K13022, JP17K01762, JP18H03160), the Descente Sports Foundation, the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan, and Graduate School of Health Sciences, Toyohashi SOZO University. (COI: Properly Declared)

Local Organizing Committee Symposium

Symposium39

Cutting-Edge Optical Imaging of Neuronal Circuits and Synapses

(Co-organized by Grant-in-Aid for Scientific Research on Innovation Area 'ABiS' of MEXT, Japan and Co-sponsored by Spectra-Physis)

(March 30, Sat., 10:00-12:00, Room D)

S39-1

Mechanical forces of spine enlargement detected by presynaptic FRET/FLIM imaging

Haruo Kasai[1,2]; Hasan Ucar[2]; Jun Noguchi[3]; Satoshi Watanabe[3]; Sho Yagishita[1,2]; Noriko Takahashi[4] (*[1]Graduate School of Medicine, The University of Tokyo, Japan; [2]Intl. Res. Ctr. for Neurointelligence* (*WPI-IRCN*)*, UTIAS, The Univ. of Tokyo Japan; [3]Natl. Ctr. of Neurol. and Psychiatry; [4]Department of Physiology, Kitasato Univ. School of Medicine, Japan*)

We study whether the mechanical force caused by the enlargement of the dendritic spines has any effects on presynaptic functions. We used the Schafer collateral (SC) innervating CA1 pyramidal neurons in hippocampal slice cultures. First, we measured the vesicle status of the presynaptic boutons of SC by using the trans-SNARE probe which measured FRET between t-SNARE (Syntaxin1A) and v-SNARE (VAMP2) by utilizing fluorescence lifetime imaging (FLIM). We found rapid increases in the FRET value when we pushed single presynaptic boutons by a glass electrode. Second, we measured glutamate release probability (Pr) from an identified single bouton, using a variant of iGluSnFR in SC, and its responses to single action potentials delivered at 0.07 Hz. We found the pushing augmented the Pr in a good correlation with the increases in the trans-SNARE formation. Lastly, we induced spine enlargement by spike-timing- dependent plasticity (STDP) at the dendritic spine using 2-photon glutamate uncaging to test the physiological relevance of the mechanical effect. We found that spine enlargement caused an increase in the trans-SNARE formation, even though we did not stimulate presynaptic axons by action potentials. Importantly, we found that the increase in the trans-SNARE formation was not detected when spines twitched and did not push the bouton, even though spines were enlarged. Thus, we have revealed that the effects of mechanical forces of spine enlargement on the presynaptic terminals. (COI: No)

S39-2

Multi-scale calcium imaging in the marmoset visual cortical network

Kenichi Ohki[1,2] (*[1]Department of Physiology, Graduate School of Medicine, University of Tokyo, Japan; [2]International Research Center for Neurointelligence* (*IRCN*)*, University of Tokyo, Japan*)

Primate neocortex analyzes visual scenes with a hierarchical neuronal network. To understand how such network interactively process visual scenes, we developed a method to monitor neuronal activity at multiple spatial scales. Based on Tet-off system (Sadakane et al., 2015), we first designed new AAV2/9 vectors which contain TLoop system (Cetin and Callaway, 2014) or two in-tandem of GCaMP, and successfully increased the level of GCaMP expression in a large volume of the marmoset neocortex.Using the improved vectors, we first performed wide-field 1-photon calcium imaging. In addition to orientation map in the primary visual cortex (V1), we found that full-field luminance increment and decrement evoked regular patches of responses in V1 (luminance polarity map). We then studied cellular activity using 2-photon imaging. In addition to orientation-selective cells, we found "non-tuned cells" that were responsive to drifting gratings but not selective for orientation, and "non-responsive cells". Interestingly, non-tuned cells selectively responded to the luminance increment, whereas non-responsive cells selectively responded to the luminance decrement. The present method is applicable to higher visual areas beyond V1. Smooth neocortex of marmosets allowed us to monitor neuronal activity in multiple brain areas spanning occipital to parietal cortices These results demonstrate usefulness of the marmoset brain to study the visual cortical network. (COI: No)

S39-3

Biochemical Signal Computation in Single Dendritic Spines

Ryohei Yasuda (*Max Planck Florida Institute for Neuroscience, USA*)

Activity-dependent changes in synaptic strength and structure are believed to be cellular basis of learning and memory. A cascade of biochemical reaction in dendritic spines, tiny postsynaptic compartments emanating from dendritic surface, underlies diverse forms of synaptic plasticity. The reaction in dendritic spines is mediated via signaling networks consist of hundreds of species of proteins. Aiming to elucidate the operation principles of such signaling networks, we have developed several new techniques to measure the key properties of the signaling components. First, based on 2-photon fluorescence lifetime imaging and highly sensitive biosensors, we have developed techniques to image signaling activity in single dendritic spines. Second, based on CRISPR/Cas9-mediated gene-editing, we have developed a technique to fuse fluorescent tags to endogenous proteins in single cells in vivo. This technique, termed SLENDR, allows us to measure the precise localization and dynamics of any proteins. Third, we have established a molecular tool to manipulate protein activity with light. Using this new optogenetic tool, we measured the temporal window of CaMKII activity required for synaptic plasticity and animal's learning. The data obtained by these techniques provides new insights into the mechanisms underlying the spatiotemporal regulation of signaling dynamics underlying synaptic plasticity and learning and memory. (COI: No)

S39-4

Super-resolution microscopy for neuroscience: new methods & applications

Valentin Nagerl (*Interdisciplinary Institute for Neuroscience, University of Bordeaux, France*)

The advent of super-resolution microscopy has created unprecedented opportunities to study the mammalian central nervous system, which is dominated by anatomical structures whose nanoscale dimensions critically influence their biophysical properties. I will present our recent methodological advances 1) to visualize the extracellular space of the brain and 2) to reveal the morphological structure and molecular arrangement of adhesive structures and synapses in live cells at the nanoscale level.

We combined 3D-STED microscopy and fluorescent labeling of the extracellular fluid to develop super-resolution shadow imaging (SUSHI) of brain ECS in living brain slices. SUSHI enables quantitative analysis of ECS structure and produces sharp negative images of all cellular structures, providing an unbiased view of unlabeled brain cells with respect to their complete anatomical context in a live tissue setting.

Current super-resolution microscopes are proficient at collecting either single molecule or morphological information, but not both. I will present a new super-resolution platform that permits correlative single molecule imaging and STED microscopy in living cells. We demonstrate that this multi-modal approach can give access to both kinds of information by revealing on a nanometer spatial scale protein localization and dynamics and cellular morphology. (COI: No)

Symposium40

Social communication through sensory information

(March 30, Sat., 10:00-12:00, Room E)

S40-1

TRPM2 in the sensation for warmth

Chun-Hsiang Tan (*Graduate Institute of Clinical Medicine, Kaohsiung Medical University, Taiwan*)

Thermosensation and thermoregulation are crucial for the survival of all animals, both in order to identify and maintain optimal thermal environments suitable for life and to avoid injuries caused by exposure to extremes of temperature. Several ion channels within the TRP family have been shown to be activated by thermal stimuli within various range, and have been proposed to serve as thermal detectors. However, identifying the mechanisms responsible for warmth detection was found to be challenging. We used a combination of calcium imaging, electrophysiology and RNA sequencing to demonstrate that the ion channel generating novel heat sensitivity independent of TRPV1, TRPV2, TRPV3, TRPV4 and TRPM3 in sensory and autonomic neurons to be TRPM2. More importantly, mice genetically deleted of TRPM2 showed a striking deficit in the detection of non-noxious warmth. The results indicate that the molecular mechanism underlying warmth sensation is mediated by TRPM2. TRPM2 has received less attention than many other members of the TRP family but is rapidly assuming importance as a key target in normal physiology and disease pathology. (COI: No)

S40-2

Physiological and behavioral changes in infants during mother-infant interaction

Sachine Yoshida (*Department of Anatomy, Faculty of Medicine, Toho University, Japan*)

Mother-infant bonding is the earliest and most essential relationship to mammalian infants since all mammalian species raise their infants with breast milk.
Mother-infant relationship consists of not only verbal but also nonverbal communication through various forms of sensory information such as tactile and visual stimulation.
We have found before that both human infants and rodent pups showed the coordinated calming responses, named the Transport Response, during maternal carrying. The Transport Response is composed of reduction of vocalization, body movement and heart rate, and facilitates the maternal carrying. Recently, we also found the developmental change in infant heart rate variability during maternal holding. We are currently working to clarify the relationship between the heart rate variability and the way of holding. I will briefly overview the current progress and discuss the developmental process of mutual communication in mother and infant using skin-to-skin contact. (COI: No)

S40-3

Evolutionary changes in the function and diversity of color vision in primates

Chihiro Hiramatsu (*Department of Human Science, Faculty of Design, Kyushu University, Japan*)

Animals utilize color vision to distinguish objects, which reflect, transmit, or emit different wavelengths of light. Fortuitous and adaptive evolutionary processes have shaped the various types of color visions in the animal kingdom. Many primates adapted trichromatic color vision that compares the activities of Long (L), Medium (M), and Short (S) wavelength cone cells in the retina, though some do not show uniform trichromacy. Here, I will review recent progress on understanding color vision in primates. Trichromatic vision in primates is believed to have evolved from the ancestral dichromatic vision through the shift from nocturnal to diurnal activity patterns. Finding reddish ripe fruits in the background of green leaves is hypothesized to be a primary driving force for the evolution of trichromacy. Theoretical studies with spectral analyses of fruits and leaves support this ideology. Field studies found behavioral differences between dichromatic and trichromatic individuals by focusing on species with polymorphism. These studies showed the benefits of trichromacy in fruit and flower foraging. The advantage of dichromacy in insect foraging has also been reported, suggesting trichromacy is not always the best color vision. Apart from foraging, trichromatic vision is also utilized to detect social signals like recognition of emotional or reproductive state through facial color. Perhaps, enjoying colorful paintings may be a function of trichromacy acquired by humans. (COI: No)

S40-4

BodySharing: How we can share our body experiences

Emi Tamaki[1,2] ([1]*Waseda University, Japan;* [2]*H2L Inc., Japan*)

BodySharing: What happens if we share our body each other?

If several persons can control one person's body, people will be able to share several experiences as a new comminication system.
In our research, it was verified what experience can be shared and what kind of problems will be encountered if two persons control one human body simultaneously.
Two users attached a new communication device, UnlimitedHand to their wrist, and muscle deformation sensors and electrical stimuli made each other's hands ready to control. In other words, it was made possible for two persons to share each person's body. We call this state BodySharing.
In this state, two users control one body to be performed an experience "Pouring water into a cup" that purpose was fixed, and another experience "Ikebana(flower arrangement)" that purpose was not fixed.
The result of this experiments showed that the sense of accomplishment and satisfaction can be shared. On the other hand, problems concerning the adjustment of force input and the intention of consensus were discovered.
This study revealed what experiences can be shared and what kind of problem would happen when BodySharing establishes. (COI: No)

International Scientific Program Committee Symposium

Symposium41

Leveraging novel techniques to research and translate synaptic transmission and plasticity (ISPP, Iran)

(March 30, Sat., 10:00-12:00, Room F)

S41-1

Withdrawn

S41-2

Addressing Therapeutic Challenges in Neuroscience with Digiceuticals

Bechara John Saab[1,2,3] (*[1]Mobio Interactive, Canada; [2]University of Zurich Psychiatric Hospital, Switzerland; [3]Royal Society of Medicine, UK*)

The combination of a richer neurobiological understanding and a ubiquity of mobile devices is opening exciting avenues for the translation of neuroscience in healthcare. In particular, Mobio Interactive has been improving the efficacy and reach of psychotherapy via computer vision, photoplesymographic imaging and deep learning in order to stimulate therapeutic neural activity in healthy individuals and patients groups around the world. This talk provides an intimate exposé of some of the technology and clinical trials underway at Mobio Interactive, diving deep into specific examples in order to highlight current limitations and future possibilities for the translation of neuroscience. (COI: Properly Declared)

S41-3

Withdrawn

S41-4

Dual effects of dopamine on synaptic plasticity in normal and hyperexcitable brain

Javad Mirnajafi-Zadeh[1]; Mahboobeh Ahmadi[1]; Bechara John Saab[2]; Yaghoub Fathollahi[1]; Nahid Roohi[1]
(*[1]Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Iran; [2]Research & Development, Mobio Interactive, Canada*)

Dopamine plays important role in different forms of synaptic plasticity. In this study we investigated the role of dopamine receptors in synaptic potentiation and cognitive behaviors following kindled seizures. Obtained results showed that in normal brain dopamine has inhibitory effect on long-term potentiation (LTP) through its D2-like receptors (D2R). However, dopamine could return the ability of LTP induction to hippocampal slices of kindled animals through D2Rs. This effect of dopamine was similar to effect of low- frequency stimulation (LFS) in kindled animals. Therefore, we checked the role of D2Rs in mediating the anticonvulsant action of LFS. Application of these receptors antagonist reduced the action of LFS. Also, LFS reduced the activity of dopaminergic neurons in ventral tegmental area of animals following seizure. In addition, optogenetically low frequency stimulation of dopaminergic fibers in ventral tegmental area reduced the cognitive impairments in kindled animals. Therefore, it may be concluded that dopamine D2Rs has important role in anticonvulsant action of LFS and their activity can prevent the seizure-induced impairments in synaptic plasticity and cognitive functions. (COI: No)

S41-5

Does exercise reverse cognitive and synaptic plasticity deficits following sleep deprivation?

Vahid Sheibani; Hakimeh Saadati; Amin Rajizadeh; Khadijeh Esmaeelpour (*Neuroscience Research Center, Kerman University of Medical Sciences, Iran*)

Inadequate sleep or sleep deprivation is a common problem in our societies. The aim of current short presentation is to review the effects of forced or voluntary exercise on cognitive dysfunctions in rats following paradoxical sleep deprivation (PSD).
Forced or voluntary exercises for 4week by treadmill or running wheel were used in rats. The multiple platform method was applied for the induction of PSD. The cognitive functions were evaluated by using behavioral and electrophysiological methods. Our results showed that physical exercise alleviated the PSD-induced learning and memory, LTP induction and maintenance impairments in Rats. Voluntary exercise before PSD improved long term learning and memory and novel object recognition memory impairments in rats. These results confirmed the negative effect of PSD on cognitive functions and forced or voluntary exercise seems to protect rats. (COI: No)

Symposium42 (Sponsored Symposium)

Physiological function of royal jelly contributing to healthy longevity - The effectiveness on Locomotive syndrome, Menopausal disorders, Infectious diseases -

(Co-sponsored by Yamada Bee Company, Inc.)

(March 30, Sat., 10:00-11:30, Room G)

S42-1

Royal Jelly Prevents the Progression of Sarcopenia

Hongmei Wu; Xue Bao; Yeqing Gu; Shunming Zhang; Ge Meng; Kaijun Niu (*Nutritional Epidemiology Institute and School of Public Health, China*)

Sarcopenia is characterized by the age-related loss of muscle mass and strength. We examined the effects of royal jelly (RJ) or protease-treated royal jelly (pRJ) treatment on the skeletal muscles in an animal model using aged mice. In vivo, RJ/pRJ treatment attenuated the decrease in the muscle weight and grip strength and increased the regenerating capacity of injured muscles and the serum insulin-like growth factor-1 levels compared with controls. In vitro, using isolated satellite cells from aged mice, pRJ treatment increased the cell proliferation rate, promoted cell differentiation, and activated Akt intracellular signaling pathway compared with controls. These findings suggest that RJ/pRJ treatment had a beneficial effect on age-related sarcopenia. In addition, we further examined the potential effects of pRJ on sarcopenia in elderly nursing home residents. One hundred and ninety-four subjects enrolled into this multicenter, randomized, double-blind, placebo-controlled study. Subjects received either placebo (Group 1), pRJ 1.2 g/d (Group 2), or 4.8 g/d (Group 3). We found that pRJ treatment might not improve, but rather attenuate the progression of decrease in muscle strength in elderly people. In addition, we have not found that pRJ intervention can achieve improvement or attenuating the decrease in physical performance. (COI: Properly Declared)

S42-2

Mitigation of postmenopausal neurological disorders by administration of royal jelly

Akira Minami (*Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Japan*)

Estrogen deficiency after menopause increases the risk of memory impairment and depression as well as metabolic syndrome and osteoporosis. The risk of depressive disorder is 2.5-times higher in the menopausal transition period than in the premenopausal period due to the association with the hormone level. The estrogen receptor (ER) has two different isoforms, ERα and ERβ. The beneficial effects of estradiol on postmenopausal neurological disorders in ovariectomized (OVX) rodents were shown to be mediated by activation of ERβ rather than by activation of ERα. In contrast, activation of ERα by hormone replacement therapy (HRT) is a risk factor for cancer. Considering the negative aspect of HRT, it would be helpful to find alternative and naturally occurring substances that minimize neurological disorders in a postmenopausal state. Royal jelly (RJ) from honeybees (*Apis mellifera*) has various medicinal effects including immune modulation, anti-fatigue, anti-tumor, anti-hypertensive and anti-osteoporosis effects. Recently, it was reported that RJ improves anxiety in postmenopausal Japanese women. Fatty acids and sterols contained in RJ have estrogenic activity and bind more selectively to ERβ than to ERα. Thus, memory impairment and depression-like behaviors in OVX rats were mitigated by RJ administration. Since activation of ERα is a risk factor for cancer, little effect on ERα could be a beneficial aspect of RJ for mitigation of postmenopausal neurological disorders. (COI: No)

S42-3

10-hydroxydecanoic acid in royal jelly elicits antigen-specific mucosal IgA response

Shogo Misumi (*Department of Environmental and Molecular Health Sciences, Faculty of Life Sciences, Kumamoto University, Japan*)

The effective antigen (Ag) uptake across gut epithelium by microfold cells (M-cells) is an important for the induction of a mucosal immune response. Here we show that medium chain fatty acid 10-hydroxydecanoic acid (10-HDAA), the ingredient of royal jelly, has a potential to induce an effective antigen-specific mucosal immune response in a cynomolgus macaque. Caco-2 monolayer assay demonstrated that 10-HDAA efficiently increased the protein expression level of a M-cell marker, glycoprotein 2 (GP2) at 3 days post-exposure. Furthermore, 10-HDAA stimulation elevated the RNA expression level of a master regulator of M-cell differentiation, Spi-B on day 1 and sequentially induced the RNA expression of GP2 with peak expression on day 2. As a result, 10-HDAA stimulation differentiated Caco-2 cells into M-like cells with characteristic morphological and efficiently increased the transcytosis efficiency. Furthermore, intranasal administration of 10-HDAA to cynomolgus macaques increased M-cells in follicle-associated epithelium (FAE) covering the luminal surface of nasopharynx-associated lymphoid tissue (NALT). Therefore, we investigated whether 10-HDAA can elevate the mucosal *in vivo* vaccine efficiency by increasing the number of M-cells. Oral administration of 10-HDAA to cynomolgus macaques significantly increased the antigen-specific IgA levels. These findings suggest that 10-HDAA exhibits mucosal adjuvant properties via stimulation of effective uptake of Ags through M-cells. (COI: Properly Declared)

Symposium43

TRP channels and inflammation/fibrosis

(March 30, Sat., 10:00-12:00, Room H)

S43-1

The regulation of TRPC5 channel activity by S-glutathionylation and S-palmitoylation

Chansik Hong[1]; Insuk So[2] (*[1]Department of Physiology, Chosun University School of Medicine, Korea; [2]Department of Physiology, Seoul National University College of Medicine, Korea*)

Redox reactions play a crucial role in the regulation of physiological functions and maintenance of cellular homeostasis. Increased oxidative stress and redox dysregulation are involved in the development of inflammation through multiple redox processes. The Ca^{2+}-permeable TRPC channel is predominantly expressed in the brain which is particularly vulnerable to oxidative stress. The molecular basis of TRPC5 channel regulation by redox reactions and their role in neurodegeneration are not known. Here, we report a mechanism of TRPC5 S-glutathionylation and S-palmitoylation, and the effect of glutathionylated and depalmitoylated TRPC5 on striatal neurons in Huntington's disease. By S-glutathionylation at Cys176/Cys178 residues, the oxidized glutathione-activated TRPC5 current results in a sustained increase in cytosolic Ca^{2+}, activated calmodulin-dependent protein kinase and the calpain-caspase pathway, ultimately inducing striatal neuronal cell death. S-palmitoylation at Cys181 residue enhances TRPC5 trafficking and membrane stability. We identified decreased levels of oxidative modification in single and combined Cys176/Cys178/Cys181 mutation, indicating that the three vicinal cysteines are dependently capable of reversible oxidation by the intracellular redox state. Collectively, these findings in controlling cross-talk between palmitoylation and glutathionylation on TRPC5 provide insight to a new therapeutic approach for neurodegenerative diseases. (COI: No)

S43-2

TRPM7 mediated fibrogenesis in heart diseases

Lixia Yue; Zhichao Yue; Albert S. Yu; Jianlin Feng (*Department of Cell Biology, Calhoun Cardiology Center, University of Connecticut School of Medicine, USA*)

The transient receptor potential melastatin 7 (TRPM7) is a unique channel protein which processes both ion channel and protein kinase functions. TRPM7 is ubiquitously expressed in various tissues and cells. Although it has been shown that TRPM7 is essential for embryonic development, the pathophysiological role of TRPM7 is not fully understood. We previously demonstrated that TRPM7 plays a role in fibrogenesis cascade. As fibrosis is a detrimental factor for a variety of heart diseases including hypertrophy and heart failure, we hypothesized that TRPM7 plays a critical role in fibrosis-associated heart failure. Using transverse aortic restriction (TAC) induced hypertrophy/heart failure model, we found that TRPM7 was highly up-regulated in TAC-induced heart failure mice. The current amplitude of TRPM7 in fibroblasts from 4 weeks TAC hearts was 1.5 fold larger than that of sham control. Deletion of *Trpm7* (TRPM7-KO) significantly increased surviving rate after TAC, enhanced heart performance of TAC mice by attenuating the reduction of ejection fraction (EF), decreasing the ratio of heart weight/body weight, and reducing fibrosis in TAC hearts. The strong protective effect of TRPM7 deletion against hypertensive hypertrophy/heart failure suggests that inhibition of TRPM7 may serve as a novel therapeutic target for fibrosis-associated heart diseases. (COI: No)

S43-3

The role of TRPM7 channel in pathogenesis of pulmonary arterial hypertension and right heart failure

Lin Hai Kurahara[1]; Keizo Hiraishi[1]; Lixia Yue[2]; Aya Yamamura[3]; Jianlin Feng[2]; Yaopeng Hu[1]; Mikiko Aoki[4]; Ryuji Inoue[1] (*[1]Department of Physiology, Fukuoka University, Japan; [2]Cardiology/Cell Biology, University of Connecticut Health Center, USA; [3]Department of Physiology, Aichi Medical University, Japan; [4]Department of Pathology, Fukuoka University, Japan*)

TRPM 7 (Transient receptor potential melastatin-7) channel is known to be deeply involved in cardiovascular remodeling, but it's role in pathogenesis of right ventricle (RV) and pulmonary artery remodeling is still unknown. We created pulmonary arterial hypertension (PAH) and right heart pressure-overload models in wild-type and TRPM7 knockout mice and compared their morphological and functional changes in pulmonary artery (PA) and RV. PAH was induced by monocrotaline pyrrole administration. The wild-type PAH mice showed a severely decreased RV function, which was markedly improved in TRPM7-knockout mice. The RV pressure-overload model was generated by pulmonary artery constriction. The degrees of RV hypertrophy and fibrosis were significantly reduced in TRPM7-knockout mice compared to wild-type mice. In addition, we found that the endothelial-mesenchymal transition in PA and abnormal PA smooth muscle cell proliferation were both suppressed by TRPM7 antagonists. TRPM7 immunoreactivity was clearly detected in the endothelium, medial wall and plexiform lesions of blood vessels from PAH lung tissues, as well as in RV. A Chinese herb medicine *ophiocordyceps sinensis* strongly suppressed a TRPM 7 channel activity in the expression system and significantly improved the compromised RV function and PA pathology in PAH mice and rats.

These results suggest that TRPM7 channel may play a vital role in cardiovascular remodeling during PAH and RV failure. (COI: No)

S43-4

Critical role of TRPC6 Targeting Hepatic Stellate Cell in Liver Fibrosis

Seung-Kuy Cha[1,2]; Kyu-Hee Hwang[1,2]; Ji-Hee Kim[1,2]; Soo-Jin Kim[1,2]; Kyu-Sang Park[1,2] (*[1]Department of Physiology, Yonsei University Wonju College of Medicine, Korea; [2]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Korea*)

Deregulation of Ca^{2+} signaling has been postulated as pathological event in fibrosis. A primary culprit for hepatic fibrosis is hepatic stellate cell (HSC) activation. Aberrant Ca^{2+} influx mediates either directly or indirectly HSC activation and leads to hepatic fibrosis. Thus, we firstly monitored Ca^{2+}-permeable channels in *in vivo* hepatic fibrosis animal models by bile duct ligation (BDL) and thioacetamide (TAA) administration. We found that TRPC6 was overexpressed in cirrhotic models. Notably, we found that expression level of TRPC6 was strongly correlated with Laennec scoring system for stating fibrosis in human liver biopsy specimens. To uncover underlying mechanism, we monitored TRPC6 activation in *in vivo* animal models and *in vitro* HSC activation model using primary HSCs culture. Expression of TRPC6 was significantly increased in hepatic fibrosis animal models and HSC activation model. Fibrotic changes and Ca^{2+} influx were ameliorated by TRPC6 knockout in *in vivo* animal model and *in vitro* HSCs, respectively. Together, these data demonstrate that TRPC6-mediated Ca^{2+} influx causes hepatic fibrosis via HSC activation. These provide a new perspective on the pathogenesis of hepatic fibrosis and offers clues for therapeutic strategies for liver cirrhosis. [NRF-2015R1D1A1A01060454 & 2017R1A5A2015369] (COI: No)

S43-5

The non-neuronal protection of transient receptor potential vanilloid 1 in vascular system

Tzong-Shyuan Lee (*Graduate Institute and Department of Physiology, College of Medicine, National Taiwan University, Taiwan*)

Transient receptor potential (TRP) vanilloid type 1 (TRPV1), a ligand-gated non-selective cationic channel, is mainly expressed in primary nociceptive sensory neurons. TRPV1 channel can be activated by heat, protons, several endogenous lipid molecules and various exogenous ligands such as evodiamine and capsaicin. Activation of TRPV1 permits calcium (Ca^{2+}) entry, which results in the elevation of intracellular Ca^{2+} and elicits responses. TRPV1 interacts with several intracellular proteins such as calmodulin and PI3K/Akt, important regulators of signaling pathways to regulate cell function. Phosphorylation of TRPV1 by protein kinases, including PI3K and CaMKII, is reported to regulate TRPV1 activity in sensory neurons. However, the non-neuronal significance of TRPV1 and its molecular mechanism in vascular system is not fully understood. The findings from our and other teams suggest that TRPV1 is expressed in endothelial cells and macrophages and plays crucial roles in regulating vascular tone, angiogenesis and the development of atherosclerosis. By use of clinical therapeutic reagents (statins and erythropoietin, capsaicin), food nutrition and traditional herbal medicine (curcumin, 14,15-epoxyeicosatrienoic acid, epigallocatechin-3-gallate and evodiamine), we confirmed the vascular protective effects of TRPV1 activation and the underlying molecular mechanism in the regulation of endothelial function and cholesterol metabolism of macrophages, as well as atherosclerosis. (COI: No)

International Scientific Program Committee Symposium

Symposium44

Cutting-edge approaches to long-lasting questions and novel aspects of inward rectifier K^+ channels -- A quarter-century anniversary of cDNA isolation (ISPP, Israel)

(March 30, Sat., 10:00-12:00, Room I)

S44-1

New insights into K^+ dependences of the strong inward rectifier potassium channel Kir2.1

Keiko Ishihara (*Division of Integrated Autonomic Function, Department of Physiology, Kurume University School of Medicine, Japan*)

Kir2.1 is the canonical member of inward rectifier K^+ channel family. The channel is constitutively open at membrane potentials around K^+ equilibrium potential (E_K) and its conductance declines steeply with membrane depolarization (i.e., strong inward rectification) due to voltage-dependent channel block by intracellular polyamines. The strong inward rectifiers show physiologically important two K^+ sensitivities; the voltage dependence of rectification shifts with E_K when the extracellular K^+ concentration ($[K^+]_{out}$) is altered, and the open conductances are proportional to the "square root" of $[K^+]_{out}$ (Hagiwara & Takahashi, 1974). These have been explained, for example, by repulsion between multiple K^+ ions within the pore and by the intracellular blocking cation interacting with those K^+ ions. However, the detailed mechanisms are still obscure, and the effects of intracellular K^+ concentration ($[K^+]_{in}$) have been controversial. We recently examined the effects of $[K^+]_{out}$ and $[K^+]_{in}$ on Kir2.1 currents and found that the $[K^+]_{out}$ dependence of the open Kir2.1 conductance is not the property of K^+ permeation, but is due to the fast channel block by external Na^+, which is competitive with external K^+. We also found that the voltage dependence of polyamine block follows the shifts in E_K when $[K^+]_{in}$ is altered, but not as perfectly as with the changes in $[K^+]_{out}$. The findings provide important constraints on the theoretical explanation for the K^+ dependence of inward rectification. (COI: No)

S44-2

The mechanism underlying rectification of ion flow in Kir2.1 and evolutionarily relevant channels

Chung-Chin Kuo (*Department of Physiology and Neurology, National Taiwan University, Taiwan*)

Voltage- and flow-dependent block of outward K+ currents by intracellular polyamines (e.g., spermine, SPM) is the major mechanism underlying the inward rectification in Kir2.1 channels. The on and off kinetics of SPM to and from the binding site are extremely slowed by point mutation E224Q, an effect completely reversed by concomitant mutations in the M2 bundle crossing region A178T or M183W. Also, the on rate carries little voltage dependence, but the off rate is consistently decelerated e-fold per ~15 mV depolarization. The SPM site responsible for the inward rectification is thus located at an electrical distance of ~0.5, probably in the central cavity delimited internally by the inner end of the M2 bundle crossing region. This region may undergo opening/closing conformational changes mimicking channel gating, which is controlled by intracellular but not extracellular cations including SPM and K+. The inner end of the bundle crossing region thus is a pivotal segment coupling channel gating to (inward rectifying) ion permeation. Similar biophysical principles of ion permeation and block also apply to evolutionally relevant channels. For example, extracellular Mg2+ block of the NMDA channel is facilitated by the flux-coupling effect *per se*, as if the poorly permeable Mg2+ is "pushed" against a barrier by permeating ions. The flow-dependent blocking effect may be an earliest form of "use-dependent" ion channel modulation, being more manifest with larger ionic fluxes. (COI: No)

S44-3

Regulation mechanisms of G-protein-gated inwardly rectifying K^+ channel by small molecules

I-Shan Chen[1,2]; Chang Liu[1,2]; Yoshihiro Kubo[1,2] ([1]*Division of Biophysics and Neurobiology, Department of Molecular and Cellular Physiology, National Institute for Physiological Sciences, Japan;* [2]*Department of Physiological Sciences, School of Life Science, SOKENDAI, Japan*)

G-protein-gated inwardly rectifying K^+ (GIRK) channels control various physiological functions. For example, GIRK1/2 heterotetramers in the brain regulate neuronal excitability; GIRK1/4 heterotetramers in the heart regulate heart rate. We previously identified a novel GIRK activator, ivermectin (IVM), and more recently a novel GIRK inhibitor, terfenadine (TER). Here we present our findings of the regulation mechanisms of GIRK channels by these small molecules. By electrophysiological recordings using *Xenopus* oocytes expressing different GIRK subunits without G-protein-coupled receptors, we observed that GIRK2 is sensitive to IVM and GIRK1 is sensitive to TER. Chimeric and mutagenesis analyses revealed the structural determinants for IVM and TER effects. For IVM effect, the slide helix, which connects the transmembrane domain 1 and the N-terminal tail domain of GIRK2, is responsible for the IVM-mediated activation. In the case of TER effect, the pore helix, which connects the selective filter and the pore region of GIRK1, contributes to the TER-mediated inhibition. Taken together, the present data show the effects of the novel activator and inhibitor on GIRK channels and the structural determinants for the regulations. The results provided us with a clue toward the elucidation of the novel gating mechanisms of GIRK channels by small molecules. (COI: No)

S44-4

The G protein coupled potassium channel in the mammalian brain

Eitan Reuveny (*Weizmann Institute of Science, Israel*)

G protein coupled inwardly rectifying K^+ (GIRK) channels control the excitability of many cell types including neurons in the peripheral and central nervous system. These channel are responsible for slow inhibitory postsynaptic potentials in the brain and for the slowdown of the heart rate following vagal nerve stimulation. The opening of these channel is controlled by the Gbg subunits of Gi/o proteins family released upon activation of G protein coupled receptors, and thus is one of the ways chemical transmission is converted to electrical ones. GIRK channel consists of 4 subunits (GIRK1-4) which form homo or hetrotetrameric functional channels. Few compositions of functional channel exist in the brain. GIRK2 can form homotetramers while GIRK1 and GIRK3 must associate with other family members to form functional heterotetrameric channels like GIRK1/GIRK2 and GIRK3/GIRK2. In my talk I will demonstrate the cellular and physiological consequences of selectively attenuating the capacity of GIRK1 heteromerization but not GIRK2 homotetramers or GIRK2/GIRK3 heterotetramers using transgenic animals. (COI: Properly Declared)

Symposium45

New molecular insights into the synaptic tagging and capture hypothesis

(March 30, Sat., 10:00-12:00, Room J)

S45-1

Behavioural and molecular insights in facilitating memory persistence

Szu-Han Wang (*Centre for Clinical Brain Sciences, University of Edinburgh, UK*)

The principle of lengthening plasticity changes through synaptic tagging and capture has also been shown effective in facilitating memory persistence. Specifically, a novel or salient behavioural event around memory encoding can enable an otherwise fading memory to last (Wang et al, 2010 PNAS). In this talk, I will cover our recent work on (1) identifying behavioural events for improving memory persistence, (2) characterising the impact of early ageing on this process (Gros and Wang, 2018, Neurobio Aging), and (3) using molecular markers to visualise the involvement of hippocampal cells in memory formation and facilitation. (COI: No)

S45-2

Inverse synaptic tagging : an inactive synapse-targeted mechanism to capture activity-induced Arc

Haruhiko Bito[1,3]; Yuichiro Ishii[1]; Hiroyuki Okuno[2] (*[1]Dept of Neurochemistry, The University of Tokyo Graduate School of Medicine, Japan; [2]Dept of Biochemistry and Molecular Biology, Kagoshima University Graduate School of Medical and Dental Sciences, Japan; [3]WPI-IRCN, The University of Tokyo Institutes for Advanced Study, Japan*)

Deciphering the intricate and interactive relationship between the information encoded in the genome and the ongoing synaptic activity is critical for understanding the molecular and cellular signaling underlying long-term memory formation and maintenance of long-lasting changes within the brain. To systematically dissect this question, we investigated the molecular basis of the signaling from synapses to the nucleus and from the nucleus to the synapses, which crucially determines the persistence of synaptic plasticity. We previously uncovered an activity-dependent protein kinase cascade CaMKK-CaMKIV that critically controls the amplitude and time course of phosphorylation of a nuclear transcription factor CREB downstream of synaptic activity, thereby activating a plethora of adaptive transcriptional responses within an active neuronal circuit. Further investigation of the cell biology associated with one CREB target gene, Arc, identified a hitherto unknown inverse synaptic tagging mechanism. Activity-induced Arc, during its diffusion into dendrites from the soma, was actively captured by inactive synapse during the late-phase of synaptic plasticity, via an enhanced affinity between Arc and Ca^{2+}/CaM-unbound CaMKIIbeta. Thus, inverse synaptic tagging may act as a brake that helps weaken non-potentiated synapses and facilitate accentuation of synaptic contrast during the maintenance phase of synaptic plasticity. (COI: Properly Declared)

S45-3

Role of p75 neurotrophin receptor in sleep deprivation induced changes in synaptic plasticity

Sajikumar Sreedharan (*Department of Physiology, National University of Singapore, Singapore*)

Sleep deprivation (SD) interferes with hippocampal structural and functional plasticity, formation of long-term memory (LTM) and cognitive function. The molecular mechanisms underlying these effects are incompletely understood. Here, we show that SD impairs synaptic tagging and capture (STC) and behavioral tagging (BT), two major mechanisms of associative learning and memory. Strikingly, mutant mice lacking the p75 neurotrophin receptor ($p75^{NTR}$) are resistant to the detrimental effects of SD on hippocampal plasticity at both cellular and behavioral levels. Mechanistically, SD increased $p75^{NTR}$ expression and its interaction with phosphodiesterase (PDE4A5). $p75^{NTR}$ deletion preserved hippocampal structural and functional plasticity by preventing SD-mediated effects on hippocampal cAMP-CREB-BDNF and RhoA-ROCK2-LIMK1-cofilin pathways. Our study identify $p75^{NTR}$ as an important mediator of hippocampal structural and functional changes associated with SD, and suggest that targeting $p75^{NTR}$ could be a promising strategy to limit the memory and cognitive deficits that accompany sleep loss. (COI: No)

S45-4

Rapid reversal of microRNA-induced silencing: a novel mechanism mediating synaptic plasticity

Ted Abel[1,2]; Alan Jung Park[4]; Xiuping Fu[3]; Aparna P. Shah[3]; Mahesh Shivarama Shetty[1,2]; Jay M Baraban[3] (*[1]Iowa Neuroscience Institute, University of Iowa Carver College of Medicine and University of Iowa, USA; [2]Department of Molecular Physiology and Biophysics, University of Iowa, USA; [3]Solomon H. Snyder Department of Neuroscience, Johns Hopkins School of Medicine, USA; [4]Mortimer B. Zuckerman Mind Brain Behavior Institute, Columbia University, USA*)

Long-lasting forms of synaptic plasticity and memory require de novo protein synthesis. Yet, how learning triggers this process to form memory is unclear. The translin/trax microRNA degrading enzyme is an attractive candidate to drive this learning-induced memory mechanism because it is located in dendrites and has been implicated in suppressing microRNA-mediated translational silencing. We have found that mice lacking translin/trax display defects in long-lasting forms of LTP, synaptic tagging, and long-term memory, processes that require de novo protein synthesis at activated synapses. Hippocampal samples harvested from these mice following learning show increases in microRNAs targeting type I activin receptors ALK4 and ALK7, members of the transforming growth factor-β receptor superfamily. Furthermore, a small molecule inhibitor that targets ALK4 and ALK7 mimics plasticity and memory deficits displayed by mice lacking translin/trax. Thus, these findings define a new memory mechanism by which learning reverses microRNA-mediated silencing to elicit de novo translation. (COI: No)

S45-5

Dopaminergic memory boostby two distinct novelty systems

Tomonori Takeuchi[1,2,3] (*[1]Department of Biomedicine, Aarhus University, Denmark; [2]The Danish Research Institute of Translational Neuroscience (DANDRITE), Aarhus University, Denmark; [3]Aarhus Institute of Advanced Studies (AIAS), Aarhus University, Denmark*)

Many people have vivid memories of the first dinner date with their partner, including details like the name of the restaurant and the food they had. In contrast, it is very difficult to remember what you had for dinner a few weeks ago. Most everyday memories may be formed automatically in the hippocampus. The key role of this memory system is to filter out unnecessary information, but keep the important memories by a mechanism that involves novelty-associated dopamine release in the hippocampus.

Recently, our studies (Takeuchi *et al*., Nature, 2016) revealed that projections from neurons in the locus coeruleus to the hippocampus can drive environmental novelty-associated enhancement of memory retention through non-canonical release of dopamine in the hippocampus, in line with the synaptic tagging and capture theory of initial memory consolidation. These studies also raise a possibility that the impact of distinct novel experiences which, by their very nature, bear minimal relationship to past experiences ('distinct novelty') may differ from novel experiences that share some commonality with past experiences ('common novelty') (Yamasaki and Takeuchi, Neural Plasticity, 2017; Duszkiewicz *et al*., Trends Neurosci, 2018). We now propose that memory of events accompanied by novelty can be selectively retained through two distinct dopaminergic mechanisms, depending on the nature of the novel experience itself. (COI: No)

Symposium46

Plasticity of inhibitory signaling in Epilepsy: New Physiological Mechanisms

(March 30, Sat., 10:00-12:00, Room K)

S46-1

Neural circuits basis of temporal lobe epilepsy

Zhong Chen; Yi Wang; Cenglin Xu (*Zhejiang University, China*)

Temporal lobe epilepsy (TLE) is a common type of epilepsy and not well controlled by current treatments, but the underlying cellular/circuit mechanisms remain unclear. The early series of our studies have proved the success of low-frequency stimulation treatment for epilepsy, which was mainly depending on the stimulation target, the stimulation frequency and stimulation time (the therapeutic-window phenomenon). Now, by using optogenetics, multiple-channel EEG analysis, imaging, electrophysiological and molecular techniques, we are continued to investigate the circuit mechanism of therapeutic deep brain stimulation, and found that entorhinal principal neurons mediate antiepileptic "glutamatergic-GABAergic" neuronal circuit for brain stimulation treatments of epilepsy. Meanwhile, we are currently focusing on the interplay of inhibitory and excitatory network in subicular microcircuits especially that related to the generation of generalized seizures (GS) in TLE, and we found that depolarized GABAergic signaling in subicular microcircuit mediates GS in TLE. This may be of therapeutic interest in understanding the pathological neuronal circuitry and further the development of novel therapeutic approaches. (COI: Properly Declared)

S46-2

Conditional upregulation of KCC2 enhances inhibition during seizures in mice

Chelsea Goulton[1]; M Watanabe[2]; D Cheung[1,2]; A Khoshaba[1]; H Indada[2]; K Eto[2,3]; H Wake[2,4]; J Nabekura[2,3]; A Moorhouse[1]

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The K^+Cl^--cotransporter (KCC2) is essential for regulating intracellular Cl^-, thus is key in sustaining $GABA_A$-receptor mediated inhibition. This study characterizes a transgenic mouse where KCC2 overexpression can be regulated by doxycycline in the diet. Elevated KCC2 was first confirmed with *in-situ* hybridization and western immunoblotting. A corresponding enhancement in membrane transport function was demonstrated using a pH sensitive fluorophore (AM-BCECF) and by measuring NH_4^+- mediated fluorescence in acute slices. The effects on neuronal excitability were assessed using *in vitro* field recordings from hippocampal CA1 in acute slices. Under basal conditions, evoked responses were unchanged, as were muscimol concentration-response relationships. Interestingly, tetanus-induced afterdischarges were virtually abolished ($p<0.01$), while 0-Mg^{2+}/high K^+ seizures had a delayed onset ($p<0.01$) and the spiking frequency was reduced ($p<0.01$). However, results *in vivo* were mixed, with clear protection against s*tatus epilepticus* induced by kainic acid (up to 50mg/kg i.p, $p<0.0001$), but not pentylenetetrazole (85mg/kg, s.c.). Overall, our data support that KCC2 overexpression may help sustain Cl^- homeostasis under hyperexcitable conditions and provide a novel strategy to preserve effective neuronal inhibition. This conditional transgenic mouse should facilitate more specific investigations into the potential of targeting KCC2 for the treatment of conditions such as epilepsy. (COI: No)

S46-3

Human epilepsy and animal model with mutations in KCC2

Atsuo Fukuda (*Department of Neurophysiology, Hamamatsu University School of Medicine, Japan*)

SLC12A5 encodes KCC2 which is the main Cl^- extruder of neurons rendering the proper inhibitory function of GABA. Thus any mutation in *SLC12A5,* if it causes dysfunction of KCC2, could be pathogenic for neurological disorders by causing deteriorated inhibition and collapse of excitation-inhibition balance.

Epilepsy caused primarily by an imbalance of excitation and inhibition may be the most relevant disease for *SLC12A5* mutation. Whole exome sequencing has revealed causal *SLC12A5* mutation in patients of intractable epilepsy, i.e., 3 patients of epilepsy of infancy with migrating focal seizures with compound heterozygous mutations in *SLC12A5*. Heterologous expression of KCC2 mutants, mimicking the patient status, resulted in $[Cl^-]_i$ level significantly higher than with wildtype KCC2, but less than without KCC2. The results indicate that even mildly impaired neuronal Cl^- extrusion in individuals could be causal to epilepsy.

Phosphorylation of KCC2 at two threonines (Thr^{906} and Thr^{1007}) which inhibits KCC2 activity, decreases in parallel with an increase in KCC2 activity and the lowering of neuronal $[Cl^-]_i$ during brain development, but the significance of this *in vivo* is unknown. Therefore we engineered mice to express two KCC2 alleles with the missense mutations Glu^{906} and Glu^{1007} (*Kcc2*$^{e/e}$) to mimic constitutive phosphorylation at these sites. *Kcc2*$^{e/e}$ demonstrated deteriorated ability to extrude Cl^-, being susceptible to status epilepticus by any sensory stimulations. (COI: No)

S46-4

Altered Cl-homeostasis during epileptogenesis

Claudio Rivera[1,2,3] ([1]*Neuroscience Center, University of Helsinki, Finland;* [2]*INMED, France;* [3]*Aix-Marseille Université, France*)

Remodelling of neuronal networks is one of the major pathophysiological processes observed in epileptic tissue and post-traumatic brain injuries. The understanding of the molecular mechanisms involved is of particular importance since this remodelling of glutamatergic and GABAergic synapses creates a powerful hyperexcitable cerebral focus that drives recurrent disabling seizures. Besides network rewiring, an abnormal depolarizing GABAergic drive has been hypothesized to participate in epileptogenic processes. This includes the deregulation of functional expression of the neuronal specific K^+-Cl^-co-transporter KCC2 and the Na^+-K^+-2Cl^-co-transporter NKCC1. We have also previously shown that depolarizing GABAergic transmission triggers the up-regulation of the pan-neurotrophin receptor, $p75^{NTR}$. We have now tested the hypothesis that the early alteration of Cl^-homeostasis following status epilepticus (SE) is a precipitating event that triggers recurrent mossy fibre sprouting *via* the activation of $p75^{NTR}$. We also examined a novel therapeutic strategy based on the transient blockade of Na^+-K^+-2Cl^-co-transporter NKCC1 early after SE to reduce ectopic sprouting and recurrent seizures in the chronic phase (i.e. several months after SE). The findings from this study define promising and novel targets to constrain reactive glutamatergic network rewiring in adult epilepsy (COI: No)

S46-5

Upregulating KCC2 as a Target for Seizure Therapies

Dennis Lawrence Cheung[1]; Chelsea Sarah Goulton[2]; Miho Watanabe[3]; Junichi Nabekura[1]; Andrew John Moorhouse[2]

([1]*Division of Homeostatic Development, National Insititute for Physiological Sciences, Japan;* [2]*School of Medical Sciences, Faculty of Medicine, UNSW Sydney, Australia;* [3]*Department of Neurophysiology, Hamamatsu University School of Medicine, Japan*)

During a seizure, the balance between excitatory and inhibitory activity is disrupted. Given its central role in Cl- homeostasis, we hypothesized that increased KCC2 activity would enhance GABAergic inhibition thus improving resistance to seizures. We tested this using a systemic kainic acid (KA) seizure model, in control and KCC2 upregulated mice. We used a tetracycline conditional expression mouse to overexpress KCC2 via withdrawal of doxycycline dietary supplementation. In this seizure model, mice underwent an escalating dose regime receiving two KA injections (5 mg/kg, IP) administered one hour apart. Behavioural seizures were terminated after the second hour by a single diazepam injection (5 mg/kg, IP). EEG was recorded throughout the entire procedure. We assessed seizure severity based on the number of seizure spikes per hour in the EEG traces and the percentage of time spent in seizure. In both measures, there was no significant difference between control and KCC2-upregulated mice post KA. However, post diazepam there was a significant reduction in both seizure measures in KCC2-upregulated mice ($n = 11$) as compared to control ($n = 10$) ($p < 0.05$ both, Kruskal-Wallis test). Our results suggest that increased KCC2 activity by itself has only a limited effect on improving the resistance threshold to seizures. However, its ability to potentiate the effectiveness of diazepam in ameliorating seizures represents a novel approach for improving current seizure pharmacotherapies. (COI: No)

Symposium47 (Sponsored Symposium)

New Frontiers in Regenerative Medicine of Renal Function

(Co-sponsored by Shinkoiwa Clinic)

(March 30, Sat., 13:30-15:00, Room F)

S47-1

Failure to sense energy depletion in chronic kidney disease

Eisei Sohara; Hiroaki Kikuchi; Shinichi Uchida (*Department of Nephrology, Tokyo Medical and Dental University, Japan*)

The kidneys consume a large amount of energy. The identification of factors that cause energy mismatch in the setting of chronic kidney disease (CKD) and the development of interventions aimed at improving this mismatch are key research imperatives. Although the critical cellular energy sensor 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) is known to be inactivated in CKD, the mechanism of AMPK dysregulation is unknown. In a mouse model of CKD, metabolome analysis confirmed a decrease in AMPK activation in the kidneys despite a high AMP: ATP ratio, suggesting that AMPK did not sense energy depletion. Similar AMPK inactivation was found in heart and skeletal muscle in CKD mice. Several uremic factors found by metabolome of each tissue were shown to inactivate AMPK. The specific AMPK activator A-769662, which bypasses the AMP sensing mechanism, ameliorated fibrosis and improved energy status in the kidneys of CKD mice, whereas an AMP analog did not. We further demonstrated that a low-protein diet activated AMPK independent of the AMP sensing mechanism, leading to improvement in energy metabolism and kidney fibrosis. These results suggest that a failure to sense AMP is the key mechanism underlying the vicious cycle of energy depletion and CKD progression and direct AMPK activation may be a novel therapeutic approach in CKD. (COI: No)

S47-2

Kidney reconstitution from iPS cells based on developmental biology

Ryuichi Nishinakamura (*Institute of Molecular Embryology and Genetics, Kumamoto University, Japan*)

The kidney develops by the reciprocal interactions between the nephron progenitor and ureteric bud. We previously identified the distinct origins of these two precursor tissues and generated the nephron progenitor from human iPS cells (Taguchi et al. Cell Stem Cell, 2014). The induced nephron progenitors readily formed glomeruli and renal tubules in vitro, and upon transplantation, human glomeruli were vascularized with the host mouse endothelial cells (Sharmin et al. J Am Soc Nephrol, 2016). These technologies were successfully applied to patient-derived iPS cells, which reproduced the initial stage of the congenital nephrotic syndrome (Tanigawa et al. Stem Cell Reports 2018). We also reported a protocol to selectively induce glomerular podocytes from human iPS cells (Yoshimura et al. J Am Soc Nephrol, 2019).

However, these conventional kidney organoids lacked the connections between the nephrons. To generate the genuine kidney structure, we established an induction protocol for the ureteric bud from pluripotent stem cells. Importantly, mouse organoids reassembled from the differentially induced ureteric bud and nephron progenitors developed the inherent structure of the embryonic kidney (Taguchi et al. Cell Stem Cell, 2017). This selective induction and reassembly strategy will be a powerful approach to recapitulate higher-order architectures in stem cell-derived organoids. (COI: No)

S47-3

Next generation Therapy for dialysis patients using iPS cells

Takashi Yokoo (*Department Internal of Medicine, Jikei University School of Medicine, Japan*)

We have been engaged in "regenerating" the whole kidney for many years. Kidney regeneration involves the following steps: 1) establishment of nephron progenitor cells from induced pluripotent stem (iPS) cells; 2) establishment of regenerating functional kidney from nephron progenitor cells; and 3) is construction of urinary excretion pathway for urine to pass to the bladder. We have already proved all three steps using different animals and provided the Proof of Concept (POC) of our strategy. Therefore, in this applied study, we will conduct the three remaining experiments before the clinical trial. We developed a system wherein only exogenous progenitor cells are mature in the niche by removing existing precursor cells under the presence of the agent by gene manipulation. Subsequently, we succeeded in establishing nephrons derived from 100% exogenous NPSs. Furthermore, by transplanting them into the living body, recruiting blood vessels is possible, and urine production was confirmed.

The final stage is to complete all three elements in the human environment. In other words, this success is based on experiments with rats and mice, so we need to prove also with NPCs derived from human iPS cell. Then we will proceed to the next step, the clinical trial with human subjects. (COI: No)

Local Organizing Committee Symposium

Symposium48

Inter-tissue communications underlying metabolic and feeding control in living body (whole day symposium) part II

(March 30, Sat., 15:10-17:10, Room A)

S48-1

Central insulin action and hepatic glucose metabolism

Hiroshi Inoue[1,2]; Yuka Inaba[1]; Emi Hashiuchi[2] (*[1]Institute for Frontier Science Initiative, Kanazawa University, Japan; [2]Graduate School of Medical Sciences, Kanazawa University, Japan*)

Insulin controls hepatic glucose production (HGP) through the hypothalamic insulin action, as well as the direct hepatic insulin action. This central insulin action regulates HGP via the vagus nerve. Central insulin action results in the suppression of the vagus nerve activity, which in turn decreases HGP. The vagal action via alpha 7 nicotinic acetylcholine receptor (A7nAchR) plays an important role in this central regulation of HGP. Activation of A7nAchR is known to suppress the expression of inflammatory cytokines in the macrophage, including IL-6. The acute suppression of hepatic vagus nerve activity releases hepatic Kupffer cells from A7nAchR-dependent suppression of inflammatory cytokines, resulting in the hepatic IL-6 increase. Hepatic IL-6 increase induces hepatic STAT3 activation, followed by the suppression of hepatic gluconeogenic genes and HGP.

Obesity and insulin-resistance is known to impair central insulin action, presumably followed by the persistent suppression of the vagus nerve activity. We have revealed that, in obese mice, the persistent suppression of the vagus nerve results in the smoldering activation of Kupffer cells and blunting of acute activation response of hepatic STAT3 by IL-6. Furthermore, IL-6-dependent STAT3 activation is depressed by hepatic ER-stress in obese mice. The impediment of central-mediated regulation of the vagus nerve may be related to both HGP increase and hepatic chronic inflammation in obesity. (COI: No)

S48-2

Contribution of the hepatokine selenoprotein P to the various pathologies of type 2 diabetes

Hirofumi Misu (*Department of Endocrinology and Metabolism, Kanazawa University, Japan*)

Contribution of the hepatokine selenoprotein P to the various pathologies of type 2 diabetes.

The liver may contribute to the onset of various pathologies of type 2 diabetes by way of the production of secretory proteins "hepatokines." By using the comprehensive gene expression analyses in human livers, we have rediscovered selenoprotein P (SeP), a transport protein of selenium with anti-oxidative capacity, as hepatokines involved in the onset of insulin resistance and hyperglycemia (**Cell Metabolism** 2010 483-495). Notably, we have recently revealed that SeP impairs health-promoting effects of exercise training by inhibiting ROS/AMPK/PGC-1α pathway in the skeletal muscle through its receptor LRP1 (**Nature Medicine** 2017 508-516). In the current study, we investigated the actions of SeP on thermogenesis in brown adipose tissue. We found that SeP knockout mice showed hyperthermia after cold exposure. UCP1 sulfenylation was increased in brown fat of SeP deficient mice. Treatment with SeP impairs noradrenaline-induced superoxide formation and elevation of cellular temperature in primary brown adipocytes. These findings indicated that the hepatokine SeP impairs thermogenesis in brown fat via anti-oxidative capacity. Further studies would develop novel diagnostic or therapeutic procedures targeting SeP to combat over-nutrition-related diseases such as type 2 diabetes. (COI: No)

S48-3

NeuroImmunoMetabolic regulation of cardiac physiology and heart failure

Ichiro Manabe (*Chiba University, Japan*)

Heart failure is a complex clinical syndrome characterized by cardiac function that is insufficient to meet systemic demand. In addition to abnormalities intrinsic to the heart, dysfunction in other organs and systemic factors greatly affect the development and consequences of HF. In particular, nearly half of chronic HF (CHF) patients also have chronic kidney disease (CKD), which increases their rate of cardiovascular mortality, suggesting cardiorenal linkage via mechanisms still poorly understood. We found that pressure overload in the heart activates renal collecting duct (CD) epithelial cells via sympathetic nerves. Within the kidneys, activated communication between CD cells, tissue macrophages and endothelial cells leads to secretion of CSF2, which in turn stimulates cardiac-resident macrophages essential for the myocardial adaptive response to pressure overload. We show that CD-specific deletion of the transcription factor *Klf5*, renal sympathetic denervation or adrenergic beta2 receptor blockade/deletion disrupts the renal response to cardiac pressure overload. Our results clearly demonstrate that dynamic interplay between the heart, brain and kidneys is necessary for proper adaptation to cardiac stress. In the heart resident macrophages control cardiac metabolism in the steady state, pointing to an immunometabolic crosstalk in the maintenance of cardiac homeostasis. (COI: No)

S48-4

JMJD1A mediates acute and chronic thermogenic responses through complementary mechanisms

Juro Sakai[1,2] (*[1]Tohoku University School of Medicine, Molecular Physiology div., Japan; [2]The University of Tokyo, RCAST, Metabolic Medicine div., Japan*)

In acute cold exposure in mammals, JMJD1A, an H3K9 demethylase, is known to up-regulate thermogenic gene expression in response to β-adrenergic signaling in brown adipose tissue (BAT). Recently, we showed that JMJD1A activity depends on phosphorylation of a serine residue (S265) but not on its demethylation activity. Mammals also have another type of thermogenesis in response to long-term cold stress, which induces the browning of subcutaneous white adipose tissue (scWAT) referred to as "beige-ing" of scWAT. Previous studies have suggested that the long-term cold stress is epigenetically memorized, although the mechanism remains unclear. Here we show that S265 phosphorylation of JMJD1A is also pivotal for beige-ing. Ablation of S265-phosphorylation of JMJD1A impaired beige fat function and insulin sensitivity in mice. In the first step (signal sensing) cold stress (which generates β-adrenergic signal) causes the phosphorylation of JMJD1A at S265, which in turn, causes JMJD1A to target beige-selective genes through the formation of a transcriptional protein complex containing PGC1α, PRDM16, and DNA-bound sequence PPARγ. In the second step (epigenetic rewriting) under prolonged stress, phosphorylated JMJD1A demethylates H3K9me2 to turn on transcription in scWAT. (COI: No)

S48-5

Metabolic adaptation and maladaptation in the adipose tissue

Shingo Kajimura (*University of California, USA*)

The adipose tissue possesses the remarkable capacity to control its size and function in response to a variety of internal and external cues, such as nutritional status and temperature. The regulatory circuits of fuel storage and oxidation in white adipocytes and thermogenic adipocytes (brown and beige fat) play a central role in systemic energy homeostasis. On the other hand, dysregulation of the pathways is closely associated with metabolic disorders and adipose tissue malfunction, including obesity, insulin resistance, chronic inflammation, and mitochondrial dysfunction. Here, I plan to discuss an overview regarding our current understandings of adipose cell metabolism in physiology and disease, and also possible strategies to rewire fat cell metabolism to improve metabolic health. (COI: No)

Local Organizing Committee Symposium

Symposium49

Frontiers in pain physiology - from detection to the survival behavior

(March 30, Sat., 15:10-17:10, Room B)

S49-1

Primary sensory neuron-secreted proteins modulate pain transmission in spinal level

Xu Zhang (*Institute of Neuroscience and State Key Laboratory of Neuroscience, CAS Center for Excellence in Brain Science, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China*)

Primary sensory neurons in dorsal root ganglia (DRG) convey peripheral nociceptive signals to spinal dorsal horn. The synaptic transmission could be regulated by neuromodulators secreted by DRG neurons. Previous work demonstrated that follistatin-like 1 (FSTL1), a secreted protein, inhibits afferent synaptic transmission by activating presynaptic Na+,K+-ATPase (NKA). Activin C secreted by DRG neurons could suppress inflammation-induced nociceptive transmission by inhibiting the phosphorylation of extracellular signal-regulated kinase (ERK) in DRG and spinal dorsal horn. Our recent work has showed that pancreatitis-associated protein (PAP) I is continuously upregulated in DRG neurons after peripheral nerve injury. PAP-I was transported to terminals and secreted in spinal dorsal horn. Both PAP-I gene knockout and intrathecal application of PAP-I antibody alleviated tactile allodynia induced by SNI. Intrathecal delivery of PAP-I enhanced sensory hypersensitivity and formalin-induced hyperalgesia. The functions could be mediated by the direct activation of microglia by PAP-I in spinal level. Inhibition of microglia activation could abolished the pro-nociceptive effect of PAP-I. Thus, PAP-I mediates the immune crosstalk between primary sensory afferents and spinal microglia after nerve injury and contributes to the maintenance of neuropathic pain. All these data suggest that DRG neurons could modulate pain transmission in spinal level by various secretory proteins. (COI: No)

S49-2

Withdrawn

S49-3

How opioids and noxious stimuli regulate delivery of nociceptive information to the amygdala

Elena Bagley (*Discipline of Pharmacology and Charles Perkins Centre, University of Sydney, Australia*)

The amygdala contributes negative emotional value to nociceptive sensory information and forms the association between an aversive response and the environment in which it occurs. Opioids are strong analgesics and reduce both the sensory discriminative and the affective component of pain but whether opioids regulate activity at the two nociceptive inputs onto the amygdala is unknown. Using whole-cell electrophysiology, optogenetics and immunohistochemistry in Sprague Dawley rats, we found that MOR activity inhibited synaptic transmission at the PB-CeLC synapses but MOR, DOR and KOR activity inhibited BLA-CeLC synapses. Given the evidence of the opioid's role in reducing pain affect, modulation of these synapses could be, in part, the site of opioid action and may become particularly important in chronic pain. In chronic pain conditions the parabrachial-amygdala synapse is potentiated however very little is known about how plasticity occurs in conditions without ongoing injury. We showed that a brief nociceptive stimulus with no ongoing injury produced long-lasting synaptic plasticity at the rat parabrachial-amygdala synapse. Furthermore, this synaptic potentiation primes the synapse so that a subsequent noxious stimulus causes prolonged potentiation of the nociceptive information flow into the amygdala. As a result, a second injury could have an increased negative emotional value and promote associative learning that results in pain related avoidance. (COI: Properly Declared)

S49-4

Lateralized amygdala plasticity independent of bilateral parabrachial activity in inflammatory pain

Yukari Takahashi[1,2]; Yuta Miyazawa[1,2]; Yae K Sugimura[1,2]; Fusao Kato[1,2] ([1]*Dept Neurosci, Jikei Univ Sch Med, Japan;* [2]*Cntr Neurosci Pain, Jikei Univ Sch Med, Japan*)

The amygdala is the core of the "defensive survival circuit", receiving direct nociception-related information via the "general alarm" system of the parabrachial nucleus. In particular, the majority of neurons in the capsular part of the central amygdala (CeC) are excited by noxious stimuli through monosynaptic glutamatergic inputs from the lateral parabrachial nucleus (LPB) and this excitatory LPB-CeC transmission undergoes robust synaptic potentiation in various pain models. It is recently reported that the right central amygdala (rCeA) is preferentially activated in response to inflammation in the either side of the hind limb. We evaluated latent cellular activation of the LPB and amygdala using c-Fos expression and the LPB-CeC synaptic transmission at 3 h and 6 h, respectively, after formalin injection to either side of the upper lip. Despite bilateral increase in c-Fos expression in the LPB and the basolateral amygdala (BLA), higher c-Fos expression and LPB-CeC synaptic potentiation were predominantly observed in the rCeA. These results suggest that the lateralized activation and synaptic potentiation in the rCeA are not simple consequences of the bilaterally enhanced LPB neuron activity and it is likely that unidentified lateralized factors determine the inflammation-associated synaptic plasticity rather than simple Hebbian plasticity of the LPB-CeC synapses in the rCeA. (COI: No)

S49-5

No pain no gain and no protection: Chronic pain protects heart from ischemia-reperfusion injury

Chien-Chang Chen; Yi-Fen Cheng; Ya-Ting Chang; Wei-Hsin Chen; Hsi-Chien Shih; Bai-Chuiang Shyu (*Institute of Biomedical Sciences, Academia Sinica, Taiwan*)

Ischemic heart disease or coronary heart disease is the leading cause of death in the world and the 2nd cause of death in Taiwan. Timely restoration of blood flow is the most effective way to rescue myocardium. However, reperfusion can also damage cardiomyocytes due to calcium overload, free radical production and inflammatory cell infiltration. Up to 25% of the population is suffering from chronic pain, especially in elder. Ischemic heart disease is also prevalent in the elderly population. However, it is unclear whether this is a relationship between chronic pain and ischemic heart diseases. Here we show that chronic neuropathic pain reduces ischemia/reperfusion injury following myocardial infarction, and this cardioprotection is induced via an anterior nucleus of paraventricular thalamus (PVA)-dependent parasympathetic pathway. Inhibition of ERK activation in the PVA abolishes neuropathic pain-induced cardioprotection, whereas activation of PVA neurons pharmacologically, or optogenetic stimulation, is sufficient to induce cardioprotection. Furthermore, neuropathic injury and optogenetic stimulation of PVA neurons reduce the heart rate. These results suggest that the parasympathetic nerve is responsible for this unexpected cardioprotective effect of chronic neuropathic pain in mice. (COI: No)

International Scientific Program Committee Symposium

Symposium50

Maternal influences on offspring development (AuPS, Australia)

(March 30, Sat., 15:10-17:10, Room C)

S50-1

Fetal origins of osteoarthritis induced by maternal xenobiotic exposure

Hui Wang[1,4]; Liaobin Chen[3,4]; Hao Kou[2,4]; Yinxian Wen[3,4]
([1]*Department of Pharmacology, School of Basic Medical Sciences, China;* [2]*Department of Pharmacy, Zhongnan Hospital of Wuhan University, China;* [3]*Department of Orthopedic Surgery, Zhongnan Hospital of Wuhan University, China;* [4]*Hubei Provincial Key Laboratory of Developmentally Originated Disease, China*)

Studies indicate that osteoarthritis belongs to metabolic syndrome (MS) and might have a fetal origin. However, the fetal origin of osteoarthritis and the intrauterine programming mechanism remains unknown. By selecting caffeine, nicotine and ethanol as xenobiotic representatives, we demonstrated that maternal xenobiotic exposure (MXE) induced intrauterine growth retardation (IUGR) and fetal cartilage dysplasia and increased susceptibility to adult osteoarthritis in IUGR offspring. The hypotheses of "two-programming" and "two-hit" underlying fetal-originated osteoarthritis were proposed. Fetal cartilage dysplasia caused by MXE (the first insult) was associated with direct damage of xenobiotics and indirect compensation of maternal glucocorticoids (GC). Maternal GC inhibited cartilage development via inducing low-functional programming of TGFβ/IGF1 signaling (the first programming). On the other, the hepatic "GC-IGF1 axis programming" (the second programming) leads to hypercholesteremia in adulthood, which increases the accumulation of cholesterol in local chondrocytes. Furthermore, postnatal environmental change (the second hit) have interactions with MXE and may exacerbate adult articular cartilage matrix imbalance and cholesterol accumulation. This study confirmed that adult osteoarthritis has a fetal origin, which provides basis for elucidating international hot issue "developmental origin of health and diseases (DOHaD)". (COI: No)

S50-2

How can maternal deprivation cause neurodevelopmental disorders?

Ken-Ichi Ohta; Shingo Suzuki; Takanori Miki (*Department of Anatomy and Neurobiology, Faculty of Medicine, Kagawa University, Japan*)

The bond between a mother and her child is crucial in neuronal brain development. Maternal deprivation in early postnatal life is known to induce stress in children and disrupts normal brain development, which can lead to neurodevelopmental disorders. However, many of its etiological mechanism have not yet been clarified.

We have examined the influence of maternal separation (MS) on brain development using a rat model. Our results showed that MS during early postnatal life causes social deficits later in life and especially decreases social recognition among social behavior. Regarding this social deficit, we additionally found that inhibitory neurons and synapses were decreased in the medial prefrontal cortex (mPFC) after MS. A sequential study revealed that activation of the mPFC was lower during social behavior tests related to social recognition in young adult rats exposed to MS. Our findings suggest that the neuronal excitation/inhibition imbalance caused by MS is attributed to decreased activation of the mPFC, which causes social deficits later in life. Given that these excitation/inhibition imbalances and social deficits are core symptoms in neurodevelopmental disorders such as autism spectrum disorders, our results indicate that maternal deprivation is a possible cause.

In this symposium, I will discuss how maternal deprivation is associated with developmental disorders in regard to the social deficits described above, including our new findings. (COI: No)

S50-3

Role of linoleic acid in offspring development: Focus on inflammation and the placenta

Deanne Helena Hryciw[1,2]; Nirajan Shrestha[3]; James SM Cuffe[3]; Olivia J Holland[3]; Amanda Cox[3]; Andrew Bulmer[3]; Anthony V Perkins[3]; Andrew J McAinch[2,4] ([1]*School of Environment and Science, Griffith University, Australia;* [2]*Institute for Health and Sport, Victoria University, Australia;* [3]*School of Medical Science, Griffith University, Australia;* [4]*Australian Institute for Musculoskeletal Science (AIMSS), Victoria University, Australia*)

Linoleic acid (LA) is an omega 6 that is increasing in bioavailability, with elevated consumption in women including those at childbearing age. Across species, the consumption of elevated LA has been demonstrated to increase inflammation, and in pregnancy, alter the sex ratio of her offspring. This study aimed to investigate whether consumption of a maternal diet with elevated LA altered maternal or fetal growth, maternal inflammation, sex-ratio or maternal metabolic indicators.

Wistar Kyoto Rats consumed a high LA diet (6.21%) or control LA diet (1.44%) for 10 weeks prior to mating. Omega 3 concentrations in both diets are matched (0.3%). Animals were sacrificed at E20, and maternal body and organ weights, fetal body and organ weights, placental weight, maternal blood and sex-ratio were determined.

Compared with maternal rats consuming a control LA diet, in maternal rats consuming a high LA diet, there was no difference in maternal body weight and organ weight, water and food consumption, impedance, circulating maternal inflammatory mediators, fetal body weight and organ weight, placental weight, or maternal and fetal blood glucose. In litters from mothers consuming a high LA diet, there was a decrease in the number of male offspring and a significant increase in prostaglandins, however leukotriene concentrations were unaltered.

Consumption of a maternal diet high in LA decreased the survival of male fetuses in the absence of a maternal inflammatory response. (COI: No)

Symposium51

Cutting-edge Research in Neural Network Dynamics

(Organized by Women in Physiology of Japan (WPJ))

(March 30, Sat., 15:10-17:10, Room D)

S51-1

State-dependent multi-sensory integration in the posterior parietal cortex

Seung-Hee Lee (*Department of Biological Sciences, KAIST, Korea*)

Sensory perception in the real world requires proper integration of different modality inputs. The process of multisensory integration is not uniform. It varies from individual to individual and changes at different behavioral states of the animal. What factors affect the multisensory integration? Here I present our recent findings on neural circuit mechanisms for audiovisual integration in the cortex. We found that the posterior parietal cortex (PPC) receives converging inputs from the primary visual and auditory cortices and plays a critical role in audio-visual integration in mice. In resolving conflicts between audition and vision, parvalbumin-positive inhibitory neurons in the PPC mediate auditory dominance over visual perception in mice. We further found that locomotion modulates this circuit and in turn modifies the multisensory perceptual behaviors in mice. Our results demonstrate inhibition in the higher association cortex is important for active integration of audition and vision in mammals and leads to unique and subjective experience of perception. (COI: No)

S51-2

Involvement of V1 neurons preferring low-contrast stimuli in difficult orientation discrimination

Rie Kimura[1,2]; Yumiko Yoshimura[1,2] ([1]*Division of Visual Information Processing, National Institute for Physiological Sciences, Japan;* [2]*Department of Physiological Sciences, SOKENDAI, Japan*)

Animals can often perceive even vague visual stimuli. To explore the neural mechanisms, we analyzed spiking activities in primary visual cortex (V1) during a difficult orientation discrimination task. The head-fixed rats were trained to push or pull a lever in response to high-contrast stimuli depending on whether the stimuli were vertical or horizontal. After learning, we decreased the stimulus contrast to make the task more difficult. We performed multiple single-unit recordings from deep layers of V1 during the task performance. Consistent with the previous reports, we observed high-contrast preferring neurons in which the firing rates during visual stimuli decreased with a reduction of the contrast. In addition, we observed a new type of low-contrast preferring neurons in which the firing rates increased with a reduction of the contrast. The low-contrast preference was rarely observed in awake rats with passive viewing or in anesthetized rats. Furthermore, low-contrast preferring neurons fired more frequently in correct-choice trials, compared with incorrect trials. The properties of low-contrast preference were common to both wide-spiking (putative excitatory) and narrow-spiking (inhibitory) neurons. Decoding to show whether the presented stimuli were vertical or horizontal had a higher accuracy using activities of neurons including low-contrast preferring neurons. These results suggested that low-contrast preference contributes to difficult visual discrimination. (COI: No)

S51-3

mGRASP for high-resolution structural and functional synapse mapping

Jinhyun Kim[1,2] ([1]*Korea Institute of Science and Technology, Korea;* [2]*University of Science and Technology, Korea*)

Many types of questions in neuroscience require the detection and mapping of synapses in the complex mammalian brain. We developed a tool, mammalian GFP reconstitution across synaptic partners (mGRASP), offers a relatively easy, quick and economical approach to this technically challenging task. Using mGRASP, we analyzed the fine structure of connectivity in dorsal hippocampal CA1 excitatory and inhibitory neurons innervated by Schaffer collaterals (SCs) and different connectivity rules in these two cell types. Furthermore, we have applied optogenetics and mGRASP, a light microscopy technique that labels synaptic contacts, to map the number and strength of defined connections. This study highlights the feasibility of combining mGRASP and optogenetics to reveal synaptic weighting of defined projections at the level of single neurons, enabling functional connectomic mapping in diverse neural circuits. (COI: Properly Declared)

S51-4

Synaptic communication from subplate neurons controls neuronal migration in the developing neocortex

Chiaki Ohtaka-Maruyama (*Neural Network Project, Tokyo Metropolitan Institute of Medical Science, Japan*)

The cerebral neocortex is responsible for higher order brain functions, such as mental activity, in humans. In the neocortex, billions of neurons are precisely arranged in an ordered 6-layered structure. This structure is formed by the sequential generation of neurons and their migration toward the brain surface in the fetal period. Various genes involved in mental disorders such as autism and schizophrenia are associated with defects in radial migration process, suggests that the elucidation of this mechanism will help in understanding these diseases. Subplate neurons are the first neurons born in the neocortex and work transiently during neocortical development. However, its role in corticogenesis has remained elusive. Recently, we found that subplate neurons actively extend processes to form transient synapses on newly born multipolar migrating neurons and send signals to control their migration. This synaptic communication leads to switch from multipolar migration to locomotion. Subplate neurons have been known to guide thalamocortical afferents and help the first neural circuit formation in the cortex. Taken together, it is suggested that subplate neurons play as an organizer that arranges multiple processes such as production of neurons, migration, axon pathfinding and synaptogenesis that are proceeding at the same time during the limited developing period. (COI: No)

Symposium52

Sports and Brain

(Co-sponsored by De Luca Foundation)

(March 30, Sat., 15:10-17:10, Room E)

S52-1

Functional organization of spinal motor map in sport athletes

Toshiki Tazoe (*Neural Prosthesis Project, Department of Dementia and Higher Brain Function, Tokyo Metropolitan Institute of Medical Science, Japan*)

While motor representation in the motor cortex is capable of reorganizing as a result of adaptation through motor training, it has yet been discovered whether a repetition of patterned voluntary movement influences the motor representation in the spinal motor circuitry. Here, we report that spinal motor map of stimulus-induced leg movement configures specific forms depending on sport experience. Magnetic stimulation was percutaneously delivered over thoraco-lumbar spinal vertebrae in athletes who engage in various sports activities involving characteristic patterns of leg movements. We found that spinal stimulation induced either walking-like bilateral alternative leg movement or hopping-like bilateral symmetric leg movement depending on the stimulus site over spinal vertebrae. Spinal motor map representing the pattern of induced leg movement was different depending on athletes. For instance, runners have large area in which walking-like alternative movement was induced whereas weight-lifters show large area in which hopping-like symmetric movement was induced. Our finding demonstrated that the human spinal motor circuitry forms functional motor map of leg movements which are relevant with the history of specific behavioral pattern of motor training. (COI: No)

S52-2

Neural Correlates of Intuitive Decision - Making in Soccer

Xiaohong Wan[1,2]; Tomohisa Nagano[3]; Keiji Tanaka[2] ([1]*School of Pychology, Beijing Normal University, China;* [2]*Cognitive Brain Mapping Laboratory, RIKEN Center for Brain Science, Wako, Japan;* [3]*Faculty of Policy Management, Keio University, Japan*)

Intuition, i.e., a quick, largely unconscious problem-solving process, constitutes the core of experts' superior capability. While mechanisms of intuition have been studied mainly in board games, the expertise of athletes also involves cognitive components. In the present study, we measured the brain activities of professional soccer players while they quickly selected the pass target in soccer games. We found a close association of the activity in the head of the caudate nucleus, which is a part of the basal ganglia, with the quick selection. The loop circuits that the basal ganglia forms with the cortex have a characteristic structure, which is advantageous for a quick selection of an action among many candidates. Together with our previous findings in shogi (Japanese chess) we suggest that the caudate head plays an essential role in experts' intuition in a wide range of domains by supporting quick selections. (COI: Properly Declared)

S52-3

The Paralympic Brain - Brain reorganization appeared in Paralympic athletes -

Kimitaka Nakazawa (*Department of Life Sciences, The University of Tokyo, Japan*)

The primary goals of Paralympic-brain and neurorehabilitation studies share the common area in the neurosciences, which aim to reveal the underlying neural mechanism in the reorganization of brain after interventions of physical rehabilitation or athletic training. We found that the brains of Paralympic athletes are reorganized uniquely in such a manner dependent on disability types and athletic-specific training. Factors playing the major roles in the reorganization are most probably use-dependent plasticity and disability-specific compensations. In this presentation the specific ipsilateral corticospinal excitation in athletes with lower limb amputee and large expansion of foot area in the archer with congenital absence of both arms will be shown with our recent results obtained from the functional brain imaging and transcranial magnetic stimulation. (COI: Properly Declared)

S52-4

Why is muscle relaxation difficult during sports?

Kouki Kato; Kazuyuki Kanosue (*Faculty of Sport Sciences, Waseda University, Japan*)

Muscle relaxation is an important aspect to make a fine control of the body in sports as well as daily life. Neuroimaging and neurophysiological studies suggest that volitional muscle relaxation from contraction is an active process requiring cortical activation, and not just the cessation of contraction. Several studies utilizing transcranial magnetic stimulation (TMS) have demonstrated that, during the relaxation phase of a particular muscle, the excitability of the corticospinal tract controlling that particular muscle is suppressed and the intra-cortical inhibition is activated just before muscle relaxation. Moreover, we recently demonstrated that muscle relaxation of one body part suppresses corticospinal activities controlling other body parts in different limbs. Therefore, muscle relaxation of one body part elicits a set of specific brain activities that extend an inhibitory influence to many parts of the body. During multi-limb movements such as those involved in sports and playing musical instruments, this influence of relaxation hinders the appropriate contraction of muscles involved in certain movements. This spread of inhibition may be the reason why muscle relaxation is so difficult, especially for beginners. In this presentation, we review the neural mechanisms of muscle relaxation mainly based on our recent work involving TMS technique. (COI: No)

Symposium53

Dynamic signaling of axon and presynaptic terminals revealed by direct recordings

(March 30, Sat., 15:10-17:10, Room F)

S53-1

Control of synaptic outputs by dynamic axonal excitability

Shin-Ya Kawaguchi[1,2,3] (*[1]Society-Academia Collaboration for Innovation, Kyoto University, Japan; [2]Graduate School of Science, Kyoto University, Japan; [3]Institute for Advanced Study, Kyoto University, Japan*)

An axon has traditionally been considered to reliably transmit action potentials to presynaptic terminals. Technical difficulties arising from the small size of the axon/presynaptic terminals have impeded the study of axonal physiology. However, recent technical advances to directly record from axonal compartments have contributed to unveil detailed mechanisms of axonal signaling. Using direct patch-clamp recording from an axon and/or presynaptic bouton labeled with EGFP in primary culture, we have studied the biophysical mechanisms of axonal and presynaptic signaling in the cerebellar circuit, mainly focusing on Purkinje cells (PCs), sole output neurons in the cerebellar cortex. Direct voltage-clamp recording from an axon/presynaptic bouton of a PC makes it possible to measure the local membrane excitability and the presynaptic transmitter release machinery by measuring exo-endocytosis of synaptic vesicles upon presynaptic Ca^{2+} influx. In this symposium, I am going to overview the dynamic modulation of axonal signal propagation and its impact on the presynaptic transmitter release machinery, contributing to the dynamic computation in the cerebellar circuit. (COI: No)

S53-2

Analog signaling in molecular layer interneurons of the cerebellar cortex

Federico F Trigo (*Brain Physiology Laboratory, France, and University Paris Descartes, France*)

In the classical view of synaptic integration, the tasks of the different neuronal compartments are sharply defined: the somatodendritic compartment gathers synaptic information; the axon initial segment sets the threshold for action potential (AP) firing; and the axon transmits the new AP to presynaptic terminals. In recent years however, several studies have uncovered substantial deviations from this simple picture, and today it is clear that individual neurons do not necessarily behave as the « Platonic neuron » described by Coombs, Eccles and Fatt in the middle 1950s.

The demonstration that somatodendritic voltage changes can be passively transmitted down the axon for a certain distance and that they affect transmitter release indicates that the axonal compartment does not only transmit a digital type of signal (the AP), but that it also transmits analog signals (subthreshold voltage changes); this has been named “mixed” (analog and digital) transmission. Experimentally, the questions of whether physiological regimes of activity are passively conducted from the soma and dendrites to the axon, how they are integrated by the cell and how they affect release have only recently been addressed.

In the present talk I will briefly introduce the analog signaling phenomenon, I will then demonstrate its presence in cerebellar interneurons and how this type of coupling affects neurotransmitter release and finally, I will discuss its significance for neuronal signaling. (COI: No)

S53-3

Presynaptic properties at lemniscal fiber terminals in the somatosensory thalamus

Mitsuharu Midorikawa; Mariko Miyata (*Department of Physiology, Division of Neurophysiology, School of Medicine, Tokyo Women's Medical University, Japan*)

Somatosensory information from the maxillary region of rodent is conveyed to the ipsilateral trigeminal nuclei via the infraorbital nerve and then to relay neurons in the contralateral ventral posteromedial thalamic nucleus (VPM) via medial lemniscal fibers. During early postnatal development, the lemniscal fibers undergoes a number of morphological and functional changes. We have shown that most VPM relay neurons become innervated by single strong lemniscal fiber after developmental synapse elimination. We also showed the functional properties of the developmental changes at the postsynaptic side. From these studies, developmental changes at the postsynaptic side of lemniscal fiber-VPM neuron synapse were well described. However, how presynaptic properties of lemniscal fiber terminals changes with development is not yet well established.

Here, we combined electrophysiological and optogenetics techniques to elucidate the properties of transmitter release from mice lemniscal fiber terminals, and clarified their detailed properties and developmental changes. (COI: No)

S53-4

Regulation of neuronal signaling by axonal ion channels and neurotransmitter receptors

Yousheng Shu (*State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, China*)

Action potentials (APs) initiate first at the axon initial segment (AIS). Because of their all-or-none feature, they are usually considered as digital signals. However, APs are not solely digital because their voltage waveforms are variable; those with longer durations may cause more Ca^{2+} entry and thus more neurotransmitter release, suggesting an analog model of signaling. In neocortical pyramidal cells (PCs), we performed whole-cell recordings from axonal blebs (cut end of the axon) and revealed an important role of voltage-gated Kv1 channels in shaping the waveform of APs. Activation of dopamine receptors regulates Kv1-mediated K^+ currents and causes changes in AP waveform. Activation of axonal $GABA_A$ receptors results in local hyperpolarization and changes in AP waveform and AP-triggered Ca^{2+} transient. In GABAergic interneurons, the parvalbumin-containing neurons and the somatostatin-containing neurons, axonal whole-cell recording also revealed the expression of $GABA_A$ receptors at their AIS. Activation of these ligand-gated Cl^- channels substantially reduces the probability of synaptically evoked APs. Together, these findings indicate that neuronal excitability and the AP waveform are subject to modulation by axonal voltage-gated and ligand-gated ion channels, suggesting a role of axonal channels in the regulation of output signals in both principal cells and inhibitory interneurons. (COI: No)

S53-5

Dynamic control of spike signaling by axonal afterdepolarization

Haruyuki Kamiya (*Department of Neurobiology, Hokkaido University Graduate School of Medicine, Japan*)

Spike propagation along the axon provides fast and reliable digital signaling in the nervous system. Recent studies, however, demonstrated that axonal spikes are subject to fine-scale modulation by neuronal activity or by influence from surrounding glia. We aimed at elucidating the modes and exact mechanisms underlying dynamic control of axonal spike signaling in the brain. To this end, we adopted direct recordings from hippocampal mossy fiber terminals, since the exceptionally large structure enables stable subcellular recordings of axonal action potentials. We focused on the mechanism underlying afterdepolarization (ADP), lasting depolarization which follows axonal spike. We revealed that slow activating Na^+ channels are partly involved in the generation of ADP on the hippocampal mossy fibers. Passive components due to slow capacitive discharge of axonal membrane also substantially contribute to ADP, suggesting that the shape and the electrical properties of the axon are critical determinants of time course of ADP. In other words, microstructure of the axon is optimized for high-fidelity propagation, since ADP inevitably lowers threshold of the subsequent spikes during repetitive stimuli. In addition, ADP may regulate transmitter release, since Ca^{2+} currents facilitated in response to the paired-pulse voltage-command mimicking axonal spike and ADP sequence. Therefore, ADP may contribute to fine-tuning of short-term plasticity through modulation of presynaptic Ca^{2+} entry. (COI: No)

Symposium54

Ca^{2+} signaling in health and disease

(March 30, Sat., 15:10-17:10, Room G)

S54-1

A multi-hierarchical study on the arrhythmogenicity of a Ca-activated cation channel TRPM4

Ryuji Inoue[1]; Yaopeng Hu[1]; Yanghua Shen[2]; Keizo Hiraishi[1]; Lin Hai Kurahara[1]; Jun Ichikawa[1]; Tomohiro Numata[1]; Xin Zhu[2]
([1]*Department of Physiology, Fukuoka University School of Medicine, Japan;* [2]*Department of Biomedical Information Technology, Aizu University, Japan*)

TRPM4 channel is a ubiquitous, Ca^{2+}-activated cation channel and effectively regulated by intracellular Ca^{2+} and membrane potential. In this study, we systematically investigated the implications of these properties in aberrant cardiac excitability by electrophysiological experiments and multi-hierarchical numerical simulations.

Voltage-dependent activation of TRPM4 channel re-evaluated by a newly-devised ionomycin-permeabilized cell-attached recording was remarkably enhanced by submicromolar Ca^{2+}. Numerical simulation incorporating the rate constants of opening and closing obtained by this new method suggested that albeit negligibly contributive to the normal heart, excessive activation of upregulated TRPM4 channels became arrhythmogenic under remodeling conditions. The same experimental and theoretical approaches applied to an arrhythmic mutant of TRPM4 channel E7K revealed that its open-to-closed transition was remarkably slowed stabilizing the open state thereby rendering the channel more arrhythmogenic.

Quantitative analysis with FRET-based PIP_2 measurement revealed that decreased PIP_2 level greatly suppressed wild-type TRPM4 activity by shifting its voltage-dependency positively but this effect was only marginal in the E7K mutant. Numerical simulations suggested that PIP_2 depletion may counteract arrhythmic changes due to wild-type TRPM4 upregulation, which appears greatly compromised in the E7K mutant.

Ref. Hu Y. *et al.* Cardiovasc Res. 113: 1243-1255, 2017. (COI: No)

S54-2

TRPP2 acts through autophagy to exert cyto-protective role in human stem cell-derived cardiomyocytes

Xiaoqiang Yao; Jun Lu (*School of Biomedical Sciences, Chinese University of Hong Kong, China*)

Human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs) provide an unlimited source of human cardiomyocytes for potential application in disease modeling, drug screening and cell-based heart therapies. These hESC- or hiPSC-derived cardiomyocytes (hESC-CMs or hiPSC-CMs) are suggested to have many properties of authentic human cardiomyocytes. In the present study, we utilized hESC-CMs as models to investigate the potential role of TRPP2 (polycystin-2 or PKD-2) in autophagy. Our study demonstrates that TRPP2 functions to promote autophagy under glucose starvation, thereby protects cardiomyocytes from apoptotic cell death. The mechanism may involve TRPP2 interaction with ryanodine receptors to alter Ca^{2+} release from sarcoplasmic reticulum (SR), consequently modulating the activity of AMPK and mTOR, resulting in alteration of autophagy and apoptosis. We suggest that this scheme of TRPP2-autophagy-apoptosis may have important pathophysiological relevance in cardiomyopathy in patients with autosomal dominant polycystic kidney disease and ischemic heart diseases.

Acknowledgment: We thank the financial support from Hong Kong Research Grant Committee 14118516, National Natural Science Foundation of China Grant 31470912, AoE/M-05/12 and TBRS T13-706/11 (COI: No)

S54-3

Ca^{2+} signaling in early fate decision of cardiac lineage cells

Huangtian Yang; Yijie Wang; Jijun Huang; Ji Liang; Liming Chu
(*Laboratory of Molecular Cardiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China*)

Ca^{2+}signals participate in various cellular processes with spatial and temporal dynamics, while their roles and downstream regulatory pathways in cell fate decisions during early development are not fully understood. Embryonic stem cells (ESCs) have an unlimited potential to proliferate in vitro and differentiate into derivatives of all three primary germ layers, including cardiomyocytes. Apoptosis is important for normal embryogenesis *in vivo*, while its regulatory mechanisms in differentiating ESCs are unclear. In addition, cardiac and hematopoietic lineages are both derived from $Flk1^+$mesoderm cells but it remains unclear how $Flk1^+$progenitor cells determine the alternative cell fate towards one of these lineages. Inositol 1,4,5-trisphosphate receptors (IP_3Rs)-mediated Ca^{2+}signals are critical for early development, buttheir precise roles and mechanisms in cell fate decisions remain largely unknown. Our studies reveal the import roles of IP3R3- regulated Ca^{2+}signals in the regulation of mesodermal$Flk1^+$cellapoptosis and IP3Rs-regulated Ca^{2+}release governs hematopoietic and cardiac divergence of Flk1+ cells via calcineurin-NFATc3-Etv2 pathways. These findings provide new insights into the important role of IP3R-mediated Ca^{2+}signals in the specific lineage fate decision during early ESC differentiation

(Grants: NSFC (81520108004, 81470422); National Key R&D Program of China (2017YFA 0103700)). (COI: No)

S54-4

Use of tetrandrine to treat flavivirus infection

Jianbo Yue; Lihong Huang (*Department of Biomedical Sciences, City University of Hong Kong, China*)

Members of the flavivirus, e.g. Japanese encephalitis virus (JEV), Zika virus (ZIKV), and Dengue virus (DENV), are among the leading causes of human and animal infectious diseases in the world. Yet, no effective clinical treatment for flavivirus infection is available. Therefore, development of effective antiviral drugs against these viruses is the top priority of public health. We found that calcium influx is required for the entry of flavivirus into cells. To screen calcium channel inhibitors affecting flavivirus infection, we developed a high-content fluorescence imaging-based assay. We identified tetrandrine, an alkaloid isolated from herbs and known calcium channel blocker, potently inhibited flavivirus infection of host cells. Tetrandrine indeed blocked the entry of flavivirus into cells. We also identified a number of its structure analogues by virtual drug screening of chemical library, and found that some of these analogues potently inhibited flavivirus infection. Currently we are developing tetrandrine and its synthetic analogues into effective therapy against flavivirus. The success of our project is of clinical importance to health care of world. (COI: No)

S54-5

Structure-function Study of TRPP Channels

Xiaodong Liu[1]; Yuxia Liu[1,2]; ([1]*Beihang University, China;* [2]*Tsinghua University, China*)

Ca^{2+} -permeable cation channels of polycystin family (TRPP) such as PKD2L1 are involved in various biological processes, presumably by responding to extracellular stimuli, *e.g.* , Ca^{2+} (ICE, influx-operated Ca^{2+} entry) or H^+ (acid-evoked off response) (Hu *et al.* Cell Reports 2015). Here, based on the structural advances in PKD2L1 (Su *et al.* Nature Communications 2018) and other TRP channels, we report an atypical ion-channel interplay, in which both the outer-pore and the cations play unprecedented roles. The primary gating is initiated at the outer pore, when Ca^{2+} ions flow through the outer-pore lined up with acidic residues. Hinted by the functional divergence between two closely-related TRPPs, i.e., PKD2L1 and PKD2, we unveil that an aromatic triad is critical for such outer-pore activation to transmit further to the lower constriction. Our findings promote the long-sought functional and mechanistic understanding of TRPP including the disease-causing PKD2. Moreover, in contrary to the classical view as passive diffusion, our data demonstrate that cation flux driven by electrochemical gradients is able to convert its intrinsic energy into the activation or facilitation of the holo-channel, potentially applicable to a broad spectrum of cation channels. (COI: No)

Symposium55

Brain pathways linking between emotion, behaviour and autonomic responses

(March 30, Sat., 15:10-17:10, Room H)

S55-1

Contribution of medullary raphé serotonergic neurons in the stress-induced autonomic responses

Yoko Ikoma[1,4]; Ikue Kusumoto[1]; Akihiro Yamanaka[2]; Youichirou Ootsuka[1,3]; Tomoyuki Kuwaki[1] (*[1]Department of Physiology, Graduate School of Medical & Dental Sciences, Kagoshima University, Japan; [2]Department of Neuroscience II, Research Institute of Environmental Medicine, Nagoya University, Japan; [3]Centre for Neuroscience, Department of Human Physiology, School of Medicine, Flinders University, Australia; [4]Super-network Brain Physiology, Graduate School of Life Sciences, Tohoku University, Japan*)

The medullary raphé nuclei are involved in controlling cardiovascular, respiratory and thermoregulatory functions, as well as mediating stress-induced tachycardia and hyperthermia. Although the serotonergic system in the medullary raphé has been suggested to be the responsible entity, specific evidence has been insufficient. In the present study, we tested this possibility utilizing an optogenetic approach. We used genetically modified mice (Tph2; ArchT mice) in which archaerhodopsin-T, a green light-driven neuronal silencer, was selectively expressed in serotonergic neurons under the regulation of Tph2 promoter. We first confirmed that an intruder stress-induced activation of serotonergic neurons in the medullary raphé was suppressed by photo-illumination via a pre-implanted optical fiber, as evidenced by the decrease of a cellular activation marker protein in the neurons. Next, we measured ECG, respiration, body temperature, and locomotor activity in freely moving mice during intruder and cage-drop stress tests, with or without photo-illumination. In the intruder test, photo inactivation of the medullary serotonergic neurons significantly attenuated tachycardia and tachypnea, but not hyperthermia or hyper locomotion during photo-illumination. Similar results were obtained for cage-drop test. We conclude that the medullary raphé serotonergic neurons, specifically mediate stress-induced tachycardia and tachypnea. (COI: No)

S55-2

Lateral habenula-ventral tegmental area pathways for emotional hyperthermia

Youichirou Yoyo Ootsuka[1]; Mariana Brizuela[1]; Steven J Swoap[2]; Anna Antipov[1]; William W Blessing[1] (*[1]Centre for Neuroscience, College of Medicine and Public Health, Flinders University, Australia; [2]Department of Biology, Williams College, USA*)

The lateral habenula (LHb), a nucleus in the epithalamus, has drawn much attention for its role in coordinating behavioural strategy to salient and adverse environment events. The LHb-mediated behavioural response is mediated via a powerful inhibitory influence on dopamine neurons in the ventral tegmental area (VTA) via GABAergic interneurons. The behavioural responses are integrated with dynamic changes in autonomic function including temperature regulation, such as emotional hyperthermia that is the increase in body temperature that occurs in response to an animal detecting a salient stimulus. We have hypothesized that the LHb is also involved in this autonomic physiological component of responses. We previously showed that activation of neurons in the LHb increases heat production in brown adipose tissue (BAT) (Physiol Rep, 3:e12297, 2015) and inhibition of the LHb neurons attenuate an increase in body temperature in response to emotional stimuli (Scientific reports 7:4102, 2017). Our recent study reveals that inhibition of neurons in the VTA activates sympathetically-mediated BAT thermogenesis and blockade GABAergic signalling in the VTA abolishes the LHb-elicited BAT thermogenesis (Am J Physiol in press). Activation of dopamine D2-like receptors attenuates emotional. A body of the evidence suggests that the LHb mediates emotional hyperthermia via the inhibitory mechanisms in the VTA. (COI: No)

S55-3

The medial amygdala is critical for endocrine and behavioural responses to emotional stress

Christopher Vincent Dayas (*School of Biomedical Sciences and Pharmacy, University of Newcastle, Australia*)

The medial amygdala (MeA) plays a key role in the generation of neuroendocrine responses to emotional stress. However, it is unclear how the MeA mediates these effects. To validate the existence of possible direct projections from the MeA to the PVN, we made injections of AAV5-hSyn-YFP into the MeA of *corticotrophin-releasing hormone* (*CRH*)-*Cre:: td-tomato*-expressing mice (*CRH-Cre::td-tomato*, n = 3-5/group). Using CLARITY and confocal imaging we found robust YFP-terminal expression making close apposition to CRH::td-tomato/+ve neurons. Next, we used a transgenic mouse line that expressed Cre-recombinase under the control of the *single-minded-one (Sim-1-Cre)* gene promoter. We targeted the *Sim-1-Cre/+ve* neurons with injections of AAV5-DIO-ChR2-YFP and made electrophysiological recordings from putative CRH cells in PVN. We identified that 100% of photostimulation light-evoked inputs (13 cells from 7 mice) onto putative CRH cells were CNQX-sensitive but picrotoxin-insensitive; confirming that *Sim-1*+ve neurons in the MeA provide glutamatergic input to the PVN and excite putative CRH cells. Further, after AAV5-DIO-ChR2-YFP injections into the MeA and fibre optic probes targeting the PVN, 473nm blue light produced a stress-like pattern of c-*fos* expression in PVN and grooming behaviour. Together these findings indicate that glutamatergic MeA neurons project to CRH PVN neurons and have the capacity to generate HPA axis and stress-like behavioural responses. (COI: No)

S55-4

Striatopallidal output pathways promoting and preventing motivated behaviour

Gavan McNally (*School of Psychology, UNSW Sydney, Australia*)

Striatopallidal pathways are critical for the processing and execution of motivated behaviors. However, the specific functional roles of these different pathways and the extent to which these pathways are segregated from each other remains poorly understood. We used an animal model of alcohol-seeking to identify the roles of specific nucleus accumbens shell (AcbSh) and ventral pallidal output pathways in promoting versus preventing relapse. First we show that relapse and abstinence are embedded within distinct output circuits of dopamine 1 receptor (Drd1) expressing AcbSh neurons that anatomically and functionally segregated. Next we show that distinct components of relapse are controlled by discrete ventral pallidum cell types embedded in distinct output pathways. These findings identify a considerable degree of cell-type and circuit-specificity in the striatopallidal control over relapse and motivated behaviour. (COI: No)

Symposium56

Optical neuroscience: reading and manipulating neural computation behind cognition, memory, and behavior

(March 30, Sat., 15:10-17:10, Room I)

S56-1

Multiscale understanding of synaptic pathology of psychiatric disorders

Akiko Hayashi-Takagi (*Lab of Medical Neurosci, IMCR, Gunma Univ, Japan*)

Various lines of evidence have suggested that synaptopathy is involved in schizophrenia. However, it is unknown whether synaptopathy is an underlying mechanism of disease or a secondary consequence. Thus, we performed a longitudinal *in vivo* 2-photon imaging analysis of the brain of a schizophrenia model (DISC1 knockdown mice) and found that this model exhibited a decrease in the density of dendritic spines, where the majority of excitatory synapses are formed. Furthermore, we found a significantly greater number of large dendritic spines in the model mice. The presence of the large spines in the schizophrenia mice model mirrors findings from another schizophrenia mice model, calcineurin knockout mice. It is well-known that there is a strong correlation between spine head size and its synaptic efficacy, whereby the large spines can generate a larger synaptic current. This led us to hypothesize that large spines can non-linearly affect the dendritic computation, causally resulting in subsequent behavioral alterations. To test this hypothesis, we use a multiscale analysis that consists of an uncaging-evoked single spine EPSC measurement and Ca^{2+} imaging to visualize the synaptic input, dendritic event, action potential, and behavioral manifestations. In addition, with use of *in vivo* optical and *in silico* manipulation of the spines in the model animals, we are now trying to causally examine what kind of synaptic pathology would underlie the pathology of disorders. (COI: No)

S56-2

Population coding of fear memory in prefrontal cortex

Masakazu Agetsuma[1,2,3]; Yoshiyuki Arai[3]; Atsushi Kasai[4]; Hitoshi Hashimoto[4]; Takeharu Nagai[4] (*[1]Division of Homeostatic Development, National Institute for Physiological Sciences Japan; [2]Japan Science and Technology Agency, PRESTO, Japan; [3]The Institute of Scientific and Industrial Research, Osaka University, Japan; [4]Graduate School of Pharmaceutical Sciences, Osaka University, Japan*)

For efficient and correct information processing in cerebral cortex, neural circuit dynamics must be spatially and temporally regulated with great precision. Medial prefrontal cortex (mPFC) of rodents has been shown important for various types of learning and memory, including fear memory, and related to various psychiatric diseases. However, it still remains unclear how population of neurons in this region enables the information processing depending on learning states, of which major problems are the complexity and heterogeneity of the prefrontal networks. Little is known especially about the mechanism underlying fear memory. Here we investigate this by chronic two-photon Ca^{2+} imaging from populations of neurons in mouse mPFC in vivo, which allows us to 1) record activities simultaneously from large number of neurons at the single cell resolution with high temporal resolution, and 2) investigate changes of neuronal responses depending on the learning states. We focus on the change in population responses of mPFC neurons for the fear memory by developing a new device to perform and test Pavlovian fear conditioning under the microscope. While many of the previous imaging studies for the mPFC relied on the invasive method, our system can minimize such damage by introducing a microprism-based observation method. We further demonstrate population coding underlying memory recall as well as extinction. (COI: No)

S56-3

SLM-based methods for 3d control and imaging in the brain

Darcy Peterka (*Zuckerman Mind Brain Behavior Institute, Columbia University, USA*)

Recording neuronal activity throughout the brain with high temporal and spatial resolution may be a critical step in understanding how the brain works. Task-based approaches allow intelligent trade-offs between resolution, speed, and signal. I will describe projective two-photon imaging methods that leverage the spatiotemperal sparseness of neural activity and use holographic multiplexing, and statistical source separation create capable platforms for high performance imaging with single cell resolution. Similar holographic platforms can also be used to activate ensembles of neurons with single cell precision, and I will describe recent efforts to improve targeting and control in awake behaving animals. (COI: No)

S56-4

Manipulation of behavioral performance by targeted activation of cortical ensembles

Luis Alberto Carrillo-Reid (*Department of Developmental Neurobiology and Neurophysiolgy, National Autonomous University of Mexico, Mexico*)

Cortical ensembles in primary visual cortex are groups of neurons whose coordinated activity represents visual stimuli. The functional connectivity of these ensembles generates an internal representation of the surrounding world. In this way, learned behavioral tasks associated with visual stimuli could be based on the recalling of neuronal ensembles from primary visual cortex. However, whether it is possible to manipulate visually guided learned behaviors by the targeted activation of cortical ensembles in primary visual cortex remains unknown. In order to study the modulation of behavioral performance induced by the selective activation of cortical ensembles with single cell resolution we used simultaneous two-photon optogenetics and imaging of neuronal populations in awake head fixed mice performing a go/no-go task. We used probabilistic graphical models to identify and target neurons with pattern completion capability belonging to cortical ensembles in primary visual cortex. The selective photoactivation of neurons belonging to cortical ensembles associated with the go signal positively modulated behavioral performance whereas the photoactivation of randomly selected neurons during the go signal negatively modulated behavioral performance. Our findings demonstrate the possibility to manipulate functional neuronal ensembles with single cell resolution and observe behavioral correlates of cortical microcircuit reconfiguration. (COI: No)

S56-5

Brain states through brainwide neuromodulation in zebrafish

Misha Benjamin Ahrens (*Howard Hughes Medical Institute, Janelia Research Campus, USA*)

Neuromodulatory systems can introduce flexibility in the way neural circuits process information and generate behavior, allowing animals to adapt to changing environments and behavioral demands. Here we extend our investigation of brainwide experience-dependent neuromodulation from the serotonergic system to other neuromodulatory systems by combining whole-brain light-sheet imaging in virtual environments with anatomically-specific delineation of neuronal populations and with brainwide activity perturbations. By searching for neuromodulatory systems activated during behavioral switches, we found that the noradrenergic system modulates behavior and tracks the outcomes of swim actions in a complementary way to the serotonergic system. Together these neuromodulatory systems enable experience-dependent modulation of brain state and behavior across different behavioral regimes. (COI: No)

Symposium57

Alternative GPCR and G-protein signaling in cardiovascular disease and therapy

(March 30, Sat., 15:10-17:10, Room J)

S57-1

The Membrane-Intracellular Organelle Interface: A Compartment for GPCR Regulation of Cell Physiology

Hemal Patel (*UC San Diego & VA San Diego, USA*)

When considering which components of the cell are the most critical to function and physiology, we naturally focus on the nucleus, to the mitochondria that regulate energy and apoptotic signaling, or to other organelles. Few people will suggest that the membrane is the most critical element of a cell. Those that consider the membrane critical will point to its obvious barrier function and numerous ion channels. What becomes evident upon closer inspection is that not all membranes are created equal, and that there are lipid-rich microdomains which are platforms for signaling. This talk will advance the novel concept that membranes, in particular caveolae, serve to couple membrane-mitochondrial interactions. These interactions are both physical and direct as well as dependent on signaling networks linking GPCR signaling to metabolism. Within this framework, the membrane then is the primary and critical regulator of stress adaptation of the cell. The talk will further advance a novel hypothesis that membrane-mitochondrial symbiosis results in a discrete compartment for GPCR-regulated cell physiology. We propose that caveolin/caveolae may serve as adaptogens for restoring homeostasis that is unbalanced in disease. Such concepts have major implications for cellular processes (repair, growth, nutrient handling) and pathophysiology (aging, diabetes, cancer, cardiovascular, neurologic) that may be therapeutically targeted. (COI: Properly Declared)

S57-2

Role of activator of G-protein signaling (AGS) 8 in neovascularization

Hisaki Hayashi; Motohiko Sato (*Department of Physiology, Aichi Medical University, Japan*)

Ligand induced activation of GPCRs causes intracellular signal via heterotrimeric G-proteins to regulate cellular fates. Recently, accessory proteins for heterotrimeric G-protein, which activate G-protein independently of GPCR activation, have been reported to be involved in cardiovascular diseases. We previously cloned activator of G-protein signaling 8, AGS8, as Gβγ subunit binding protein from a cDNA library established from rat ischemic heart model with extensive collateral development. We examined a role of AGS8 in vascular development and its potential as therapeutic application. In cultured human endothelial cells, AGS8 knockdown by siRNA inhibited vascular endothelial growth factor (VEGF)-induced tube formation, cell growth, migration, and phosphorylation of VEGFR-2 and downstream molecules. Further study indicated that AGS8-Gβγ complex, which binds to VEGFR-2, was involved in the trafficking of VEGFR-2 from cytoplasm to the cell membrane. Local production of VEGF causes choroidal neovascularization (CNV), which is associated with development of age related macular degeneration (AMD), a major cause of vision loss among aged people. AGS8 was expressed in murine CNV experimental model with distinct distribution, particularly in laser-induced lesions. Significantly, AGS8 knockdown suppressed the development of CNV. Altogether, these data indicate involvement of AGS8 in VEGF signaling and suggest potential of AGS8 as a therapeutic target for AMD. (COI: No)

S57-3

Uncovering new GPCR signaling pathways in prostaglandin E_2-mediated vascular inflammation

Utako Yokoyama; Al Mamun; Hiromi Taro; Yoshihiro Ishikawa (*Cardiovascular Research Institute, Yokohama City University, Japan*)

Chronic inflammation is a hallmark of many cardiovascular diseases, including abdominal aortic aneurysm (AAA). The importance of immune cells in inflammation is well-recognized, whereas that of tissue-constituent cells, such as vascular smooth muscle cells (VSMCs), remains largely unknown. Here, we demonstrate that VSMCs play a primary role in sustaining inflammation, via prostaglandin E receptor EP4. In a mouse model, in which EP4 is overexpressed in VSMCs (EP4-Tg), most of the mice died of aneurysmal rupture within two weeks after angiotensin-II infusion while no death in non-transgenic littermates. Ly6C^{hi} inflammatory monocyte infiltration was markedly enhanced in EP4-Tg aorta, with increases in matrix metalloprotease-9 and cyclooxygenase-2. Inflammatory monocytes were likely recruited by SMC-derived interleukin-6 (IL-6), because EP4 stimulation of VSMCs, obtained from either EP4-Tg or human aorta, produced large amounts of IL-6 via transforming growth factor beta activated kinase 1 and nuclear factor kappa B signaling pathways. Administration of either anti-IL-6 receptor antibody or EP4 antagonist to EP4-Tg drastically decreased Ly6C^{hi} monocyte infiltration and prevented AAA. These results indicate that sustained vascular inflammation is triggered by EP4 in VSMCs, forming malignant positive feedback with immune cells, leading to AAA deterioration. Targeting EP4 in VSMCs may be an effective strategy to treat AAA. (COI: No)

S57-4

Age-dependent dimer formation of AT1R and P2Y6R promotes angiotensin II-induced hypertension

Akiyuki Nishimura[1]; Caroline Sunggip[2]; Takuro Numaga-Tomita[2,3,4]; Motohiro Nishida[1,2,3,4] ([1]*Department of Translational Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kyushu University, Japan;* [2]*National Institute for Physiological Sciences* (*NIPS*)*, National Institutes of Natural Sciences Japan;* [3]*Department of Creative Research, Exploratory Research Center on Life and Living Systems* (*ExCELLS*)*, National Institutes of Natural Sciences Japan;* [4]*School of Life Sciences, SOKENDAI Japan*)

The angiotensin (Ang) type 1 receptor (AT1R) contributes to maintain vascular homeostasis but also promotes hypertension. Ang II promotes hypertrophy of adult vascular smooth muscle cells (VSMCs), resulting in hypertension, whereas Ang II induces physiological proliferation in neonatal VSMCs. However, how VSMCs determine the responsiveness to Ang II under different developmental conditions is mostly unclear. We found that the purinergic P2Y6 receptor (P2Y6R), an inflammation-inducible G protein-coupled receptor, promoted Ang II-induced hypertension in mice. Mice with a deletion of P2Y6R did not show Ang II-induced increase in blood pressure and vascular remodeling. AT1R and P2Y6R formed stable heterodimers, which enhanced G protein-dependent vascular hypertrophy but reduced βArrestin recruitment and βArrestin-dependent vascular proliferation. Pharmacological disruption of AT1R-P2Y6R heterodimers by the P2Y6R antagonist MRS2578 suppressed Ang II-induced hypertension in mice. Furthermore, P2Y6R abundance was developmentally increased in VSMCs, and the increased AT1R-P2Y6R dimer converted AT1R-stimulated signaling in VSMCs from βArrestin-dependent physiological proliferation to G protein-dependent pathological hypertrophy. These results suggest that increased formation of AT1R-P2Y6R heterodimers with age may increase the likelihood of hypertension induced by Ang II. (COI: No)

S57-5

A novel physiological role of tetrahydrobiopterin, a key GTP metabolite, in cardiovascular system

Jin Han[1]; Hyoung Kyu Kim[1]; Ippei Shimizu[2]; Tohru Minamino[2]; Bernd Nilius[3] ([1]*Cardiovascular and Metabolic Disease Center, Inje University, Korea;* [2]*Department of Cardiovascular Biology and Medicine, Niigata University Graduate School of Medical and Dental Sciences, Japan ;* [3]*KU Leuven, Department of Cellular and Molecular Medicine, Belgium*)

Diabetic cardiomyopathy (DCM) is a major cause of mortality and morbidity in diabetes mellitus patients. Although tetrahydrobiopterin (BH4), a GTP metabolite, shows therapeutic potential as an endogenous target in the cardiovascular system, its effect on myocardial cells and mitochondria in DCM and the underlying mechanism are unknown.

We tested whether BH4 deficiency is involved in DCM and if supplementation restores mitochondrial and heart function in late-stage DCM. The transcription of three BH4 synthesis-regulating genes was compared in cardiac tissues of patients with low or normal ejection fractions (EFs).

Forty-eight-week-old type 2 diabetic rats were divided into BH4 treatment and control groups. BH4 levels and the functions of heart and mitochondria were assessed in diabetic or control rats. Sepiapterin reductase knockout (*Spr*$^{-/-}$) mice, a model of BH4 deficiency, were used to determine the mechanism of the therapeutic effect of BH4 on DCM.

Relative to control rats, diabetic rats, as well as *Spr*$^{-/-}$ mice, had cardiac contractility, hypertrophic remodeling, and mitochondrial dysfunction, which recovered with BH4 supplementation. BH4 directly bound to CaMKK2 and activated downstream pathways in cardiomyocytes.

BH4 is a novel therapeutic target for recovering the left ventricular contractility and structural remodeling in DCM via direct binding and activation of CaMKK2 signaling pathways. (COI: No)

Symposium58

Zinc physiology and pathophysiology

(March 30, Sat., 15:10-17:10, Room K)

S58-1

Role of the zinc homeostatic system in skin and skeletal muscle development

Toshiyuki Fukada (*Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan*)

Zinc is an essential trace element that is required for a variety of cellular functions, and unbalanced zinc homeostasis results in health problems. Recent studies have highlighted that zinc acts as a signaling mediator: zinc signal, which is controlled via zinc transporters, and participates in health and disease conditions **(1)**. This symposium will aim to share the updated information about the roles of zinc homeostasis and zinc signaling in physiology and pathophysiology.

The first manifestations that appear under zinc deficiency are skin defects **(2)**. It should be also noted that about 60% of whole zinc in body is kept in skeletal muscle. However, the zinc-related molecular mechanisms underlying normal skin and muscle development, as well as the mechanism by which disturbed zinc homeostasis causes their disorders, have not been clarified yet.

In this symposium, I will provide an overview of the relationships between zinc dysregulation and skin disorders, by focusing on the roles of zinc transporter ZIP7 for dermis **(3)**, and ZIP10 for epidermis and hair follicle **(4)**. I also address the zinc homeostatic system contributes skeletal muscle formation and function via zinc signaling mediated by ZIP13.

References:
1: ***International Journal of Medical Sciences*** 18: 2708, 2017
2: ***Nutrients*** 10: 219, 2018
3: ***Journal of Investigative Dermatology*** 137: 1682-1691, 2017
4: ***Proc. Natl. Acad. Sci. USA*** 114:12243-12248, 2017
(COI: No)

S58-2

Zn^{2+} sensitivity of Hv1 channel: an evolutionary perspective

Adisorn Ratanayotha[1,2]; Takafumi Kawai[1]; Yasushi Okamura[1]
([1]*Laboratory of Integrative Physiology, Department of Physiology, Graduate School of Medicine, Osaka University, Japan;* [2]*Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand*)

Voltage-gated proton channel (Hv1) mediates proton (H^+) across the cell membrane. Hv1 has been documented to be associated with functions of various immune cells, particularly neutrophils where Hv1 plays an essential part in producing reactive oxygen species (ROS) during respiratory burst and pathogen elimination. Hv1 orthologs from different species share sensitivity to extracellular Zn^{2+}, which acts as a channel gating modifier suppressing Hv1-derived H^+ currents at physiological condition. However, the potency of extracellular Zn^{2+} suppression on Hv1 is distinct among species. Mammalian orthologs, such as human and mouse Hv1s, maintain a high Zn^{2+} sensitivity, while nonmammalian Hv1s are comparatively resistant. This phenomenon appears to reflect variation in Zn^{2+}-coordinating residue within the extracellular linker region in Hv1. Notably, in our comparative study between mouse and zebrafish Hv1s, we have found that the serum Zn^{2+} concentration is much higher in zebrafish than in mouse, evidently as a compensation to the lower Zn^{2+} sensitivity. Because Zn^{2+} is physiologically significant to functions of Hv1s, these findings raise the possibility that Zn^{2+} sensitivity was acquired following a change in the serum Zn^{2+} concentration during evolution in order to maintain its functions in native cells.
(COI: No)

S58-3

How does zinc signaling control the fate determination of beige fat cells?

Ayako Fukunaka (*Institute for Molecular & Cellular Regulation Gunma University, Japan*)

Obesity and its associated metabolic diseases are caused by a prolonged imbalance between energy intake and energy expenditure. Adipose tissues are the major control site of energy balance, which comprises two functionally distinct cell types; white fat cells and brown fat cells. White fat cells store excess energy, whereas brown fat cells specialize in energy expenditure. In addition, brown fat-like cells have also been found within white adipose tissue (WAT). These inducible brown fat-like cells are known as beige fat cells, which are increased by cold exposure (a process also known as adipocyte browning). Therefore, the identification of signaling pathways that regulate the acquisition of beige fat cell properties by WAT have gained attention from the therapeutic viewpoints against obesity.
We have previously reported that zinc signaling via the zinc transporter ZIP13 negatively regulates the adipocyte browning pathway (Fukunaka A, et al,*PLoS Genet*., (2017)). Here, we demonstrated that the unique amino acid region of ZIP13 facing the cytosol, named intracellular loop2 (IntL-2), which is distinct from the other ZIP family members, is involved in the inhibition of adipocyte browning. Furthermore, we identified binding molecules that associate with the IntL-2 of ZIP13. We are now clarifying the molecular mechanism as to how these IntL-2 binding proteins regulate adipocyte browning via ZIP13. (COI: No)

S58-4

Physiology and biochemistry of zinc enzymes

Taiho Kambe (*Graduate School of Biostudies, Kyoto University, Japan*)

More than one-third of newly synthesized proteins are targeted to the early secretory pathway, a considerable proportion of which requires zinc as an essential factor for their structural and catalytic functions. Zinc homeostasis in the secretory pathway is tightly controlled within narrow boundaries, which suggests that zinc metallation would be performed under strict control. Here, physiological significance of the activation of zinc-requiring ectoenzymes by zinc is discussed, focusing on molecular mechanisms underlying the activation.
Of zinc-requiring ectoenzymes, ENPP1, ENPP3, NT5E/CD73, and TNAP are involved in extracellular adenine-nucleotide metabolism, which suggests that zinc deficiency significantly affects the metabolism, and thus purinergic signalling. Actually, zinc deficiency delays both extracellular ATP clearance and adenosine generation in both rats and cell culture models, indicating that zinc modulates extracellular adenine-nucleotide metabolism. These findings provide a novel insight into why zinc deficiency results in diverse symptoms. Interestingly, some of these ectoenzymes become active by being metallated with zinc during the secretory process, which is mediated by ZNT5-ZNT6 heterodimers and ZNT7 homodimers, while others are not. Clarification of molecular mechanisms of zinc-requiring ectoenzyme activation would be increasingly important, because they are involved in important physiological functions. (COI: No)

Symposium59

Contribution of microglia in health and disease of the brain

(March 30, Sat., 15:10-17:10, Room M)

S59-1

Deciphering the origins of repopulated microglia in the central nervous system

Bo Peng (*Chinese Academy of Sciences, China*)

New-born microglia rapidly replenish the whole brain after selective elimination of most microglia in adult mice. Previous studies reported that repopulated microglia were largely derived from microglial progenitor cells expressing Nestin in the brain. However, the origin of these repopulated microglia has been hotly debated. In this study, we investigated the origin of repopulated microglia by a series of fate mapping approaches. We first excluded the blood origin of repopulated microglia via parabiosis. With different transgenic mouse lines, we then demonstrated that all repopulated microglia were derived from the proliferation of the few surviving microglia. Though with a transient pattern of Nestin expression in newly forming microglia, none of repopulated microglia was derived from Nestin-positive non-microglial cells.

In addition to repopulated brain microglia, we investigated the origins of repopulated microglia in the retina and found that the repopulated retinal microglia were not derived from the residual microglia in the retina. Instead, they had two distinct origins: the center-emerging microglia were derived from residual microglia in the optic nerve and the periphery-emerging microglia were derived from macrophages in the ciliary body/iris. Therefore, we identified novel origins of retinal microglia by using a model of microglial repopulation. In conclusion, our findings expand the understanding on the origins of microglia in the brain and retina. (COI: No)

S59-2

Microglia in Post-stroke Axon Remyelination and Tissue Repair

Dandan Sun (*Department of Neurology, University of Pittsburgh, USA*)

Activation of microgliais involved in brain injury and tissue repair.Upon brain lesion, microglia are rapidly activated and release inflammatory cytokines, hindering axonal regeneration and oligodendrocyte maturation. Simultaneously, adaptive function of microglia is activated by releasing restorative cytokines/growth factors and clearing tissue debris through phagocytosis and promotes remyelination. Therefore, regulation of a switch between proinflammatory and adaptive phenotypes of microglia/macrophage is important for oligodendrocyte differentiation, remyelination, as well as remodeling synapses. We recently discovered that microglial Na/H exchanger 1 (NHE1) transports H+ efflux to maintain optimal H+ homeostasis and to promote sustained NADPH oxidase isoform 2 (NOX2) stimulation and cytokine release in activated microglia. Selective deletion of microglial *Nhe1* in mice reduced inflammation, enhanced APC+ mature oligodendrocyte counts and remyelination, and improved neurological functional outcome after ischemic stroke.
Taken together, our study demonstrated that microglia played an important role in neuroinflammation, white matter demyelination, and poststroke neurological dysfunction. Activation of microglial NHE1 protein is critical for this process. NHE1 protein emerges as a potential therapeutic target for
neuroinflammation and white matter tissue repair after ischemic stroke. (COI: No)

S59-3

Roles of lipid receptors expressed by microglia in traumatic nerve injury

Hiroshi Kiyama (*Department of Functional Anatomy & Neuroscience, Nagoya University Graduate School of Medicine, Japan*)

Microglia is a key player in the surveillance system of brain environment under healthy condition, whereas under pathological condition microglia are activated and play paradoxical roles such as neuroprotective and neurotoxic. Traumatic or inflammatory peripheral nerve damage induces changes not only in the damaged-neurons but also in microglia located nearby the neurons in CNS. The injured-neuron non-autonomous responses elicited by surrounding microglia appear crucial, and the failure of the communication between injured neurons and microglia or a devastating signal released from microglia would result in an exacerbation of neuron damages. To reveal functional significance in the intercellular communications between damaged neurons and microglia, we have carried out transcriptomic analysis including the G-Protein Coupled Receptor (GPCR) screening using hypoglossal nerve injury model, and demonstrates that expressions of some receptors for lipid mediators such asTREM2/DAP12 andsome GPCRs were induced by microglia in response to nerve injury. Most of these receptors are associated with a prolongation of inflammatory responses, phagocytosis, and neuropathic pain. In this talk I would like to introduce recent perspectives on the role of these molecules in microglia and their contribution to neural diseases. (COI: No)

S59-4

Sex- and age-dependent effect of thyroidism on microglia and brain function

Mami Noda (*Kyushu University, Graduate School of Pharmaseutical Sciences, Japan*)

Thyroid hormones (THs) are essential for the development and function of the central nervous system (CNS). In adult CNS, both hyperthyroidism and hyperthyroidism, the prevalence in female being >10 times higher than that in male, may affect psychological condition and potentially increase the risk of cognitive impairment and neurodegeneration including Alzheimer's disease. Since we have reported the non-genomic effects of L-tri-iodothyronine (3, 3', 5–triiodothyronine; T3) on microglial functions and its signaling in cellular level using primary cultured mouse microglia, we analyzed morphological changes in glial cells in cortex and hippocampus in mouse models of hyperthyroidism and hypothyroidism. The changes in immunofluorescence intensities for Iba1 and GFAP were sex- and age-dependent in both hyperthyroidism and hypothyroidism. Behavioral changes in young mice in thyroidism were also sex-dependent. We further examined the morphology of the perforant path-granule cell synapses in the mouse dentate gyrus, using FIB/SEM (focused ion beam/scanning electron microscopy). A three-dimensional reconstruction of dendritic spines revealed that changes in spine density and spine volume were also sex-dependent in young mice with hyperthyroidism. These results may help to understand physiological and/or pathophysiological functions of THs in the CNS and how hyper- and hypothyroidism affects behavioral and psychological conditions in sex- and age-dependent manner. (COI: No)

Symposium60

Hibernation and Torpor in mammals

(March 31, Sun., 8:00-9:30, Room C)

S60-1

Daily torpor in mice as a model of active hypometabolism

Genshiro A Sunagawa (*Laboratory for Retinal Regeneration, RIKEN Center for Biosystems Dynamics Research, Japan*)

Some mammals enter a hypometabolic state either daily torpor (minutes to hours in length) or hibernation (days to weeks), when reducing metabolism would benefit survival. The metabolic rate is reduced to 1~30% of normal rates and the animal result in severe hypothermia surprisingly without any tissue damage. The mechanisms for such hypothermia-resistance and hypometabolism-resistance is not understood. In 2016, we have developed a method to induce torpor stably in mice (Sunagawa GA and Takahashi M, *Sci Rep*, 2016) and this introduced modern techniques in genetics such as genetical engineering to the field of mammalian hypometabolism research. Recently, we found that two genetically close inbred mouse strains C57BL/6J (B6J) and C57BL/6N (B6N) have distinct torpor phenotypes. This led us to hypothesize that the torpor phenotype in mice is regulated by relatively few genes or gene loci. We analyzed the soleus muscles from 38 B6J mice in torpid and non-torpid conditions and identified 287 torpor-specific genes. Among the torpor specific genes, a transcription factor ATF3 was found highly expressed during torpor deprivation and that its binding motif was enriched in torpor-specific promoters. Our results demonstrate that mouse daily torpor combined with powerful genetic tools are useful for studying active hypometabolism. (COI: No)

S60-2

Hypothalamic control of mouse daily torpor

Hiroshi Yamaguchi; Luis De Lecea (*Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, USA*)

Hibernators save energy by entering torpor, a state characterized by active hypometabolism and low body temperature, to survive harsh environmental conditions in winter. Torpor can be found in a wide variety of homeothermic animals including birds, rodents and primates. Laboratory mice go into short torpid state called daily torpor when they are fasted at cold ambient temperature. Although it is presumed that torpor is regulated by the central nervous system, the exact neuronal mechanism by which mice regulate daily torpor remains unclear. Using immediate early gene mapping and optogenetic manipulation, we found that the activities of neurons in the medial preoptic hypothalamus and the dorsomedial hypothalamus are essential for the induction of daily torpor in mice. (COI: No)

S60-3

Cold-inducible RNA-binding protein may participate in cold tolerance in hibernating hamsters

Yasutake Shimizu[1,2]; Yuuki Horii[1]; Hiroki Shimaoka[1]; Takahiko Shiina[1] (*[1]Department of Basic Veterinary Science, Laboratory of Physiology, The United Graduate School of Veterinary Sciences, Gifu University, Japan; [2]Center for Highly Advanced Integration of Nano and Life Sciences (G-CHAIN), Gifu University, Japan*)

Hibernators can maintain regular heartbeat even under severe hypothermic condition less than 10°C during hibernation. We hypothesized cold-inducible RNA-binding protein (CIRP) might participate in the tolerance to hypothermia, since CIRP plays important roles in protection of various types of cells against harmful cold temperature. In the present study, we investigated expression patterns of CIRP mRNA in the heart in hibernating hamsters. To induce hibernation, hamsters were kept in a cold room. The RT-PCR analysis revealed that CIRP mRNA is constitutively expressed in the heart of non-hibernating euthermic hamsters with three alternative splicing variants. The short form contained open reading frame for full-length CIRP. In contrast, the long form was inserted sequences containing a stop codon, suggesting production of C-terminal deletion isoform of CIRP. In the hibernating hamsters, only the short product was amplified. These results indicate that CIRP expression is regulated at the levels of alternative splicing, which would permit a rapid expression of functional CIRP during entering hibernation. Artificial hypothermia, in which body temperature was rapidly decreased by using anesthesia combined with cooling, did not mimic the hibernation-specific shift in splicing. Considering that the artificial hypothermia caused tissue injury of the heart, the hibernation-specific splicing of CIRP mRNA would be important for tolerance to hypothermia. (COI: No)

S60-4

IPSCs from hibernators: a way to study hibernation-related cell protection mechanisms

Jingxing Ou; Wei Li (*National Eye Institute, National Institute of Health, USA*)

Hibernating mammals survive profound hypothermia (<10°C) without injury, a remarkable feat of cellular preservation that bears significance for potential medical applications. However, mechanisms imparting cold-resistance, such as cytoskeleton stability, remain elusive. Using the first iPSC line from a hibernating mammal (13-lined ground squirrel), we uncovered cellular pathways critical for cold-tolerance. Comparison between human and ground squirrel iPSC-derived neurons revealed differential mitochondrial and protein quality control responses to cold. In human iPSC-neurons cold triggered mitochondrial stress, resulting in reactive oxygen species overproduction and lysosomal membrane permeabilization, contributing to microtubule destruction. Manipulations of these pathways endowed microtubule cold-stability upon human iPSC-neurons and rat (a non-hibernator) retina, preserving its light responsiveness after prolonged cold exposure. Furthermore, these treatments significantly improved microtubule integrity in cold-stored kidneys, demonstrating the potential for prolonging shelf-life of organ transplants. Thus, ground squirrel iPSCs offer a unique platform for bringing cold-adaptive strategies from hibernators to humans in clinical applications. (COI: No)

S60-5

Systemic body remodelling preceding hibernation in a mammalian hibernator, Syrian hamster

Yoshifumi Yamaguchi[1]; Daisuke Anegawa[1,2]; Yuya Sato[1,2]; Yuichi Chayama[2]; Lisa Ando[2]; Shuji Shigenobu[3]; Yutaka Tamura[4]; Masayuki Miura[2] (*[1]Institute of Low Temperature Science, Hokkaido University, Japan; [2]Department of Genetics, Graduate School of Pharmaceutical Science, The University of Tokyo, Japan; [3]National Institute of Basic Biology, Japan; [4]Fukuyama University, Japan*)

Mammalian hibernation is an adaptive strategy to survive during the cold period with little or no food by dramatic suppression of thermogenesis and extensive utilization of stored fat as a fuel. In small-bodied mammals, hibernation period involves multiday hypothermic deep torpor and normothermic periodic arousal. Deep torpor is characterized by the profound suppression of metabolism, body temperature, heart rate, and locomotive activity, which cannot be achieved by non-hibernators such as mice and humans. It has long been suggested that hibernators prepare for hibernation by remodeling their bodies from the summer phenotype to the winter phenotype during fall, the pre-hibernation period (pre-HIB). However, the nature of the body remodeling remains elusive. To understand this, we examined Syrian golden hamsters (*Mesocricetus auratus*), which begin to hibernate when they are transferred from the warm, summer-like conditions to the prolonged cold, winter-like conditions. We found that after several months of the cold pre-HIB, they exhibited dramatic changes in their skeletal muscles and white adipose tissues, including skeletal muscle fiber-type shift and enhancement of lipid metabolisms, prior to hibernation. In addition, the cold tolerance was also enhanced during the hibernation period. These results suggest that Syrian hamsters undergo systemic body remodeling adaptive for hibernation in an environment-dependent manner. (COI: No)

Symposium61

The Social Brain: Recent Progress in Understanding Molecules and Networks of Social Behavior

(March 31, Sun., 8:00-9:30, Room D)

S61-1

Non-genomic action by gonadal steroids drives social behaviours

Nandini Vasudevan (*School of Biological Sciences, University of Reading, UK*)

Estrogen is essential for the display of sex-typical social behaviours via both genomic and the lesser understood nongenomic signalling pathway. Estrogens regulate transcription by binding to classical, intracellular nuclear receptors such as the estrogen receptor a (ERa) and ERb but also signal rapidly and non-genomically via membrane estrogen receptors (mERs) that activate kinases and calcium flux. Previously, our laboratory demonstrated that a third pathway that we termed coupled or integrated signalling exists where rapid non-genomic signaling by 17b-E potentiates transcription via the phosphorylation of the ERa. Although ERa and ERb are present on the cell membrane, novel candidate mERs include the GPER1/GPR30 that is present in several brain nuclei involved in social behaviours. The identity and function of the mER remains controversial. Recently, we demonstrated that GPER1 activation is sufficient for lordosis behaviour and regulates spinogenesis in hypothalamic nuclei, suggesting that this is a receptor capable of initiating the non-genomic signalling part of the coupled signalling pathway. Contrary to the need for transcription for lordosis behaviour, in the male mouse GPER1 activation can rapidly decrease anxiety and regulate the level of a dendritic spine marker, PSD-95 in the hypothalamus. We also show that estrogen possibly produced by the brain itself can rapidly drive aggression; underlying mechanisms will be discussed. (COI: No)

S61-2

Neuroendocrine Regulation of Neural Networks for Social Behavior

Sonoko Ogawa (*Laboratory of Behavioral Neuroendocrinology, University of Tsukuba, Japan*)

We have been studying neuroendocrine mechanisms of social behavior by focusing on the role of estrogen receptors (ERs). In a series of studies using adeno-associated viral vector mediated RNA interference methods, we found that two types of ERs, ERa and ERb, are differentially involved in the regulation of sex-typical expression of social behavior depending on brain site(s) and/or time in development. For instance, maternal caring behavior in postpartum females was reduced by a lack of ERa in the medial preoptic area (MPOA), whereas the levels of aggression toward male intruder mice were decreased by ERb knockdown in the medial amygdala (MeA) but increased by knockdown in the MPOA. Testosterone in males also acts on ERs after being aromatized in the brain. ERain the VMN plays a role in the induction of both sexual and aggressive behavior, whereas in the MPOA it is only involved in sexual behavior. Interestingly, ERa activation in the MeA during pubertal period is crucial for male mice to fully express their male-type social behavior in adulthood. On the other hand, ERb in the MeA might play a crucial role in the control of sexual preference. In this talk, we will first overview these findings by focusing on neural network of social behavior. We will then discuss more recent studies on the effects of manipulation of neuronal activity of ERa or ERb expressing neurons with the use of pharmacogenetic methods. (Supported by KAKENHI #15H05724 to SO) (COI: No)

S61-3

Serotonin interactions with the gonadotropin-inhibitory hormone system during social isolation

Tomoko Soga (*Brain Research Institute, School of Medicine and Health Science, Monash university, Malaysia*)

Social isolation deregulates the neuroendocrine system, the neurotransmitters system, the reproductive pathways and sociosexual behavior. It also increases the risk of developing mental disorders. The brain serotonergic system plays an important role in coordinating behavioral and neuroendocrine effect of social stress responses. In recent years, animal models have been developed to understand social isolation induced neuronal dysfunctions and behaviour changes that might be similar to those occurring in the course of the development of depression. I will discuss the work we have done to develop a rat depression model. The rats were exposed to social isolation for 6 weeks to understand the brain serotonergic systems and the regulation of its target neurons. Social isolation resulted in low serotonin synthesis gene expression in the dorsal raphe and less fiber projections of serotonin in the hypothalamic nucleus. In this talk, I will focus on gonadotropin-inhibitory hormone (GnIH) neurons in the dorsomedial hypothalamic nucleus, which have an inhibitory effect on reproductive functions. The serotonergic regulation of GnIH neurons elucidates the neuronal mechanisms of reproductive dysfunction in a lifelong stress paradigm. (COI: No)

S61-4

The Neurobiology of Pair Bonding in Monogamous Prairie Voles

Larry James Young[1,2] ([1]*Center for Social Neural Networks, University of Tsukuba, Japan;* [2]*Center for Translational Social Neuroscience, Department of Psychiatry and Behavioral Sciences, Emory University, USA*)

Socially monogamous prairie vole has provided an extraordinary opportunity to explore the neural and genetic mechanisms underlying complex social behaviors, including pair bonding. Oxytocin receptor (OXTR) signaling in the brain's reward centers is critical for pair bond formation. Diversity in expression patterns of OXTR in the brain contribute to diversity in social behaviors across and within species. In prairie voles, oxytocin links the neural encoding of the social signature of the partner with the rewarding aspects of mating through interactions with dopamine and by coordinating communication across a neural network linking social information with reward. Genetic polymorphisms robustly predict natural variation in OXTR expression in the striatum, which predict pair bonding behavior and resilience to neonatal social neglect. We have also explored the capacity of prairie vole to display empathy-like behavior, specifically consoling behavior toward stressed partners. This consoling response is abolished blocking OXTR in the anterior cingulate cortex, a region involved in human empathy. Finally, loss of a bonded partner results in the development of depressive-like "grieving" behavior, which is alleviated by oxytocin replacement. I will present data suggesting that similar mechanisms are involved in romantic love in humans and may be useful targets for improving social cognition in psychiatric disorders such as autism. (COI: No)

Symposium62

Integrative neural processing of sound information in the higher auditory centers

(March 31, Sun., 8:00-9:30, Room E)

S62-1

Excitatory and inhibitory neural circuits in the auditory midbrain

Munenori Ono (*Department of Physiology, Kanazawa Medical University, Japan*)

The inferior colliculus (IC) is an integrative auditory center in the midbrain. The IC is comprised of intricate neural circuits, which receive inputs from ascending, descending and intrinsic inputs. In the neurons of the auditory midbrain, multiple synaptic inputs are integrated and transformed into spike activities as an output. Thus, information processing is achieved through the integration of synaptic inputs in the IC. In particular, recent studies have shown that the interaction of the excitatory and inhibitory synaptic inputs is critical in shaping neural responses to sound in the IC. In this presentation, I will discuss how the excitatory and inhibitory neural circuits are organized in the IC. Both excitatory and inhibitory neurons in the IC have subtypes based on their morphology and firing properties, and are organized in the tonotopic lamina structure. The spontaneous firing rate is higher in the inhibitory neurons than in the excitatory neurons, which might promote the signal transmission with less noise. Both cell types have diverse response properties to sound: they share the similar frequency tunings, but not temporal spike patterns, in the local circuits (microdomain). These results suggest that each microdomain might work as a distinct frequency channel, and it may generate temporally diverse excitatory and inhibitory outputs to preferred sound, which might form a sequentially structured population activity as it has been proposed in the sensory neocortex. (COI: No)

S62-2

Characterization of the secondary auditory field in the mouse auditory cortex

Hiroaki Tsukano (*Department of Neurophysiology, Brain Research Institute, Niigata University, Japan*)

Tonotopy is an essential functional organization in the auditory cortex (ACx), and it is crucial to reveal how tonotopy is relayed to higher-order ACx such as the secondary auditory field (A2). The source of tonotopy reflected in the primary ACx (A1) is the incoming frequency-related topographical projections from the ventral division of the medial geniculate body (MGv). However, circuits that relay this functional organization to A2 have yet to be identified. In our recent tracing study conducted using mice, we discovered a new pathway that projects directly from the caudal part of MGv to A2, while the middle part of MGv projects to A1. Tonotopy was established in A2 even after primary fields including A1 were removed. These data suggest that tonotopy in A2 can be established solely by thalamic input. Moreover, the structural nature of differing thalamocortical connections was consistent with the functional organization of the target regions in ACx. Retrograde tracing revealed that the region of MGv input to a local area in A2 was broader than the region of MGv input to A1. Consistent with this anatomy, two-photon calcium imaging revealed that neuronal responses in the thalamocortical recipient layer of A2 showed wider bandwidth and greater heterogeneity of the best frequency distribution than those of A1. These findings demonstrate a new thalamocortical pathway that relays frequency information to A2 on the basis of the MGv compartmentalization. (COI: No)

S62-3

Acute restraint stress alters sound-evoked neural responses in the rat auditory cortex

Ma Lanlan; Jiaozhen Zhang; Ling Qin (*Department of Physiology, China Medical University, China*)

Psychophysical studies showed that stress exposure induced a transient, stress-induced hypersensitivity to sounds. However, the underlying neural mechanism remains unresolved. Thus, in this study, we explored the neural activities of the auditory cortex (AC) in response to restrait stress. By comparing the electrical activities of the same rat before, during and after immobilization, we found in most cases, acute restraint stress enhanced neural responses evoked by sound, but in a minority of neurons, restraint stress suppressed the responses. The immobilization-induced enhancement was more frequently found in the neurons that originally had a low responsibility to sound stimuli. Although an increase of response magnitude, decrease of response latency, and extension of bandwidth of tuning curve (BW),the spontaneous firing rate and best frequency (BF) remained unchanged. Restraint stress also increased the synchronization ability to sound slimuli. (COI: No)

S62-4

Sound representation of long-lasting sustained activity in rat auditory cortex

Tomoyo Isoguchi Shiramatsu; Hirokazu Takahashi (*Research Center for Advanced Science and Technology, The University of Tokyo, Japan*)

To date, auditory transient activities responding to the onset of a sound have been well investigated and cortical subfields and tonotopic representation in these subfields have been well characterized. However, sustained activities that follow transient activities have received less attention because these activities do not exhibit distinct, reproducible, and time-locked responses in their amplitude to be characterized by grand averaging. To address this gap, we first tried to decode sound frequency from densely mapped sustained activities, and investigated whether and how these activities represent sound information. A microelectrode array with a grid of 10 × 10 recording sites was implanted in the rat auditory cortex, and recorded sustained activities. Then SLR was applied to discriminate the sound-induced band-specific power or phase-locking value (PLV) from those of spontaneous activities. Consequently, SLR succeeded decoding indicating that these characteristics of sustained activity represent frequency information. With these characteristics, we further demonstrated that sustained activities represent sound information beyond frequency; PLV became stronger after classical aversive conditioning, indicating that sustained activities represent emotional valence of sound, and texture of sound such as consonance and dissonance of chord is also represented in PLV, setting the groundwork for further investigation of auditory sustained activity. (COI: No)

Symposium63

Implication of tonic inhibition for Brain function

(March 31, Sun., 8:00-9:30, Room F)

S63-1

Function of cerebellar tonic inhibition

Bo-Eun Yoon (*Department of Molecular Biology, Dankook University, Korea*)

Proper brain function requires a balanced excitation and inhibition in synaptic transmission through regulation of neuronal excitability. Neuronal excitability is regulated by inhibitory synaptic transmission, which occurs primarily through GABAergic signaling. It has been reported that interactions between tonically released GABA and extrasynaptically localized high affinity $GABA_A$ receptors mediate tonic inhibition, which effectively inhibits neuronal excitability.

We have previously reported that cerebellar tonic inhibition is mediated by astrocytic GABA release through bestrophin 1(Best1) from Bergmann glia and lamellar astrocytes.

We have further reported that astrocytic GABA is synthesized by the astrocytic mitochondrial enzyme monoamine oxidase B (MAOB) via the putrescine degradation pathway.

However, in vivo function of the astrocytic GABA mediated tonic inhibition has not been elucidated.

Here, we investigated the modulation of neuronal excitability, synaptic transmission, and motor performance in the cerebellum by manipulating the level of astrocytic tonic GABA using various genetic and pharmacological tools. (COI: Properly Declared)

S63-2

Pathophysiological impact of diverse deregulation of tonic inhibition in Angelman syndrome

Kiyoshi Egawa[1]; Atsuo Fukuda[2] ([1]*Department of Pediatrics, Hokkaido University School of Medicine, Japan;* [2]*Department of Neurophysiology, Hamamatsu University School of Medicine, Japan*)

Angelman syndrome (AS) is a neurodevelopmental disorder caused by loss-of-function of the *UBE3A* gene. Motor dysfunction is one characteristic feature of AS. We previously reported that tonic inhibition is specifically decreased in cerebellar granule cells (CGCs) of *Ube3a* deficient mice. As its mechanisms, we showed that UBE3A controls degradation GABA transporter 1 (GAT1) and its deficiency induces a surplus of GAT1 that results in decreased GABA levels. Decreased tonic inhibition increases its own excitability of CGSs and alters the firing pattern of Purkinje cells. Administering low doses of THIP, a selective agonist for δsubunit-containing $GABA_A$ receptor, improved the aberrant firing pattern and ataxic motor dysfunctions *in vivo*. These results indicate that the decreased tonic inhibition in CGCs contributes to motor dysfunctions in AS. Because expression pattern of $GABA_A$ receptor subunits or GABA transporters differs by brain regions, the deregulation of tonic inhibition can be also variable by brain regions in AS. Indeed, we found that expression pattern of GAT1 in the thalamus was comparable to controls and tonic inhibition of the thalamic relay neuron was maintained in *Ube3a* deficient mice, while tonic inhibition was decreased in pyramidal neurons of the hippocampus and the cortex. We speculate that this imbalance, rather than global decrease, of tonic inhibition may have a pathophysiological impact for various nervous symptoms including motor dysfunctions in AS. (COI: No)

S63-3

Critical role of tonic GABA from reactive astrocytes in neurodegeneratve diseases

C Justin Lee (*Institute for Basic Science, Korea*)

Recently, we have demonstrated the channel-mediated release mechanism of the major inhibitory transmitter GABA from astrocytes in cerebellum involving Best1 channel and that the source of tonic inhibition in cerebellum is astrocytic GABA. We subsequently identified the biosynthetic pathway for GABA in astrocyte to be the putrescine degradation pathway leading to GABA production, in which the monoamine oxidase B (MAOB) is the key enzyme for GABA production. This series of studies implicates that astrocytes by tonically releasing the major inhibitory transmitter GABA, can exert strong inhibitory drive to the brain activity, raising a profound possibility that astrocytes can directly participate in cognitive processes via astrocytic GABA. Utilizing these newly identified molecular targets, Best1 and MAOB, we further demonstrated that tonic GABA inhibition in cerebellum is critical for motor coordination. In parallel, we have revealed the pathological role of astrocytic GABA, especially in Alzheimer's disease (AD) and Parkinson's disease (PD). We provide compelling pieces of evidence that hippocampal or SNpc astrocytes aberrantly produce GABA via MAOB and release through Best1 channels in AD or PD, respectively. The aberrant GABA from astrocytes inhibits neighboring neuronal activity to impair memory in AD or to cause motor deficit in PD. We continue to investigate the role of aberrant GABA from reactive astrocytes in white matter stroke and recovery after spinal cord injury. (COI: No)

S63-4

Best1-mediated tonic GABA release alleviating seizure susceptibility in kainate-induced epilepsy

Jin Bong Park (*Department of Physiology, College of Medicine, Chungnam National University, Korea*)

Tonic activation of extrasynaptic $GABA_A$ receptors is under the tight regulation of ambient GABA level to maintain the excitation/inhibition (E/I) balance in the brain. Any slight shift in the E/I balance is expected to cause a serious pathological condition, such as epileptic seizure. However, the pathophysiology of astrocytic GABA release has been remained elusive in epileptic seizures. Here, we demonstrated that pharmacological or genetic intervention of the GABA-permeable bestrophin-1 (Best1) channel prevented the generation of tonic $GABA_A$ inhibition, thus disinhibited the CA1 pyramidal neuronal firing and augmented the seizure susceptibility in kainic acid (KA)-injected epileptic mice. Astrocytes-specific overexpression of Best1 in KA-injected Best1 knock-out mice fully restored the generation of tonic $GABA_A$ inhibition, thus efficiently suppressed the seizure susceptibility. Our findings demonstrate that tonic GABA release from reactive astrocytes contributes to the maintaining of E/I balance in epileptic hippocampi, thus, serving as a good therapeutic target against the excitatory shift of E/I balance in epileptic seizure. (COI: No)

Symposium64

New insights into the cellular and molecular mechanisms of neurological diseases using experimental model systems

(March 31, Sun., 8:00-9:30, Room G)

S64-1

Modulatory roles of Pnn in glial apoptosis induced by disrupted energy homeostasis during ischemia

Sujira Mukda (*Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University, Thailand*)

Ischemic stroke caused by sudden loss of blood flow in the brain is the major form of cerebral stroke, resulting in damage or death of brain cells. Since the neuronal cell death can cause several pathological consequences, preventing its occurrence and understanding its underlying mechanism become necessary. Therefore, the anti-apoptotic role of Pnn, a serine-arginine related protein, was investigated in rat primary cortical astrocytes under ischemic condition. The expression level of Pnn was increased immediately after exposure to oxygen-glucose deprivation (OGD) and then declined during the reoxygenation period. Changes in Pnn levels were closely related to apoptosis-associated proteins, including Bax, Bcl2, and cleaved caspase3. Suppression of Pnn expression using siRNA promoted OGD-induced cell apoptosis and inhibited cell proliferation, especially during reoxygenation. These findings implied that Pnn may play a critical role in regulating apoptosis in primary cortical astrocytes under ischemic condition, and might be a novel therapeutic target for ischemic stroke treatment. (COI: No)

S64-2

Emerging the synaptopathology-based therapies in the environmental-toxin induced rat model of autism

Hui-Ching Lin (*Department and Institute of Physiology, National Yang-Ming University, Taiwan*)

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social communication and abnormal emotional responses. The amygdala and prefrontal cortex (PFC) have been reported in regulating socio-emotional behaviors and cognitive function in individual with ASD. The autism-like phenotypes of valproic acid (VPA)-exposed offspring have been observed the imbalances between synaptic excitation and inhibition (E/I) neurotransmission. Here, we investigated the possible treatment involved in regulating the autistic behaviors and synaptic plasticity in VPA-exposed offspring. First, we observed that less sociability, increased anxiety, abnormal fear memory and enhanced long-term potentiation (LTP) in amygdala of VPA-exposed offspring. Second, we found that NMDAR-dependent long-term depression (LTD) were absent from the amygdala associated with enhancement of levels glycogen synthase kinase 3β (GSK-3β) phosphorylation in the VPA-exposed offspring. Third, we demonstrated that the autistic behaviors, impaired NMDAR-dependent LTD and higher dendritic spine density reversed by NMDAR partial agonist D-cycloserine (DCS). Additionally, PFC deep brain stimulation (DBS) improves sociability and anxiety via modulation of the serotonin (5-hydroxytryptamine, 5-HT) system in VPA-exposed offspring. These results provided the first evidence of possible signaling that rescue of the ASD-like phenotype and synaptopathology in VPA-exposed offspring. (COI: No)

S64-3

The roles of microglial on the molecular mechanism of painful diabetic neuropathy in the rat

Idris Long[1]; Che Aishah Nazariah Ismail[2]; Che Badariah Ab Aziz[2]; Rapeah Suppian[1] (*[1] School of Health Sciences, Health Campus, Universiti Sains Malaysia, Malaysia; [2]School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Malaysia*)

This study aimed to explore the roles of microglia on the expression of NR2B NMDA receptor, BDNF and DREAM proteins, pro-inflammatory cytokines and oxidant-antioxidant status in the pathogenesis of PDN in the spinal cord of streptozotocin-induced diabetic rats. The diabetic rat model was induced by intraperitoneal injection of streptozotocin (STZ). Tactile allodynia was assessed on Day-0 (baseline), Day-14 (pre-intervention) and Day-22 (post-intervention). Minocycline at dose 80 μg and 160 μg were given intrathecally from Day-15 until Day-21. On Day-23, formalin test was conducted. Then spinal cord lumbar enlargement region was collected for immunohistochemistry, Western Blot (WB) and enzyme-linked immunoabsorbent assay (ELISA) analyses. Diabetes induction significantly increased tactile allodynia and nociceptive behavior accompanied by augmented expression of spinal NR2B NMDA receptor, OX-42, BDNF and DREAM proteins, TNF-α and MDA level and decreased catalase and SOD enzyme activity. Both doses of minocycline treatment, however, decreased tactile allodynia and nociceptive behavior followed by suppression of expression of spinal NR2B NMDA receptor, OX-42, BDNF and DREAM proteins, TNF-α and MDA level and increased catalase and SOD enzyme activity. This study revealed the important roles of microglia in the modulation of protein expression, pro-inflammatory and oxidative stress levels in the spinal cord of streptozotocin-induced painful diabetic neuropathy rats. (COI: No)

S64-4

Role of PI3K/Akt signaling in experimental brain stem death: Modulations by FLJ10540 and PTEN

Ching-Yi Tsai (*Institute for Translational Research in Biomedicine, Chang Gung Memorial Hospital, Taiwan*)

Despite great advances in contemporary medicine, brain stem death still remains enigmatic and its cellular and molecular mechanisms unsettled. The main theme of this lecture is that PI3K/Akt cascade, the classical tumorigenic signaling, is actively engaged in brain stem death. Our results were based on a clinically relevant animal model that employs the pesticide mevinphos as the experimental insult in Sprague-Dawley rats to mimic brain stem death in patients died of organophosphate poisoning. The neural substrate investigated is the rostral ventrolateral medulla (RVLM), the origin of a "life-and-death" signal detected from arterial pressure that reflects brain stem cardiovascular dysregulation that takes place before death. Activation of PI3K/Akt signaling in the RVLM upregulates the NF-κB/NOS II/peroxynitrite cascade, resulting in impairment of brain stem cardiovascular regulation that leads to the loss of the "life-and-death" signal in experimental brain stem death. This process is reinforced by FLJ10540, a PI3K-association protein; and is counteracted by PTEN, a negative regulator of PI3K/Akt signaling. The concept that a classical signaling pathway in tumorigenesis is also an active player in cardiovascular dysregulation in brain stem death promulgates the notion that rather than focusing on a particular disease condition, a new vista for future therapeutic strategy against both fatal eventualities should target this common cellular cascade. (COI: No)

Symposium65

Intervention factors of neuronal irregular development: from gut bacteria to mental situation via chemicals

(March 31, Sun., 8:00-9:30, Room H)

S65-1

Development of in vitro developmental neurotoxicity testing

Yasunari Kanda; Daiju Yamazaki (*Division of Pharmacology, National Institute of Health Sciences (NIHS), Japan*)

It is a growing concern that exposure to chemicals during prenatal period might cause developmental abnormalities in the central nervous system of children. According to the current OECD guideline, developmental neurotoxicity (DNT) has been examined using experimental animals. However, in vivo DNT assessment are problematic from the viewpoint of time-consuming, cost, and high throughput. Here we reported in vitro DNT testing method. We found that positive compounds inhibited neural differentiation capability in human iPS cells. We also tried to develop in vitro DNT testing method using multi-electrode array system in rat cortical neurons. In the symposium, we have discussed the current status and future perspectives of in vitro DNT testing method. (COI: No)

S65-2

Prenatal maternal depression and stress on infant temperament at: A disaster research in the USA

Yoko Nomura[1,2,3,4,10]; Kei Davey[5]; Patricia Pehme[1,2]; Jackie Finik[1,6]; Wei Zhang[1,7]; Melissa Haung[1,2]; Jessica Buthmann[1,2]; Kathryn Dana[1,2]; Yasunari Kanda[8]; Sachiko Yoshida[9]; Kenji J Tsuchiya[10] (*[1]Queens College, The City University of New York, USA; [2]Graduate Center, The City University of New York, USA; [3]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, USA; [4]Advanced Science Research Center, Japan; [5]Bryn Mawr College, USA; [6]CUNY Graduate School of Public Health, USA; [7]New Jersey City University, USA; [8]Division of Pharmacology, National Institute of Health Sciences, Japan; [9]Department of Environmental and Life Sciences, Toyohashi University of Technology, Japan; [10]Department of Child and Adolescent Psychiatry, Hamamatsu University School of Medicine, Japan*)

The study examined the effects of *in-utero* exposure to maternal depression and Superstorm Sandy, a hurricane that hit metropolitan New York in 2012, on infant temperament at 6 months. Temperament was assessed using the Infant Behavior Questionnaire-Revised. Maternal depression was measured by the Edinburgh Postnatal Depression Scale. The main effects and the interaction of maternal depression and Sandy exposure on infant temperament were examined using Multivariable General Linear Model. Results show that prenatal maternal depression was associated with lower emotion-regulation and greater distress. Stratification and interaction analyses suggested that the adverse effects of prenatal maternal depression on problematic temperament were amplified by *in-utero* Sandy exposure. The study underscores the importance of providing prenatal screening and treatment for maternal depression during pregnancy, while simultaneously identifying high-risk families who may have suffered from disaster-related traumas in order to provide necessary services. As the frequency of natural disasters may increase owing to climate change, it is important to understand the consequences of *in-utero* stress on child development and to formulate plans for early identification. (COI: No)

S65-3

Language development is affected by maternal postpartum depression, not by unwanted pregnancy

Kenji J Tsuchiya[1,2]; Sona Sanae Aoyagi[2]; Yoko Nomura[1,3,4,5,6]; Sachiko Yoshida[7]; Tomoko Nishimura[1,2]; Damee Choi[1,2]; Taeko Harada[1,2]; Toshiki Iwabuchi[1,2]; Ryuji Nakahara[1]; Akemi Okumura[1,8] (*[1]Research Center for Child Mental Development, Hamamatsu University School of Medicine, Japan; [2]United Graduate School of Child Development, Hamamatsu University School of Medicine, Japan; [3]Department of Psychology, Queens College, City University of New York, USA; [4]Graduate Center, City University of New York, USA; [5]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, USA; [6]Advanced Science Research Center, CUNY, USA; [7]Department of Environmental and Life Sciences, Toyohashi University of Technology, Japan; [8]Department of Child and Adolescent Psychiatry, Hamamatsu University School of Medicine, Japan*)

This study investigated whether postpartum depression (**PPD**) and unwanted pregnancy (**UP**) of mothers would be associated with delayed expressive language development of the offspring during infancy and childhood.

This longitudinal, observational study was conducted as a part of the Hamamatsu Birth Cohort for Mothers and Children, a population-representative sample. A total of 969 neonates and the mothers were enrolled. Maternal PPD was measured by the Edinburgh Postnatal Depression Scale, and UP was measured through maternal interviews. Expressive language development was measured by the Mullen Scales of Early Learning. Six time points were monitored (10, 14, 18, 24, 32, and 40 months postpartum). The associations of PPD and UP with expressive language development were analyzed using multiple linear regression and growth curve analysis.

Results indicate children of mothers with PPD, emerging only during 1 to 3 months postpartum, had significantly lower scores of expressive language at 18 months and beyond, with a score reduction of approximately 0.6 SD. The similar effect was not found in PPD emerging during the first months postpartum, nor was it found in UP.

Exposure to late-onset maternal PPD may lead to persistent decline in the rate of expressive language development in the offspring during infancy and early childhood, highlighting the significance of monitoring for late-onset PPD to facilitate early detection and intervention. (COI: No)

S65-4

Meconium microbiota is associated with maternal anxiety experienced during pregnancy

Jianzhong Hu[1]; Jenny Ly[2]; Wei Zhang[2]; Yonglin Huang[2]; Vivette Glover[4]; Inga Peter[1]; Yasmin L Hurd[5,6,7]; Yoko Nomura[2,3,5] (*[1]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, USA; [2]Department of Psychology, Queens College, City University of New York, USA; [3]Graduate Center, City University of New York, USA; [4]Institute of Reproductive and Developmental Biology, Imperial College London, UK; [5]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, USA; [6]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, USA; [7]Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, USA*)

Little is known about whether a mother's psychological state during pregnancy influences her offspring's microbiome. This study examined whether maternal anxiety, depression, and stress during pregnancy is associated with the diversity of meconium microbiome, the first internal discharge, in 75 newborns from an existing birth cohort study. The meconium microbiome was profiled using multi-barcode16S rRNA sequencing at V3-V4 hyper-variable region followed by taxonomic assignment to the green gene 16S references at 97% similarity and diversity analysis at the genus level. Results showed that the meconium contained diversified microbiota, and greater pregnancy-related anxiety was significantly associated with a less diverse meconium microbiota community (p-value=0.001). At the specific taxa level, greater pregnancy-related anxiety was correlated with a lower level of the *Enterococcaceae* family (p-value=2e-4, Spearman rho=-0.43). These findings enhance our understanding of the role that prenatal maternal mood may play in early life bacteria colonization. (COI: No)

S65-5

Developmental neurotoxicity and immune abnormality with chemicals and stress exposure on the rat

Sachiko Yoshida[1]; Yukiko Fueta[2]; Susumu Ueno[3]; Yuko Sekino[4]; Yoko Nomura[5]; Yasunari Kanda[6] (*[1]Department of Environmental and Life Sciences, Toyohashi University of Technology, Japan; [2]Department of Environmental Management and Control, School of Health Sciences, University of Occupational and Environmental Health, Japan; [3]Department of Occupational Toxicology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan; [4]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan; [5]Department of Psychology, Queens College, City University of New York, USA; [6]Division of Pharmacology, National Institute of Health Sciences, Japan*)

The prenatal environment prepares the developing fetus for conditions in the postnatal world. Environmental chemicals and stress in-utero have potential effects on the developmental neurotoxicity (DNT), leading to neurobehavioral outcomes such as attention deficit hyperactivity disorder and autism.

We have established the ASD-model rat with the administration to embryonic day 16 p.o. (VPA; 600mg/kg or CPF; 10 mg/kg of mother weight, respectively), and observed their cerebellar development. VPA- or CPF-administrated rat showed the excess development of Purkinje cells and excess folds in the V to VI lobules of the cerebellar vermis in within 2 two weeks after birth. This alteration is similar to human early ASD cerebellum. Conversely, we observed little developmental abnormality of Purkinje cells in a2.0 mg/kg tributyltin (TBT)-administrated rat, whereas TBT is the candidate of the neurodevelopmental toxins. 500 mg/kg glyphosate, the candidate of the environmental toxin, induced similar developmental abnormality in cerebellum and sometimes offspring showed motor ataxia. Food-restricted stress in-utero showed little behavioral abnormality in offspring, however, their thymuses were significantly shrunk. The shrinking or swelling of thymuses were observed in chemical- or stress-treated offspring, and this evoked us the developmental immune abnormality. It is important to coordinate this physiological knowledge of animal models with psychobiology of a human. (COI: No)

Symposium66

Inflammation and Atherosclerosis

(March 31, Sun., 8:00-9:30, Room I)

S66-1

Flow and Atherosclerosis - Roles of MicroRNAs

Jeng-Jiann Chiu (*National Health Research Institutes, Taiwan*)

Atherosclerosis develops predictably in regions of the arterial tree, where the wall shear stress is generated by complex patterns of blood flow. Hemodynamic characteristics, including disturbed flow patterns and shear stress, determines the location of lesions and contributes to the pathogenesis of atherosclerosis. Laminar blood flow in the straight part of the arterial tree and high shear stress modulate cellular signaling and endothelial cell (EC) function, and protect against atherogenesis. In contrast, disturbed flow in branches and curvatures of the arterial tree and the associated oscillatory low shear stress enhance leukocyte infiltration of the arterial wall and thus are atherogenic. However, little is known about the effect of disturbed flow on ECs, especially on their interactions with smooth muscle cells (SMCs), whose phenotypic switching is significantly implicated in atherosclerosis and neointimal lesion formation. Our recent studies using microRNA (miR) assay, in vitro EC-SMC co-culture flow system, experimental animal models, and human specimens from patients with coronary artery disease (CAD) have identified several miRs to be involved in the formation and progression of atherosclerosis. Our findings help the discovery of new molecular targets and elucidation of functional mechanisms underlying atherosclerosis, thereby facilitating the development of new approaches for therapeutic interventions. (COI: No)

S66-2

Nectin-Like Molecules as Novel Regulators in Angiogenesis and Atherosclerosis

Yoshiyuki Rikitake (*Laboratory of Medical Pharmaceutics, Kobe Pharmaceutical University, Japan*)

Nectin and nectin-like molecule (Necl) are immunoglobulin superfamily cell adhesion molecules, which interact *in trans* and *in cis* with a variety of proteins on the cell surface. Here we review our studies on the roles of Necls and afadin, an adaptor protein of nectin, in angiogenesis and atherosclerosis. In Necl-5-knockout or endothelium-specific afadin-knockout mice, recovery of blood flow after hindlimb ischemia and VEGF-induced neovascularization in implanted Matrigel plugs are impaired. Necl-5 interacts with VEGF receptor 2 (VEGFR2) and enhances Tyr951 phosphorylation of VEGFR2 and interaction with integrin $\alpha_v\beta_3$, leading to activation of Rap1 and Akt, eventually enhancing network formation, migration and survival of vascular endothelial cells. Activated Rap1 binds to afadin and regulates interaction of afadin with phosphatidylinositol 3-kinase, facilitating Akt activation. Afadin also interacts with ArhGAP29, a GAP for RhoA, which inhibits RhoA-ROCK signaling, eventually enhancing network formation and migration. On the other hand, Necl-4 also interacts with VEGFR2 and inhibits VEGFR2 Tyr1175 phosphorylation through PTPN13, eventually reducing migration and proliferation of confluent vascular endothelial cells. Necl-5-knockout mice exhibit reduced intimal thickening following carotid artery ligation because of reduced dedifferentiation, migration and proliferation of vascular smooth muscle cells. Thus, Necls are novel regulators in angiogenesis and atherosclerosis. (COI: No)

S66-3

YAP promotes angiogenesis via STAT3 in endothelial cells

Ding Ai (*Department of Physiology, Tianjin Medical University, China*)

Rationale: Angiogenesis is a complex process regulating endothelial cell (EC) functions. Emerging lines of evidence support that Yes-associated protein (YAP) plays an important role in regulating the angiogenic activity of ECs.
Objective: To specify the effect of EC YAP on angiogenesis and its underlying mechanisms.
Method and Results: In ECs, vascular endothelial growth factor reduced YAP phosphorylation time- and dose-dependently and increased its nuclear accumulation. Using Tie2Cre-mediated YAP transgenic (Tie2Cre-YAP^{Tg}) mice, we found that YAP promoted angiogenesis in the postnatal retina and tumor tissues. Mass spectrometry revealed signal transducer and activator of transcription 3 (STAT3) as a potential binding partner of YAP in ECs. Western blot and immunoprecipitation assays indicated that binding with YAP prolonged interleukin 6–induced STAT3 nuclear accumulation by blocking chromosomal maintenance 1-mediated STAT3 nuclear export without affecting its phosphorylation. Moreover, angiopoietin-2 (Ang2) expression induced by STAT3 was enhanced by YAP overexpression in ECs. Finally, a selective STAT3 inhibitor or Ang2 blockage partly attenuated retinal angiogenesis in Tie2Cre-YAP^{Tg} mice.
Conclusions: YAP binding sustained STAT3 in the nucleus to enhance the latter's transcriptional activity and promote angiogenesis via regulation of Ang2. (COI: No)

S66-4

Integrin-YAP/TAZ-JNK cascade mediates atheroprotective effect of unidirectional shear flow

Yi Zhu (*Department of Physiology, Tianjin Medical University, China*)

YAP and TAZ, effectors of the Hippo pathway, have been identified as mediators for mechanical stimuli1. However, the role of YAP/TAZ in haemodynamics-induced mechanotransduction and pathogenesis of atherosclerosis remains unclear. Here we show that endothelial YAP/TAZ activity is regulated by different patterns of blood flow, and YAP/TAZ inhibition suppresses inflammation and retards atherogenesis. Atheroprone-disturbed flow increases whereas atheroprotective unidirectional shear stress inhibits YAP/TAZ activity. In vivo endothelial-specific YAP overexpression exacerbates, while CRISPR/Cas9-mediated Yap knockdown in endothelium retards, plaque formation in ApoE−/− mice. We also show several existing anti-atherosclerotic agents such as statins inhibit YAP/TAZ transactivation. On the other hand, simvastatin fails to suppress constitutively active YAP/TAZ-induced pro-inflammatory gene expression in endothelial cells, indicating that YAP/TAZ inhibition could contribute to the anti-inflammatory effect of simvastatin. Furthermore, activation of integrin by oral administration of MnCl2 reduces plaque formation. Taken together, our results indicate that integrin–Gα13–RhoA–YAP pathway holds promise as a novel drug target against atherosclerosis. (COI: No)

Symposium67

The potential roles of NMDAR in neurological and neuropsychiatric disorders: new findings and therapeutic targets

(March 31, Sun., 8:00-9:30, Room J)

S67-1

Roles of D-serine, an endogenous co-agonist of NMDAR in psychiatric and neurodegenerative disorders

Hisashi Mori (*Department of Molecular Neuroscience, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Japan*)

The mammalian brain contains high levels of freeD-serine, which acts as an endogenous co-agonist of the *N*-methyl D-aspartate-type glutamate receptor (NMDAR). The synthesis of D-serine from L-serine is catalyzed by serine racemase (SRR). Studies using SRR knockout (KO) mice have provided compelling evidence that the majority of D-serine is produced by neuronal SRR and that an appropriate level of D-serine is required for NMDAR-mediated neurotransmission and long-term potentiation of synaptic transmission in the hippocampus. In this symposium, I will present the roles of SRR/D-serine in many psychiatric and neurodegenerative disorders including depression, post-traumatic stress disorder (PTSD), epilepsy, and pain transduction revealedby using SRR-KO mice. (COI: Properly Declared)

S67-2

The therapeutic potentials and underlying mechanism of sarcosine and RS-D7 in schizophrenia

Wen-Sung Lai[1,2,3] ([1]*Department of Psychology, National Taiwan University, Taiwan;* [2]*Graduate Institute of Brain and Mind Sciences, National Taiwan University, Taiwan;* [3]*Neurobiology and Cognitive Science Center, National Taiwan University, Taiwan*)

Schizophrenia, a severe mental illness, affects 1% of population worldwide. Common symptoms associated with schizophrenia include positive, negative, cognitive, and mood-related symptoms. To date, there is no drug for the treatment of negative and cognitive symptoms. It has become an unmet medical need. In recent years, compelling animal and clinical studies support the NMDA receptor hypofunction hypothesis of schizophrenia and suggest some promising therapeutic agents. Notably, several NMDA-enhancing agents, especially through glycine modulatory site (GMS) of NMDA receptor, resulted in a significant reduction of psychotic and cognitive symptoms in some schizophrenic patients. Given that NMDA-mediated signaling pathways has been implicated in cognitive/social functions and that GMS is a potential therapeutic target for enhancing activation of NMDA receptors, it is of great interest to investigate the effect of GMS modulators and their underlying mechanisms. In this talk, our recent findings in sarcosine and RS-D7 will be briefly introduced and revealed. A series of cell-based assays and mouse studies was conducted and indicated their efficacy, therapeutic effects, and underlying mechanism. Taking advantage of RS-D7 can be converted through a market drug RS-D7pro, our team further demonstrated RS-D7's significant therapeutic potentials in negative/cognition symptoms of schizophrenia and other neuropsychiatric disorders in our proof-of-concept clinical studies. (COI: No)

S67-3

The roles of NMDA receptors in regulating real-time motor control and parkinsonian motor behaviors

Ming-Kai Pan[1,2] ([1]*Department of Medical Research, National Taiwan University Hospital, Taiwan;* [2]*Department of Neurology, College of Medicine, National Taiwan University, Taiwan*)

NMDA receptors are best known by their plasticity generating roles. In terms of basal ganglia motor control, NMDA receptors play crucial roles in shaping striatal neuronal activities via long-term plasticity changes. These changes modulate motor behaviors in both physiological and pathological states. Interestingly, NMDA receptors also play important roles in real-time motor control and will be the main theme of this talk. We will first cover the circuitry mechanism of NMDAergic motor control and differential roles of NMDA receptors subtypes in motor execution. We will also cover the abnormalities in these NMDAergic pathways and their contributions in parkinsonian motor behaviors. Finally, we will discuss briefly about current evidence and therapeutic potentials of NMDA receptor modulators in parkinsonian motor behaviors. (COI: No)

S67-4

Novel mechanism of Ketamine's rapid action through the cytoplasmic domain of the NMDA receptor

Noboru Komiyama (*Centre for Clinical Brain Sciences, University of Edinburgh, UK*)

Major depressive disorder (MDD) is a debilitating illness that affects approximately 1 in 5 people worldwide. Currently there are only limited pharmacological treatments available for MDD due to low rates of efficacy and undesirable side effects. Ketamine, a non-competitive NMDA receptor antagonist has recently been shown to have a rapid-acting and long-lasting antidepressant effect in MDD patients, including those with treatment-resistant depression. However, ketamine has undesirable psychotomimetic side effects, is a drug of abuse, and its precise mechanism of action is still not well understood.

To try to develop future therapeutic strategies we have been investigating the molecular mechanism underlying ketamine's antidepressant effect.

Using novel mutant mouse models containing mutations in the c-terminal domain (CTD) of the NMDA receptor, we have identified a specific requirement for the CTD of the GluN2B subunit for a rapid action of ketamine. We have also found that the CTD of the GluN2B subunit is essential for formation of NMDA receptor macromolecular complexes in vivo. These findings indicate that the CTD of the GluN2B subunit is the area of the NMDA receptor responsible for coupling to intracellular signaling cascades that are important for ketamine's action, suggesting there may be downstream targets among the GluN2B-CTD interacting molecules suitable for pharmacological intervention. (COI: Properly Declared)

Symposium68

Pulmonary hypertension and inflammation: the interdependent processes triggered by each other

(March 31, Sun., 8:00-9:30, Room K)

S68-1

Withdrawn

S68-2

Monocrotaline Induces Pulmonary Hypertension By Targeting the Extracellular Calcium-Sensing Receptor

Qinghua Hu (*Department of Pathophysiology, Tongji Medical College, China*)

Monocrotaline has been widely used for years to establish animal model of pulmonary hypertension. The long-standing mystery about monocrotaline-held specificity towards the pulmonary endothelium versus systemic vasculature and its molecular target remains undetermined. Our experiments identified that monocrotaline bound with and released from erythrocytes at the physiological levels of partial pressure of oxygen in venous and arterial blood, respectively and aggregated on pulmonary artery endothelial cells. The nuclear magnetic resonance screening and cellular thermal shift assay demonstrated the binding of monocrotaline to extracellular calcium-sensing receptor. Monocrotaline dimerized extracellular calcium-sensing receptor, triggered the mobilization of calcium signaling and damaged pulmonary artery endothelial cells in extracellular calcium-sensing receptor-dependent manner. Finally, monocrotaline-induced pulmonary hypertension in rats was significantly attenuated or abolished by the inhibitor, the general or lung knockdown or knockout of extracellular calcium-sensing receptor. In conclusion, monocrotaline binds to erythrocytes in venous blood and releases from erythrocytes at the lung, then aggregates on and activates extracellular calcium-sensing receptor of pulmonary artery endothelial cells, triggers endothelial damage primarily and ultimately induces pulmonary hypertension. (COI: No)

S68-3

Endothelial Cell Integrin β4 Knockout Attenuates LPS-Induced Murine Acute Lung Injury

Weiguo Chen; Zhigang Hong; Patrick Belvitch; Jeffrey R Jacobson (*Department of Medicine, University of Illinois at Chicago, USA*)

Previously studies showed that integrin β4 plays vital role in murine acute lung injury (ALI). We hypothesized that strategies aimed precisely at inhibiting integrin β4 (ITGB4) signaling may prove to be more effective maneuver. **Methods**: We generated endothelial ITGB4 knockout mice by crossing ITGB4 flox/flox and Tie2-Cre mice. These animals were then subject to ALI induced by lipopolysaccharide prior to assessments of lung injury by bronchoalveolar lavage (BAL) fluid cell counts, protein, cytokine levels as well as lung histologic evaluation. In separate experiments, EC-ITGB4 KO mice were administered LPS to induce ALI followed by ventilation to evaluate the lung compliance. **Results:** In our LPS-ALI model, EC-ITGB4 KO mice were found to have significantly decreased total cell counts and protein levels compared to ITGB4 flox/flox mice ($p< 0.01$). BAL cytokine levels after LPS were also reduced in EC-ITGB4 KO mice compared to ITGB4 flox/flox mice, including IL-6, KC and TNFα ($p< 0.01$). These findings were corroborated by evidence of decreased inflammatory cell infiltration and interstitial edema on lung histology in EC-ITGB4 KO mice. Finally, measurements of lung function after LPS confirmed significantly decreased compliance in ITGB4 flox/flox mice compared to EC-ITGB4 KO mice. **Conclusions:** Our findings support a critical role for EC ITGB4 in the elaboration of murine ALI as EC-ITGB4 KO mice were found to have significantly decreased susceptibility to lung injury after LPS. (COI: No)

S68-4

The regulation of pulmonary immunity and stress response by airway expressed adhesion molecules

Xiaoqun Qin; Chi Liu; Yang Xiang; Yurong Tan; Xiangping Qu; Huijun Liu (*Department of Physiology, Xiangya School of Medicine, Central South University, China*)

Asthma is a complex disease characterized by Th2 inflammation, typically impaired airway epithelial cells and airway hyper-responsiveness. Our previous work have demonstrated that there are altered expression of adhesion molecule on asthma airway epithelial cells, including downregulated integrin β4, Catenin α like 1(CTNNAL1) and increased intercellular adhesion molecule 1(ICAM-1). Disruption of integrin β4 mediated adhesion between epithelial cells and basal membrane resulted in specific Th2 cell recruitment and promoted Th2-type allergic inflammation. Meanwhile, integrin β4 defects conferred aggravated airway inflammation and airway hyper-responsiveness through the differential expression of specific cytokine and chemokine after HDM stress. CTNNAL1 engage in maintaining the structural integrity and suppressing the inflammatory adhesion of neutrophils, T lymphocytes and eosinophils to airway epithelium. The silence of CTNNAL1 led to down-regulation of structural adhesion related E-cadherin, integrin β1 and integrin β4 while down-regulation of inflammation related ICAM-1. In summary, cell adhesion molecules have been identified to be engaged in the regulation of pulmonary immunity and stress response. As the adhesion molecules have many types with diverse functions, they would be the attractive targets for novel therapeutic approaches for lung disease.

(Supported by NSFC: 81670002,31671188) (COI: No)

Symposium69

Optogenetics: Contributions to Physiology and Medicine Beyond Brain Circuit-Breaking

(March 31, Sun., 8:00-9:30, Room L)

S69-1

Using optogenetics to elucidate the function of pancreatic delta cells

George J. Augustine (*Nanyang Technological University, Singapore*)

Little is known about the role of somatostatin-secreting delta cells in the islets of Langerhans in regulating blood glucose homeostasis *in vivo*. Using *in vivo* optogenetics and high-speed Ca^{2+} imaging, we found that delta cells possess dynamic filopodia that enable these cells to reach a large number of beta cells within the islet and release somatostatin. This anatomical arrangement provides for efficient and precise fine-tuning of beta cell activity. Under pre-diabetic conditions, the delta cell exhibits morphological changes that may result from a compensatory attempt to maintain paracrine regulation of the beta cell. These observations provide an integrated picture of how delta cells serve as important modulators of beta cell activity within the pancreatic islet under physiological conditions, thus preventing hypersecretion of insulin. (COI: No)

S69-2

Optical control of the genome

Moritoshi Sato (*Graduate School of Arts and Sciences, The University of Tokyo, Japan*)

The genome consists of more than 20,000 genes and is essential for most of biological phenomena. To understand these biological phenomena, including diseases, approaches that enable optical control of the genome are required. Recently, we developed an optogenetic tool, named photoactivatable Cas9 (PA-Cas9), based on split-Cas9 fragments and photo-inducible dimerization proteins (Magnet system). PA-Cas9 allows direct editing of DNA sequence of the genome by light stimulation. Additionally, we developed a light-inducible, RNA-guided programmable system (Split-CPTS2.0) for endogenous gene activation based on the CRISPR–Cas9 system. We demonstrated that Split-CPTS2.0 allowed rapid and reversible targeted gene activation by light. Using this tool, we exemplified optical control of neuronal differentiation of human induced pluripotent stem cells (iPSCs). In addition to the CRISPR–Cas9-based optogenetic tools, we also have developed a photoactivatable Cre–*loxP* system. This tool allows optical control of DNA recombination reaction in an internal organ even by external, noninvasive illumination using LED light source. Genome engineering technology and optogenetics technology have emerged as different technologies from each other so far. Our studies described above merge these emerging research fields together. (COI: No)

S69-3

Optogenetic study of cell polarity - a simple assay

Takao Nakata (*Department of Cell Biology, Tokyo Medical and Dental University, Japan*)

Optogenetics has been called attention as a tool to remote control neuronal activity at fine spatiotemporal resolution from outside. This is a wonderful merit of Optogenetics, but it appears more experimental merits than we initially expected. We would like to introduce our attempts to make use of them in the field of cell biology. Today, I would like to use Optogenetics tools to make an assay system for spontaneous polarity formation in COS7 cells. Retrospectively, it could not be achieved without the Optogenetics tools.

We found CIP4 (CDC42 binding protein 4/Toca1) was a good candidate and focused it. CIP4-FP localization have been studied extensively by 2-photon microscope and found in cell margins but moving fraction was not observed.

We succeed in visualizing moving fraction of CIP4 along the cell margin bi-directionally, relatively short distance, suggesting its transport by myosin-actin. We introduced PA-CDC42 switch in this assay, because we thought discrepancy of product /intermediate ratio was the cause of lost transport intermediate. Thus, introducing switches, we can start assembly at low product state. Furthermore, its property suggested its role in spontaneous polarity formation. Combing with lifeactactinmarker, we showed how the actin assembly machinery assemble on cell margin. We found new fan-like structure intermediate was formed. (COI: No)

S69-4

Glial optogenetics for understanding the cross talk between metabolism and information processing

Ko Matsui (*Super-network Brain Physiology, Graduate School of Life Sciences, Tohoku University, Japan*)

Consciousness is lost within a minute from blood stream loss. This fact alone shows that complex information processing in the brain requires constant and precise supply of metabolic energy. Such super-network interaction between metabolism and information is likely to be the key link to body and mind connection. Neurons are never in direct contact with vascular components in the brain and astrocytes elements always come in between. Using electrophysiological and fluorescent imaging techniques, we have studied how neurons communicate with astrocytes. Super-fast communication pathway with kinetics comparable to neuronal synaptic transmission has been identified and it requires vesicular and ectopic releases of glutamate from presynaptic elements. We have also been studying the communication flowing in the opposite direction, from astrocytes to neurons, using specific and optogenetic activation of astrocytes. Classical transmitter can be released from astrocytes which appears to influence the function of the brain and lead to changes in behavior and learning. In addition, metabolites, which are not classically categorized as transmitters, also seem to be released from astrocytes. Metabolic alterations by optogenetic manipulation of astrocytes have a large impact on the information flow in neurons. In this talk, I will introduce our recent findings concerning this cross talk between metabolism and information processing. (COI: No)

S69-5

Organelle-optogenetics - direct manipulation of intracellular Ca^{2+} dynamics by light

Hiromu Yawo[1]; Toshifumi Asano[2]; Hiroyuki Igarashi[3]; Toru Ishizuka[1] ([1]*Department of Integrative Life Sciences Developmental Biology and Neurosciences, Tohoku University Graduate School of Life Sciences, Japan;* [2]*Department of Cell Biology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), Japan;* [3]*Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Robarts Research Institute, Western University, Canada*)

As one of the ubiquitous second messengers, the intracellular Ca^{2+}, has been revealed to be a pivotal regulator of various cellular functions. Two major sources are involved in the initiation of Ca^{2+}-dependent signals: influx from the extracellular space and release from the intracellular Ca^{2+} stores such as the endoplasmic/sarcoplasmic reticulum (ER/SR). To manipulate the Ca^{2+} release from the stores under high spatiotemporal precision, we established a new method termed "organelle optogenetics". That is, the Ca^{2+}-permeable channelrhodopsin, such as ChRGR, was specifically targeted to the ER/SR. The expression specificity as well as the functional operation of the ER/SR-targeted ChRGR ($ChRGR_{ER}$) was evaluated using mouse skeletal myoblasts (C2C12): (1) the $ChRGR_{ER}$ co-localized with the ER-marker KDEL; (2) no membrane current was generated by light under whole-cell clamp of cells expressing $ChRGR_{ER}$; (3) an increase of fluorometric Ca^{2+} was evoked by the optical stimulation (OS) in the cells expressing $ChRGR_{ER}$ in a manner independent on the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$); (4) the $\Delta F/F_0$ was sensitive to the inhibitor of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and (5) the store-operated Ca^{2+} entry (SOCE) was induced by the OS in the $ChRGR_{ER}$-expressing cells. The use of organelle optogenetics would reveal the physiological significance of intracellular Ca^{2+} dynamics under spatiotemporal precision. (COI: No)

Symposium70

Contribution of brain research to the understanding of the physiology, psychology and communication of acute and chronic pain

(March 31, Sun., 8:00-9:30, Room M)

S70-1

Imaging pain in the human brain: classical debates revisited with new methods

Pierre Rainville[1,2] (*[1]Department of Stomatology, University of Montreal, Canada; [2]Centre de recherche, Institut universitaire de gériatrie de Montréal, Canada*)

Modern medical imaging methods have provided unprecedented means to investigate brain mechanisms involved in pain perception and modulation. These advances provide unambiguous evidence that acute nociceptive pain consistently activates a distributed brain network targeted by the spino-thalamo-cortical pathways and involving somatosensory areas (SI, SII and posterior insula) as well as classical limbic cortices (anterior insula and mid-cingulate cortex). Non-pharmacological interventions and various psychological factors modulate activity within this network consistent with changes in pain self-reports or other pain-evoked responses. The classical approach of functional localization further suggests that different sub-components of the pain matrix contribute at least partly distinct aspects of the pain experience but the notion of pain specificity remains controversial. Whole brain pain signatures are now providing a sensitive mean to decode acute pain states but, again, their specificity is debated. In parallel with these developments in functional imaging of acute pain, studies on chronic pain states have clearly demonstrated abnormalities in prefrontal and limbic areas that may reflect individual predispositions and/or effects of chronic pain on the brain. These findings are consistent with a role for biological, psychological and social factors in the development of chronic pain and illustrate how brain imaging methods provide powerful integrative tools to study pain. (COI: No)

S70-2

The cerebral correlates of pain decoding: from overexposure to other people's pain to empathy

Philip L. Jackson (*School of Psychology, Laval University, Canada*)

Observing other people in pain leads to change in the level of activity of brain regions involved in the actual experience of pain. A number of studies of what is now called pain empathy have shown that individual differences in the observer, the characteristics of the person in pain, as well as contextual and social factors play important roles in determining the extent of this shared representation of pain. Moreover, whether such vicarious experience of other people's pain is related to one's level of empathy and subsequent helping behaviours remains unclear. This presentation will present recent findings showing how empathy and its cerebral correlates can be modified after different types of "overexposure", including exposure to facial expressions of pain in a laboratory setting and in situ pain exposure in healthcare professionals. Novel methods of modifying responses to other people's pain such as non-invasive brain stimulation and interaction with virtual agents will also be discussed as potential means through which empathy could be optimized. The underlying framework proposes that optimized levels of empathy imply benefits for both the sufferer and the caregiver. (COI: No)

S70-3

Improving cognitive pain inhibition using neuromodulation of the dorsolateral prefrontal cortex

Alice Wagenaar-Tison (*Department of Chiropratic, Université du Quebec à Trois-Riviéres, Canada*)

Pain constitute an alarm system to signal actual or potential tissue damage and allow protection of the body. Nociceptive stimuli are therefore salient and intrinsically capture attention. However, nociceptive processing and pain perception are regulated by top-down processes and depends on the allocation of attentional resources. The dorsolateral prefrontal cortex (DLPFC) is a cerebral structure playing a key role in cognitive functions, especially in attentional control and working memory. Working memory is involved in stimulus selection and orientation of attention. Depending on cognitive goals and priorities, attention may thus be directed away from pain. Working memory performance can be improved using transcranial direct current stimulation (tDCS) over the left DLPFC. In these conditions, cognitive pain inhibition is improved during the execution of a cognitive task and participants report less pain. In this talk, studies on neuromodulation of the left DLPFC will be discussed in relation to interactions between pain and cognition. The clinical relevance of this work will also be presented with regards to working memory improvement and pain management in different clinical populations. (COI: No)

S70-4

Influence of inflammation on cardiac responses to skeletal muscle stimulation

Nobuhiro Watanabe; Harumi Hotta (*Department of Autonomic Neuroscience, Tokyo Metropolitan Institute of Gerontology, Japan*)

Patients with chronic musculoskeletal pain reportedly have more risks to accompany with cardiovascular diseases. However, a causal relationship between these two conditions has not been clarified. In this presentation, we will introduce our studies examining cardiac responses to mechanical stimulation of intact and inflamed muscles in anesthetized rats. A calf was mechanically pressed over the skin by using a stimulation probe with flat plane of contact area. The stimulation of intact muscles increased heart rate (HR) when pre-stimulus HR was lower and decreased HR when pre-stimulus HR was higher. Such HR changes were not affected by bilateral vagus nerve transection but abolished by severance of cardiac sympathetic nerves. Then, we recorded mass discharges from the inferior cardiac sympathetic nerve. The stimulation increased or decreased cardiac sympathetic nerve activity (CSNA) dependent on the pre-stimulus CSNA levels. These results suggest that CSNA contributes to HR responses induced by calf pressure stimulation. Inflammation is a factor of pain chronicity. Next, we examined the influence of acute myositis on HR and CSNA responses to the pressure stimulation. The stimulation of an inflamed calf altered HR and CSNA. Such changes were larger than those of contralateral non-inflamed calf stimulation. These results suggest that acute myositis exaggerated cardiac responses to skeletal muscle stimulation due to an increase in cardiac sympathetic nerve responses. (COI: Properly Declared)

Local Organizing Committee Symposium

Symposium71

Toward understanding the neural basis of memory

(Co-organized by the Japan Neuroscience Society)

(March 31, Sun., 10:30-12:30, Room A)

S71-1

Robustness and Flexibility of Neuronal Ensembles in Memory

Naoki Matsuo (*Graduate School of Medicine, Osaka University, Japan*)

It is a fundamental question how memories are represented in the brain. A prevailing hypothesis suggests that memory is encoded by a cooperative activity of specific subset group of neurons. However, identifying these neurons supporting a given memory is challenging because these neuronal ensembles are likely sparsely distributed within the brain. To circumvent this difficulty, we have previously developed a transgenic system in mice that allows us to manipulate neurons activated during a relevant behavior. In the system, the expression of a given transgene is regulated by neuronal activity via the promoter of *c-fos* gene, whose expression is rapidly and transiently induced in response to neuronal activity, and is also dependent on a tetracycline inducible expression system. Activities of the tagged ensembles of neurons during contextual fear learning using this system have been shown to be sufficient and necessary for the contextual fear memory expression, demonstrating a direct evidence that individual memories reside in the activities of specific spatially distributed neuronal populations within neuronal networks.
Memories are not immutable but are persistent as well. How can we explain these conflicting phenomena with neuronal ensemble activities? I will introduce our studies using mouse genetics, in vivo calcium imaging, and behavioral analyses to understand the nature of robustness and flexibility of neuronal ensembles in memory. (COI: No)

S71-2

Association and identity of memory

Kaoru Inokuchi (*Faculty of Medicine, University of Toyama, Japan*)

Memories are not stored in isolation from other memories but are integrated into associative networks. At the same time, each memory has its own identity. Using two amygdala-dependent behavioral paradigms in mice, we found that a small population of co-shared neurons mediates the link between memories. Next, we addressed the question of how the brain defines specific memory identity out of intermingled memories stored in a shared cell ensemble. Complete amnesia of a given fear memory did not affect another linked fear memory encoded in the shared ensemble. Optogenetic potentiation or depotentiation of the plasticity at synapses specific to one memory affected the recall of only that memory. Thus, sharing engram cells underlies the linkage between memories, while synapse-specific plasticity guarantees the identity and storage of individual memories. (COI: Properly Declared)

S71-3

Understanding Synaptic Basis of Learning and Memory

Bong-Kiun Kaang (*School of Biological Sciences, Seoul National University, Korea*)

Learning is the process by which we obtain the information about the world; memory is the process by which that information is stored. Long-term memory formation requires translational as well as transcriptional regulation in the brain. We are interested in understanding what molecular and cellular mechanisms underlie memory storage. I will present our recent structural and functional approaches to reveal enhanced structural and functional connectivity between engram cells in the hippocampus during memory formation. Our data demonstrated that enhanced structural and functional connectivity between engram cells across two directly connected brain regions in the hippocampus forms the synaptic correlate for memory formation as was prophesied by D.O. Hebb 70 years ago. (COI: No)

S71-4

Social memory engram in the hippocampus

Teruhiro Okuyama (*Institute for Quantitative Biosciences (IQB), The University of Tokyo, Japan*)

For social animals, it is crucial to remember and recognize different conspecific individuals (i.e., having “social memory”), and exhibit appropriate social behaviors, such as preference behavior or avoidance behavior, to each individual. In humans, lesion of the hippocampus leads to multiple memory deficits including social memory, suggesting that the hippocampus, at least in part, stores memory information on the individual as well as other components of episodic memory such as spatial or temporal memory.

Since mice naturally tend to spend more time interacting with novel mice, rather than familiar mice (social discrimination behavior), we can quantify the degree of memory of individuals by calculating the total duration of time spent with novel versus familiar individuals. Using the social discrimination behavioral assay, we recently demonstrated that vCA1 pyramidal neurons in the hippocampus store social memory (social memory engram). Even if the memory seemed lost after long separation periods, optogenetic activation of the engram can fully restore that social memory. Additionally, artificial association between social engram encoding the memory of a specific individual with fear or reward events can elicit avoidance from or preference to that individual, respectively. (COI: No)

S71-5

Hippocampal encoding of spatial information of self and other

Shigeyoshi Fujisawa (*RIKEN Center for Brain Science, Japan*)

A prominent theory states that the hippocampus provides internal representations of spatial maps of external worlds, embodied by assemblies of place cells which encode positional information of an animal. However, how spatial information of external agents such as moving objects or other animals is represented in spatial maps of the hippocampus is still to be unraveled. Here we investigate whether and how the hippocampus represents spatial information of external agents, by examining activities of hippocampal neurons while an animal is observing another individual in the same environment. We newly developed spatial observation task, which is carried out with two rats, a demonstrator (‘the other’) and an observer (‘the self’), in a T-maze. Central for this task is that a correct choice (i.e., left or right arm) for the observer rat is determined by the choice of the demonstrator, that is, the observer is required to watch carefully the trajectory of the other. Using this behavioral task, we have performed large-scale extracellular recordings from CA1 of the observer rats. We found a group of neurons which jointly represented the spatial information of the other and self during the task behavior. Our results suggest that hippocampal cell assemblies can also map the spatial information of the other, as well as that of the self. (COI: No)

International Scientific Program Committee Symposium

Symposium72

Neurobiology of reward system in the Brain (ISPP, Iran)

(March 31, Sun., 10:30-12:30, Room B)

S72-1

Effects of Stress on Brain Reward Centres and Circadian Rhythms

Dipesh Chaudhury (*New York University Abu Dhabi (NYUAD), United Arab Emirates*)

Phasic activity in the brain reward centres such as the ventral tegmental area (VTA) encode for reward related behaviours. Surprisingly we have also observed that phasic activity in the VTA encodes for depression-like behaviours in mice exposed to the chronic social defeat stress (CSD) paradigm. Strikingly, during encoding of the depressive phenotype, we found differential firing dynamics of VTA neurons projecting to the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC). The circuit mechanisms by which VTA DA neurons are modulated during the encoding of depression-related behaviours is unknown. The lateral habenula (LHb), a nucleus that functionally integrates signals between limbic forebrain and monoaminergic hindbrain regions, sends robust projections to the VTA. Furthermore, the LHb is known to encode for motivation-, reward- and depression-related behaviours. We have used electrophysiological and optogenetic approaches to investigate the role of LHb neurons projecting to VTA (LHb-VTA) and Dorsal Raphe Nucleus (LHb-DRN) to investigate the role of these 2 circuits in modulating mood. Furthermore, in light of the observations that circadian rhythms are disrupted in patients suffering from major depressive disorders, we are investigating the cellular and molecular changes in the neural circuits linking the LHb, DRN and the circadian master clock, the suprachiasmatic nucleus (SCN) in mice exposed to the CSD stress paradigm. (COI: No)

S72-2

Roles of Parvalbumin interneurons in ventral hippocampus in social behavior and memory

Jing Liang[1,2] ([1]*Institute of Psychology, Chinese Academy of Sciences, China;* [2]*Department of Psychology, University of Chinese Academy of Sciences, China*)

Ventral hippocampus (vHip) has been implicated in social memory, which is important for animals that exhibit social interactions. Abnormal vHip activity is frequently observed in patients with psychiatric disorders and may contribute to social memory impairments. Specially, Parvalbumin-expressing (Pv) neurons, a main subtype of GABAergic interneurons, occupy a crucial place for the balance of the precise excitation/inhibition ratio (E/I ratio). However, it remains unknown how Pv interneurons in vHip regulate social behaviors and social memory. Here, by using tetanus toxin-mediated neuronal inactivation, cell-type-specific optogenetic modulation and *in vivo* calcium imagine, we show that Pv interneurons in the vHip of a mouse play a necessary role in social memory retrieval. Activity suppression of this type of neurons in vHip disrupted social recognition but induced no effect on social inaction. Moreover, the mice with optogenetic activation of Pv neurons in vHip spent similar time on exploring a previous unencountered (novel) mouse and a familiar one. Finally, the Pv neurons exhibited higher activities when a mouse explored a novel conspecific than it interacted with a familiar mouse. Overall, our study indicates that Pv interneurons in vHip play an important role in social memory retrieval and a manipulation that reduces vHip Pv interneurons activities could be beneficial for rescue impaired social memory. (COI: No)

S72-3

Brain Orexinergic System and Reward-related Behaviors

Abbas Haghparast (*Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Iran*)

Behavior of mammals is a resultant of action of three large functional systems of the brain: arousal, reward and cognition systems. Reward is defined as an integrated set of hedonic (i.e., pleasurable) and motivational processes occurring at both conscious and unconscious levels and eliciting cognitive and behavioral responses. Studies of the neurobiology of reward are important to advance affective neuroscience, and provide insights into the several psychopathologies including drug addiction, eating disorders, obsession and depression. Orexins (hypocretins) are novel neuropeptides that have the important role in a variety of behaviors ranging from arousal, reward and cognition. Reward functions are associated specifically with orexin neurons in lateral hypothalamus (LH). Previous studies showed that lesion of the LH can be caused the reduction of preference scores in morphine-induced conditioned place preference (CPP). Our study showed that chemical stimulation of the LH and activation of its orexinergic neurons solely could induce CPP. These neuropeptides modulate some types of high-motivated reward seeking behaviors. Now is clear that orexin interacts with the mesolimbic pathway to produce rewarding effects. Orexins modulate the mesolimbic dopamine system during reinforcement. In this part we discuss more about the recent research highlights that clear the role of brain orexinergic system in the reward circuitry. (COI: No)

S72-4

Early detection and intervention on methamphetamine addiction: Towards biobehavioral markers

Yonghui Li (*Institute of Psychology, Chinese Academy of Sciences, China*)

The prevalence rate of methamphetamine use disorders in Southeast Asian countries is on a dramatic rise recent years, constituting a significant public health concern and economic burden. Early detection and intervention is very important to the treatment on addiction. The presentation will introduce the neurocognitive profiles of the individuals with methamphetamine use disorders during their abstinence periods, combined with self-report, questionnaire, cognitive task and psychophysiological and EEG recording technologies. Our focus is to measure the cue reactivity when the addicts are exposed to different kinds of virtual drug cues and contexts, aims to identify the biobehavioral markers for early detection of methamphetamine addiction, and develop early intervention methods by modifying the addictive memory during its reconsolidation phase with virtual reality technology to reduce the cue reactivity and craving. (COI: No)

S72-5

Specificity in the Role of Different Metabotropic Glutamate Receptor Subtypes in Reward Circuitry

Abdolrahman Sarihi[1]; Nahid Roohi[1]; Negar Baharloui[1]; Mahsaneh Vatankhah[1]; Abass Haghparast[2] ([1]*Neurophysiology Research Center, Hamadan Uni. of Med. Sci., Iran;* [2]*Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Iran*)

Metabotropic glutamate receptors (mGluRs) mediate an important effect in modulation of the rewarding properties of morphine. The effects of intra-nucleus accumbens (NAc) injection of mGluR2/3, 5 and 7 antagonist/agonist drugs on the acquisition, expression, extinction and reinstatements of morphine induced Conditioned Place Preference (CPP) in the rats were studied. The results showed that bilateral intra-accumbal administration of MTEP (mGluR5 antagonist) significantly attenuated the acquisition of morphine-induced CPP in a dose-dependent manner in addition blockade of mGluR5 reduces rewarding properties of morphine. The injection of LY379268, (mGluR2/3 agonist) into NAc markedly decreased the acquisition of morphine-induced CPP in a dose-dependent manner, it was determined that only at the highest dose, injection of LY379268 into the NAc considerably attenuated the expression of morphine CPP. The findings also suggested that activation of mGlu2/3 receptors in the NAc dose-dependently blocked both establishment and maintenance of morphine-induced CPP. Moreover, recently our results revealed that administration of AMN082,(a selective mGluR7 allosteric agonist) significantly facilitates the extinction and inhibits reinstatement of morphine-induced CPP. The results confirmed the specificity in the role of different metabotropic glutamate receptor subtypes in reward circuitry as well as the role of this system as a potential therapeutic target for treating addiction. (COI: No)

Symposium73 (Sponsored Symposium)

New Twists in Understanding Taste

(Co-sponsored by AJINOMOTO CO., INC.)

(March 31, Sun., 10:30-12:30, Room C)

S73-1

Gingival solitary chemosensory cells serve as immune sentinels to protect against periodontitis

Robert F. Margolskee (*Monell Chemical Senses Center, USA*)

Taste-like solitary chemosensory cells (SCCs) mediate innate immune responses in many tissues. These cells use Tas2r "bitter" receptors and downstream "taste" components to detect pathogens and trigger host defenses. We found SCCs in gingival tissue where they regulate the oral microbiome and protect against periodontitis. We found 10 of 35 Tas2rs and taste signaling components Gnat3, Plcß2 and TrpM5 to be expressed in mouse gingiva; Gnat3, Plcß2 and TrpM5 are co-expressed in gingival SCCs. Skn1a$^{-/-}$ mice lack gingival SCCs. Compared to wildtype, Gnat3$^{-/-}$ mice had accelerated naturally occurring alveolar bone loss. Knockout mice lacking Gnat3, TrpM5 or Skn1a displayed enhanced ligature-induced periodontitis compared to wildtype controls. Gnat3$^{-/-}$ mice had higher bacterial loads on their periodontal ligatures, up-regulated levels of pro-inflammatory cytokines, lower levels of antimicrobial peptides in their gingiva, and an altered oral microbiome. Topical application to the gingiva of bitter denatonium benzoate stimulated gingival production of defensins and reduced alveolar bone loss in wildtype but not in Gnat3$^{-/-}$ mice. In sum, mouse gingival SCCs likely respond to bacterial signals via Tas2rs and coupled signaling components to trigger secretion of antimicrobial peptides and innate immune responses to prevent overgrowth of pathogenic oral bacteria and regulate gingival microbial composition. Gingival SCCs may provide a useful target for treating periodontitis. (COI: No)

S73-2

Structural basis of amino acid-perception by T1r taste receptors

Atsuko Yamashita (*Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan*)

Amino acids are important nutrients and perceived as savory flavors (umami) by the taste sensory system. The amino acid perception begins with chemical recognition and signal transduction by Taste receptor type 1 (T1r) residing in taste cells. T1rs are conserved in vertebrates, and generally the T1r1/T1r3 heterodimers, and T1r2/T1r3 in fish also, respond to amino acids.

The amino-acid recognition by T1rs attributes to their extracellular ligand-binding domains (LBDs). Recently, we solved the first crystal structure of a taste receptor, T1r2a/T1r3LBD from medaka fish. Our biophysical analyses showed that the protein exhibits binding to various amino acids and concomitant conformational changes. The receptor forms specific interactions with the core groups in amino acids, such as α-amino and carboxyl groups as well as α-carbon and hydrogen, in the L-configuration. The interactions are considered to induce a conformational change of the protein, and might result in receptor activation. The structural characteristics are likely conserved among T1r receptors responding to amino acids, and play a key role in their chemical recognition and signal transduction. In contrast, an α-substituent group of amino acids is accommodated as a hydrated state in a large space in the ligand-binding pocket, explaining the broad ligand specificity of the protein. The structural characteristics at the region in the pocket may determine the amino acid specificity of each T1r receptor. (COI: No)

S73-3

Ion channel synapses of the taste bud

Akiyuki Taruno[1,2]; Zhongming Ma[3]; Makoto Ohmoto[4]; Mizuho A. Kido[5]; Michael G. Tordoff[4]; Ichiro Matsumoto[4]; J. Kevin Foskett[3] (*[1]Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine, Japan; [2]JST, PRESTO, Japan; [3]Department of Physiology, University of Pennsylvania, USA; [4]Monell Chemical Senses Center, USA; [5]Department of Anatomy and Physiology, Saga University, Japan*)

Taste cells in the taste bud relay taste information to sensory neurons through cell-to-cell communications. However, the neurotransmission machinery of sweet-, bitter-, and umami-sensing type II taste cells has been elusive because they do not possess classical synaptic structures including synaptic vesicles, yet transmit information to neurons by releasing ATP as a neurotransmitter. In response to taste stimuli, type II cells fire action potentials leading to non-exocytotic release of ATP, suggesting the possibility that a voltage-gated ion channel acts as a conduit for ATP release. However, none of known ATP-permeable channels possesses the properties required for action potential-dependent activation. Thus, the chemical neurotransmission mechanism in type II cells has remained unknown. Recently, we discovered a novel voltage-gated non-selective ion channel composed of CALHM1 and 3, and identified it as the *bona fide* ATP-release channel in type II cells required for perception of sweet, bitter, and umami tastes. Previously, exocytosis of synaptic vesicles was the only neurotransmitter-release pathway for action potential-mediated neurotransmission. However, our discoveries here provide the first demonstration of an ion channel-mediated mechanism of rapid chemical neurotransmission, which we call "ion channel synapse" in contrast to the classical "vesicular synapse." The discovery, structure and function of ion channel synapses in the taste bud will be reviewed. (COI: No)

S73-4

Novel taste sensory pathways for sugars and fatty acids in the mouse periphery

Yuzo Ninomiya[1,2]; Keiko Yasumatsu[1]; Shusuke Iwata[1]; Ryusuke Yoshida[3] (*[1]Division of Sensory Physiology, R&D Center for Five-Sense Devices, Kyushu University, Japan; [2]Monell Chemical Senses Center, USA; [3]Department of Oral Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan*)

Previous studies in mice demonstrated that sweet (T1R2+T1R3), umami (T1R1+T1R3), bitter (T2Rs), salty (ENaCs) and sour (PKDs/ASICs etc) receptors are expressed in the different set of taste bud cells, respectively. Such molecular evidence suggest that 5 fundamental taste qualities might be sensed by dedicated taste bud cells that relay quality information to gustatory nerve fibers. However, we and others showed that a significant portion of taste cells and nerve fibers respond to multiple taste qualities, while sweet- and umami-responsive taste cells and nerve fibers are segregated into at least two types according to their susceptibilities to gurmarin, a T1R3 inhibitor. Recent studies showed that as well as T1R2+T1R3, the primary sweet sensor for sugars and noncaloric sweeteners, glucose transporters (GLUTs, SGLTs) and the ATP-gated K^+ (KATP) channels were expressed in T1R3-expressing cells. We also found a subset of nerve fibers that respond to sugars but not to noncaloric sweeteners. Sugar responses of the fibers are inhibited by Phloridin, a SGLT inhibitor, or Diazoxide, a KATP channel opener. These results suggest existence of T1Rs-independent cellular and neural pathways for sugars. Furthermore, our recent single fiber response analysis also showed potential existence of sensory pathways for fatty acids via GPR120 receptors. The results of the above experiments on novel taste sensory pathways for sugars and fatty acids will be described. (COI: No)

International Scientific Program Committee Symposium

Symposium74

The consequences of preterm birth, intrauterine growth restriction and hypoxia-ischemia (PSNZ, New Zealand)

(March 31, Sun., 10:30-12:30, Room D)

S74-1

Therapeutic potential of recombinant human erythropoietin for hypoxic-ischaemic encephalopathy

Simerdeep Kaur Dhillon; Guido Wassink; Christopher A Lear; Joanne O Davidson; Alistair J Gunn; Laura Bennet (*The University of Auckland, New Zealand*)

Hypoxic-ischaemic encephalopathy (HIE) occurs in 1-4/1000 babies born at term and in upwards of 120/1000 babies born very preterm, and is associated with mortality and neurodevelopmental disability. Brain injury evolves over time post-HI insults, providing window of opportunity for treatment. Therapeutic hypothermia has become standard-care for term infants with HIE, however, it is only partially protective (number needed to treat is ~8) and currently, there is no treatment available for preterm infants. Thus, the focus of current research is to find neurotherapeutics for preterm infants and to explore additive treatments to further improve outcomes of term infants treated with therapeutic hypothermia. Human recombinant erythropoietin (rEpo) is currently considered one of the most promising neuroprotection treatments. Neonatal rat studies have shown that rEpo treatment after HI attenuates acute cell death, aids in the long term neurorepair and improves functional outcomes. rEpo is now being tested as combination treatment with hypothermia in term infants with HIE, and as a prophylactic treatment in preterm infants to improve neurodevelopment. This talk will discuss preclinical and clinical evidence for neuroprotection with rEpo, its optimal dose and treatment regimen, and recent data from fetal sheep studies using a translatable model of brain injury to examine neuroprotection and potential systemic side effects with different treatment regimens of rEpo. (COI: Properly Declared)

S74-2

A vessel's a vessel, no matter how small: microvascular tone regulation in the preterm neonate

Rebecca Maree Dyson[1,2]; Ian MR Wright[3]; Max J Berry[1,2]

([1]*Department of Paediatrics & Child Health, University of Otago Wellington, New Zealand;* [2]*Centre for Translational Physiology, University of Otago Wellington, New Zealand;* [3]*Illawarra Health and Medical Research Institute, University of Wollongong, Australia*)

Cardiovascular compromise is associated with poor outcome in the preterm neonate, with gestational age and male sex as independent risk factors for hypotension, developmental injury and death. Recent work has highlighted the microvasculature as important in the development of cardiovascular compromise in the preterm. We aimed to further characterise microvascular changes that occur in the preterm newborn, identify potential windows for therapeutic intervention, explore the mechanisms underlying this dysfunction, and understand the contribution of this early dysregulation to whole-of-life cardiovascular risk following preterm birth.

We propose a hypothetical model of gasotransmitter-dependent vasodilatation in the preterm newborn, including a role for oxidative stress in driving dysregulation. Our findings suggest varying roles for and interactions of the three gasotransmitters – allowing for regional and temporal control of blood flow: in male preterm neonates increases in NO are associated with increases in H_2S and microvascular blood flow. Studies in ex-preterm humans and animals suggest ongoing microvascular dysfunction in the ex-preterm, and that this may be related to structural differences which persist beyond the immediate postnatal period. (COI: No)

S74-3

High prevalence, low severity problems with neurodevelopment after common complications of pregnancy

Julia B Pitcher; Jago M Van Dam (*Robinson Research Institute, Adelaide Medical School, University of Australia, Australia*)

Conditions in the mother that increase systemic inflammation during pregnancy are increasingly being implicated with poorer neurodevelopmental outcomes (motor, cognitive, psycho-behavioural) for the offspring. These maternal pro-inflammatory conditions include diabetes (gestational and pre-existing), maternal overweight/obesity, and infection (e.g. chorioamnionitis, funisitis). However, the mechanisms by which inflammation during pregnancy alter brain development in the fetus, neonate and longer term, are still poorly understood. This currently limits our ability to identify and appropriately treat mothers and infants/children at greatest risk. While this exposure of the fetus to inflammation may largely not result in frank disability postnatally, the sheer burden of disease (in western countries, up to 50% of women are overweight or obese during pregnancy) suggests it presents a significant public health issue. We have been using non-invasive brain stimulation techniques to characterize neuroplasticity and cortical development in human adolescents either born preterm or exposed to gestational diabetes in utero. Our findings suggest inflammation might be the common factor underlying a subtle yet significant impact on neurodevelopmental outcomes, since the outcomes are similar regardless of the actual pregnancy condition (prematurity or maternal diabetes). (COI: No)

S74-4

Longer half-life phosphodiesterase 5 inhibitor, tadalafil therapy for fetal growth restriction

Tomoaki Ikeda (*Department of Obstetrics and Gynecology, Mie University Graduate School of Medicine, Japan*)

Fetal growth restriction (FGR) is a common complication of pregnancy that is associated with a variety of adverse perinatal outcomes There is also no proven fetal therapy for FGR. We investigated tadalafil, longer half-life, high sensitivity PDE5 inhibitor. [Clinical randomized multicenter study] Cases were randomized into receive either the conventional FGR management or the treatment with tadalafil (20mg/day) until delivery. FGR defined as below -1.5 SD of Japanese standard in 20 to 33 weeks of gestation. 45 cases randomly assigned to tadalafil treatment group, and 44 cases assigned to conventional treatment group. Fetal growth velocity in 2 weeks after tadalafil treatment significantly more increased compared control (17.9 g/day, IQR 13.6-23.4 vs 15.6 g/day, IQR 9.2-21.2; p=0.04). There were significant differences in prolongation of GA, limiting to cases that were registered at less than 32 weeks of GA (52.4± 28.9day vs 36.8± 26.8day, p=0.03) There were 7 cases (16%) of death in conventional treatment group, while only one death (2%) occurred in tadalafil group: stillbirth/neonatal death/infant death 4/1/2 vs. 0/1/0 (p=0.03). [animal study] C57BL/6 mice were subjected to L-NAME from 11 days postcoitum (pdc) to produce FGR model. Tadalafil, administered from 13 dpc dilates the placental maternal blood sinuses, which leads to increase placental growth factor production, and contributes to facilitate fetal growth and improve maternal systolic blood pressure. (COI: Properly Declared)

Symposium75

Ca2+-permeable channels of excitable and non-excitable cells in health and disease

(March 31, Sun., 10:30-12:30, Room E)

S75-1

Ca-secretion coupling at mammalian CNS synapses

Takeshi Sakaba (*Graduate School of Brain Science, Doshisha University, Japan*)

Ca is known to regulate transmitter release via triggering fusion of synaptic vesicles to the plasma membrane. We have used the calyx of Held synapse in the auditory brainstem and hippocampal mossy fibers synapses for studying Ca-dependence of transmitter release. By using electrophysiology, we have shown that probability of transmitter release is not uniform at the calyx of Held. Release time course is much slower at mossy fibers, consistent with low release probability. By using total internal reflection microscopy, we have monitored dynamics of synaptic vesicles. Vesicle dynamics are very similar between these two synapses except for release kinetics, suggesting that coupling between Ca channels and vesicles are the main determinant of the difference between two synapses. Moreover, cAMP, an important regulator of synaptic plasticity, increases release probability at two synapses. At the calyx synapse, release probability is increased by changing intrinsic Ca sensitivity of vesicle fusion. In contrast, at the mossy fiber synapse, it is increased by changing coupling between Ca channels and synaptic vesicles. Therefore, basic release mechanisms may be canonical, but may be adapted to particular synapses to some extent. Supported by JSPS core-to-core program A. Advanced Research Networks. (COI: No)

S75-2

CELF1 mediates connexin 43 mRNA degradation in dilated cardiomyopathy

Guey-Shin Wang[1]; Kuei-Ting Chang[1]; Ching-Feng Cheng[2,3]; Pei-Chih King[1] ([1]*Institute of Biomedical Sciences, Academia Sinica, Taiwan;* [2]*Department of Medical Research, Tzu Chi General Hospital, Taiwan;* [3]*Department of Pediatrics, Tzu Chi University, Taiwan*)

Cx43 (connexin 43) is the major cardiac gap junction protein that modulates electric coupling, and its reduction is often associated with dilated cardiomyopathy (DCM), the most common cause of heart failure. Under pathological conditions, reduced Cx43 expression is a hallmark of the transition from adaptive or compensatory stage to decompensation and heart failure. The causal mechanism is largely unknown. CELF1 (CUGBP, Elav-like family member 1) is a multifunctional RNA binding protein regulating pre-mRNA alternative splicing, translation and mRNA degradation. Elevation of CELF1 has been implicated in cardiac pathogenesis of myotonic dystrophy type 1 (DM1), a CTG expansion disorder. However, how elevated CELF1 leads to cardiac dysfunction remains unclear. We showed that CELF1 mediated Cx43 mRNA degradation by binding to Cx43 and interacting with an exoribonuclease RRP6 in an RNA-independent and nucleus-specific manner. Increased CELF1 expression accompanied with RRP6 upregulation and downregulation of CELF1-mediated targets including Cx43 was detected in 3 mouse models of DCM: DM1, CELF1 overexpression, and myocardial infarction. Importantly, using a heart-specific Celf1-knockout mouse model, we showed that Celf1 depletion in infarcted heart ameliorated the contractility dysfunction and preserved Cx43 mRNA level. The results suggest a mechanism for increased CELF1 expression downregulating Cx43 mRNA leveland a pathogenic role for increased CELF1 expression in the DCM heart. (COI: No)

S75-3

Fine tuning of neuronal $Ca_V1.3$ channels functions by alternative splicing and A-to RNA editing

Hua Huang; Tuck Wah Soong (*Department of Physiology, National University of Singapore, Singapore*)

Post-transcriptional modification mechanisms such as alternative splicing and RNA editing diversify the mRNA repertoire of neuronal voltage gated calcium channels $Ca_V1.3$. Extensive alternative splicing particularly in the C-terminus yields long form ($Ca_V1.3_{42}$) and short form ($Ca_V1.3_{42a}$) with drastically different biophysical and pharmacological properties. Alternative usage of splice site within exon 43 on the other hand modulate channel expression via generation of splice variants with premature stop codon that could be degraded via mRNA surveillance mechanism such as non-sense mediated mRNA decay. More recently, we discovered that $Ca_V1.3$ mRNA are subject to neuronal-selective A-to-I RNA editing by ADAR2. Editing yields non-synonymous amino acid changes within the IQ domain of the channel, leading to attenuation of calcium dependent regulation of the channel. Further study revealed that editing of $Ca_V1.3$ mRNA relies on a 40 bp RNA duplex formed between exon 41 and an evolutionarily conserved editing site complementary sequence (ECS) located within the preceding intron. Screening of potential repressors of ADAR2 pinpoints serine/arginine-rich splicing factor 9 (SRSF9) as a potent inhibitor of $Ca_V1.3$ A-to-I editing. Mechanistically, the inhibitory effect of SRSF9 required direct binding of pre-mRNA transcribed from intron 40. Selective down-regulation of SRSF9 in neurons provides a basis for the neuron-specific editing of $Ca_V1.3$ transcripts. (COI: No)

S75-4

Glomerular disease-associated mutations impair Ca^{2+}-dependent inactivation of TRPC6 channels

Masayuki X Mori[1]; Onur K Polat[1]; Yasuo Mori[1]; Masatoshi Uno[2]; Hidehito Tochio[2] ([1]*Department of Synthetic Chemistry and Biological Chemistry, Kyoto University, Japan;* [2]*Department of Biophysics, Kyoto University, Japan*)

Receptor-activated TRPC6 channels are suppressed by intracellular Ca^{2+} through the negative feedback process known as Ca^{2+}-dependent inactivation (CDI), in which calmodulin (CaM) has been proposed to play a critical role. However, it is largely unknown how CaM triggers CDI in TRPC6. Here, we revealed that a single CaM molecule bridges CaM-binding domains (CBDs) of two TRPC6 subunits to induce CDI. This 1:2 stoichiometry for Ca^{2+}-dependent binding of CaM to TRPC6 CBDs requires indistinguishable contributions of N- and C-lobes, as well as the coiled-coil assembly formed between two TRPC6 subunits via the segment adjacent to the CBD. In fact, deletion of this coiled-coil segment markedly slows CDI of TRPC6 currents. Gain-of-function TRPC6 mutations associated with autosomal dominant focal segmental glomerulosclerosis (FSGS) in the coiled-coil segment severely impair CDI and induce "FSGS-like" morphological changes in podocytes. Our study provides a novel mechanistic and pathophysiological insights into the CDI of TRPC6 channels. (COI: No)

S75-5

Structural basis of regulation of the endolysosomal calcium channel TRPML3

Jian Yang[1,3]; Minghui Li[1]; Xiaoyuan Zhou[2]; Deyuan Su[1]; Huan Li[3]; Xueming Li[2] ([1]*Biological Sciences, Columbia University, USA;* [2]*School of Life Sciences, Tsinghua University, China;* [3]*Kunming Institute of Zoology, China*)

The mucolipin transient receptor potential channels (TRPML1-3) localize primarily in endosomes and lysosomes. They conduct Ca^{2+} and Na^+ currents from the lumen to the cytoplasm and play important roles in the endocytic pathway. Mutations of TRPML1 cause human diseases, and dysfunction of TRPML3 causes severe defects in mice. The activities of TRPML channels are regulated endolysosomal Na^+, Ca^{2+}, pH and PIP_2. To better understand the molecular mechanisms of TRPML channel function and regulation, we determined the cryo-EM structures of full length human TRPML3 in the apo, agonist-bound, and low-pH-inhibited states. The agonist ML-SA1 binds between S5 and S6 and opens an S6 gate. Low pH induces large conformational changes in many regions. A polycystin-mucolipin domain (PMD) forms a luminal cap atop the transmembrane domain. S1 extends into PMD and connects directly to a luminal pore-loop, which undergoes dramatic conformational changes in response to low luminal pH. S2 extends intracellularly and interacts with several intracellular regions. These unique structural features, combined with electrophysiological studies, reveal a new allosteric mechanism thereby luminal Na^+, pH and PIP_2 regulate TRPML3 by changing S1 and S2 conformations. Our structures reveal unique and interesting structural designs and provide blueprints for understanding and exploring TRPML channel function, regulation, pathogenesis and therapeutic strategies. (COI: No)

International Scientific Program Committee Symposium

Symposium76

Physiome for organ function (KPS, Korea)

(March 31, Sun., 10:30-12:30, Room F)

S76-1

Image-based modeling of flow and transport processes at organ level

Vartan Kurtcuoglu (*Institute of Physiology, University of Zurich, Switzerland*)

Advances in imaging modalities such as magnetic resonance imaging (MRI), computed tomography and multi-photon excitation (MPE) microscopy enable the probing of flow and transport processes in vivo. However, each modality has shortcomings that need to be overcome by either supplementation of the acquired images with further data, or by augmentation of the images by computational modeling. MPE, for example, has limited penetration depth but high resolution, while MRI has lower resolution, but allows for full organ scale imaging. In this talk, I will give two examples of image-based modeling of flow and transport processes.

In the kidneys, the spatial distribution of oxygen partial pressure (pO_2) is of relevance for the control of erythropoietin (EPO) production. It is hypothesized that in chronic kidney disease, changes in renal pO_2 lead to a reduction in EPO, thereby causing anemia. To test this hypothesis, we have produced an organ-scale computational model of oxygen transport in the kidneys based on μCT data.

In the brain, metabolites are removed in part across the blood brain barrier, and in part they are cleared along with cerebrospinal fluid (CSF). To assess the relative importance of the latter, we have generated a set of computational models based on MRI and MPE data, showing that CSF bulk flow may be less important for metabolite clearance than assumed so far. (COI: No)

S76-2

In silico screening system for drug-induced arrhythmogenic risk

Seiryo Sugiura[1]; Jun-Ichi Okada[1]; Takashi Yoshinaga[2]; Junko Kurokawa[3]; Takumi Washio[1]; Tetushi Furukawa[4]; Kohei Sawada[2]; Toshiaki Hisada[1] (*[1]UT-Heart Inc., Japan; [2]Eisai Co., Ltd., Japan; [3]University of Shizuoka, Japan; [4]Tokyo Medical and Dental University, Japan*)

Increasing time and cost required for drug discovery is now becoming even a threat to the public heath by reducing the productivity of the innovative new drug development. In particular, because a significant proportion of failure in drug R&D is caused by cardiotoxicity, a new paradigm in cardiotoxicity testing is eagerly needed. We have developed a novel cardiotoxicity testing system combining a patch clamp and the multi-scale heart simulator "UT-Heart", in which human 12-lead ECG is reproduced based on the molecular model of human electrophysiology. Using this system we succeeded in predicting the arrhythmogenic risk of 12 test drugs (Okada et al. Sci Adv 2015), but the requirement of high-performance computer hampered its widespread use. To circumvent the use of expensive computer system, we then developed an open access ECG database which covers a wide range of drug effects consisting of nearly 10,000 patterns of multi-ion current effects (Okada et al. Brit J Pharmacol 2018). In the presentation, we will show the framework of the screening system and the use of database. (COI: Properly Declared)

S76-3

Model based interpretation of diabetes and prediabetes

Chaehun Leem; Young Boum Lee; Jeong Hoon Lee; Ki Hwan Hong; Pham Duc Duong (*Department of Physiology University of Ulsan College of Medicine/Asan Medical Center, Korea*)

Oral glucose tolerance tests (OGTTs) were commonly used to diagnose diabetes mellitus (DM). The changes on blood glucose (Glu) and insulin (Ins) by OGTTs contain information of the intestinal absorption, hepatic control of Glu and Ins, pancreatic Ins secretion and peripheral tissue Glu and Ins control. We developed an OGTT model with Ins dynamics and Glu dynamics. Simplex and Levenberg-Marquardt algorithms were then used to fit the data obtained from 44 normal subjects (24 males and 20 females), 8 Diabetic subjects, and 28 PreDiabetes subjects. All subjects agreed to participate with a written consent. We found clear gender differences in the intestinal Glu absorption kinetics, Glu sensitivity in the pancreas, maximal Ins production capacity and endogenous Glu production. The differences between normal and DM subjects in Glu and Ins, such as Ins resistance, Ins secretion and the relative roles of Glu disposal in each organ, were demonstrated clearly and quantitatively in a time-dependent manner. This study revealed the quantitative dynamic interaction between Glu and Ins using OGTT data and revealed organ function during the OGTT. Using this approach, we could identify the dysfunctional organs for Glu and Ins regulation. Data produced using this model will allow a personalized and targeted approach for health issues related to Glu and Ins. (Supported by the grant No. NRF-2015M3A9B6028310, NRF-2014M3A9D7034366 & 10068076 from MSIT) (COI: No)

S76-4

A virtual stenosis method to predict plaque progression in coronary arteries

Eun Bo Shim[1]; Kyung Eun Lee[1]; Eun Seok Shin[2] (*[1]Department of Mechanical and Biomedical Engineering, Kangwon National University, Korea; [2]Department of Cardiology, School of Medicine, University of Ulsan, Korea*)

Predicting the sites in coronary arteries that are susceptible to plaque deposition is essential for the development of clinical treatment strategies and prevention. We hypothesized that the possibility of plaque deposition at a specific site in the coronary artery is associated with wall shear stress (WSS) and fractional flow reserve (FFR). Here, we proposed a new biomarker called the stenosis susceptibility index (SSI) using the FFR and WSS derived using virtual stenosis method. To validate the clinical efficacy of this index, we applied the method to actual pilot clinical cases. Using virtual stenosis method, we computed maximum WSS and FFR according to the variation in stenotic severity at each potential stenotic site and then determined the most susceptible sites for plaque deposition by comparing SSI values between the potential sites. In conclusion, sites susceptible to plaque deposition can be identified using the SSI index. (COI: No)

International Scientific Program Committee Symposium

Symposium77

Advances in the role of adipocyte in health and disease (CPS, Taiwan)

(March 31, Sun., 10:30-12:30, Room G)

S77-1

Physiological Role and Therapeutic Potential of Thermogenic Fat

Yu-Hua Tseng (*Joslin Diabetes Center, Harvard Medical School, USA*)

Obesity is a major risk factor to develop many of the most common medical conditions, such as type 2 diabetes, cardiovascular disease, and even some cancers. Increased fat mass is the main characteristic of obesity. However, not all fat tissues are created for energy storage. Brown fat and brown-like beige fat are specialized for thermogenic energy expenditure. Owing to the immense capacity of brown/beige fat to combust fuels for heat production and the presence of thermogenic fat in adult humans, increasing the amount or activity of brown or beige fat has been considered as an appealing approach for the treatment or prevention of obesity and related metabolic disorders. Recently, emerging evidence demonstrates that brown/beige fat also serves as an endocrine organ, which secretes a number of molecules to regulate whole-body metabolism. Using a highly sensitive liquid chromatography coupled with mass spectrometry analysis, we have identified novel lipid mediators derived from brown or beige adipose tissue, whose concentrations are increased in the circulation of cold challenged humans and mice. These signaling lipid species act as an indicator and stimulator of brown fat activity and negatively correlates with body mass index and insulin sensitivity. Mechanistic studies demonstrate that the cold-induced lipids can regulate glucose and fatty acid metabolism. These findings suggest novel therapeutic pathways for the treatment of metabolic disorders by mimicking cold. (COI: No)

S77-2

Adipose tissue stiffness in the development of metabolic diseases

Yau-Sheng Tsai[1]; Ann Huang[2]; Yi-Shiuan Lin[2]; Yu-Wei Chiou[2]; Hsi-Hui Lin[2]; Ming-Jer Tang[2] (*[1]Institute of Clinical Medicine, National Cheng Kung University, Taiwan; [2]Department of Physiology, National Cheng Kung University, Taiwan*)

Obesity, defined as an expansion of white adipose tissue, is also accompanied by interstitial fibrosis and overexpression of extracellular matrix (ECM) in adipose tissue in recent studies. We hypothesize that obesity induces adipose tissue ECM crosslinking and stiffening, leading to adipocyte dysfunction. First, we applied a collagen gel system to investigate the effect of ECM stiffness on adipogenesis and mature adipocyte functions. Our results showed that increased ECM stiffness impaired adipogenesis; and blunted the sensitivity to insulin and lipolytic cues, as well as secretion of adipokines, in differentiated adipocytes. In contrast, increased ECM stiffness exacerbated the sensitivity to inflammatory stimulation. Next, we found that *ob/ob* adipose tissue exhibited an increased signal in the polarized view of picrosirius red stained section, as well as increased crosslinks and aggregated collagen in second harmonics generation (SHG) imaging. These results suggest that ECM crosslinking was upregulated in the adipose tissue of obese mice. *Ex vivo* crosslinking inhibition in *ob/ob* adipose tissue explants increased insulin sensitivity, adiponectin expression and lipolytic activity. Finally, *in vivo* crosslinking inhibition reversed metabolic impairments and adipocyte functional signature genes in *ob/ob* mice. Taken together, our study underscores that adipose tissue crosslinking and stiffening may orchestrate metabolic disorders resulting from adipocyte dysfunction in obesity. (COI: No)

S77-3

Modulation of adipokine biosynthesis and secretion in adipocytes

Juu-Chin Lu[1,2]; Yu-Ting Chiang[1]; Chia-Yun Lu[1]; Ying-Yu Wu[1] (*[1]Department of Physiology and Pharmacology, Chang Gung University, Taiwan; [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospital, Taiwan*)

The adipose tissue is recognized as an endocrine organ due to its active secretion of many peptide hormones and cytokines, collectively named adipokines, which regulate many important physiological functions. Among these adipokines, adiponectin and leptin are primarily secreted by adipocytes to regulate insulin sensitivity and energy balance, whereas monocyte chemoattractant protein-1, a chemokine that is associated with obesity-linked chronic diseases, is also secreted by immune cells. Although dysregulation of the adipokine production is known to associate with many metabolic diseases, the underlying mechanisms remain incompletely understood. We used 3T3-L1 adipocytes to elucidate the molecular mechanisms by which adipokine biosynthesis and secretion are modulated. We found that different secretory mechanisms might mediate the secretion of individual adipokines. Next, we examined if cholesterol modulated adipokine biosynthesis and secretion, given that the adipocytes store a significant amount of non-esterified cholesterol, and obese adipocytes were characterized by a redistribution of cholesterol with depleted cholesterol in the plasma membrane. We found that modulation of cholesterol altered signaling events, lipid raft integrity, and lipid metabolite profiles in adipocytes, which might account for the dysregulation of adipokine biosynthesis and secretion. Finally, the involvementof a protein kinase in regulating adipokine biosynthesis and secretion will be discussed. (COI: No)

S77-4

Novel structures and functions of adiponectin receptors

Toshimasa Yamauchi (*Department of Diabetes and Metabolic Diseases, The University of Tokyo, Japan*)

Adiponectin (Ad) is an antidiabetic adipokine, which binds to its receptors AdipoR1/R2, leading to activation of AMPK/SIRT/PGC-1 and PPAR pathways, respectively. Recently, small-molecule AdipoR agonist was shown to ameliorate diabetes and increase exercise endurance, and at the same time prolong shortened lifespan in obesity. Although AdipoRs are predicted to contain seven-transmembrane (7TM) domains, with an internal N-terminus and an external C-terminus, which is opposite to GPCRs. In this study, we successfully determined crystal structures of human AdipoR1/R2, and found that overall structures of AdipoRs are indeed distinct from those of GPCRs. The seven-transmembrane domain of both AdipoR1 and AdipoR2 was shown to have a cavity with a zinc-binding site, which contains unidentified extra electron densities. It was thus suggested that these electron densities may represent potential substrates for AdipoR hydrolytic activity or their products. Very recently, we discovered an alternative structure of AdipoR. The intracellular part of helix V has robustly moved outwards, which has now provided a larger opening of the internal cavity to the cytoplasm, which may represent an open form of AdipoR. This study should open new avenues toward elucidation of an unprecedented paradigm of signal transduction and development and optimization of AdipoR agonists. (COI: Properly Declared)

Symposium78

"Ins" and "outs" of smooth muscle

(March 31, Sun., 10:30-12:30, Room H)

S78-1

Novel mechanism of electrical rhythmicity in smooth muscle

Nick John Spencer (*College of Medicine and Public Health, Flinders University, Australia*)

Rhythmic electrical depolarisations in smooth muscle of the gastrointestinal (GI) tract are usually thought to be generated by myogenic pacemaker cells, called Interstitial Cells of Cajal (ICC). In this study, we reveal a new mechanism in the GI-tract that is responsible for the generation of rhythmic electrical depolarisations in smooth muscle, but is generated by rhythmic firing from the Enteric Nervous System (ENS). We used high resolution neuronal imaging of the ENS with simultaneous calcium imaging from the neighbouring smooth muscle layers in isolated intact whole mouse colons. In whole colons, neurogenic contractions propagated along the whole colon every 2-4 minutes that were abolished by hexamethonium. The electrical activity in the smooth muscle underlying each contraction consisted of repetitive depolarisations at 2Hz that were abolished by hexamethonium. Imaging from the ENS revealed that each neurogenic contraction was due to the emergence of temporally-synchronized firing of large populations of neurons at 2Hz. During the intervals between contractions, the ENS exhibited desynchronized neuronal firing between ganglia. The emergence of temporally-coordinated firing of large populations of enteric neurons represents a unique neural motor pattern outside the central nervous system. This is the first evidence of rhythmic firing in the central or peripheral nervous systems that is directly responsible for the generation of electrical rhythmicity in smooth muscle. (COI: No)

S78-2

Regulation of spontaneous contractile activity of the bladder muscularis mucosa

Russ Chess-Williams; Christian Moro (*Centre for Urology Research, Bond University, Australia*)

The bladder mucosa contains a thin layer of muscularis mucosa that on a weight basis develops contractions greater than those of the detrusor smooth muscle. It also develops spontaneous contractile activity thought to be mediated via interstitial cells similar to intestinal interstitial cells of Cajal. Mucosal spontaneous contractile activity is enhanced by stretch of the tissue and this response is regulated by the adjacent urothelial layer. These epithelial cells when stretched release acetylcholine which increases mucosal contractility and this might represent an additional site of action for muscarinic antagonists used in the treatment of the overactive bladder.

A number of neurotransmitters and hormones also regulate spontaneous activity of the mucosa and several treatments targeting the detrusor muscle may also have actions on the mucosa.

The physiological functions of the mucosal contractile activity is unclear, but it may be responsible for folding of the mucosa when the bladder is empty, or it may prime afferent nerves and sensitise mechanosensory processes. Also, in the overactive bladder, where gap junctions are increased, the contractile activity may pass to the detrusor muscle thus causing bladder overactivity.

In conclusion, the bladder mucosa possesses pacemakers that generate spontaneous contractile activity. Contractions are regulated by the urothelium and a number of receptors that will be affected during treatment for urinary tract conditions. (COI: No)

S78-3

Regulation and dysregulation of airway smooth muscle contractility

Jane Elizabeth Bourke (*Biomedicine Discovery Institute, Department of Pharmacology, Monash University, Australia*)

Airway smooth muscle (ASM) plays major functional roles within the lung. Spontaneous phasic contractions of ASM *in utero* are implicated in airway development, while intrinsic airway tone and contractions of ASM throughout life in response to physiological stimuli determine airway caliber. Contraction in response to diverse neurotransmitters and circulating mediators is predominantly mediated by Ca^{2+} mobilization from intracellular ASM stores and Ca^{2+} sensitivity of the ASM contractile apparatus rather than by the influx of extracellular Ca^{2+}, and can be opposed by circulating adrenaline as well as epithelial-derived relaxing factors.

Airway contractility is dysregulated in many lung diseases, including asthma, where increased contractile responses are promoted by the combination of elevated levels of bronchoconstrictors, increased ASM in the airways and the influence of airway inflammation. While bronchodilators and anti-inflammatory agents can reduce symptoms and oppose some pathophysiological changes in asthma, their efficacy is limited in severe disease and they do not provide a cure. This presentation will describe signalling mechanisms regulating ASM contraction in health and disease and the identification of novel therapeutic approaches targeting ASM that offer promise for asthma and other chronic lung diseases. (COI: No)

S78-4

New insights into understanding labour contractions in women

Helena C. Parkington[1]; Mary A. Tonta[1]; Ranga I. Siriwardhana[1]; Penelope J. Sheehan[2]; Harold A. Coleman[1]; Shaun P. Brennecke[3] ([1]*Department of Physiology, Monash University, Australia;* [2]*The Royal Women's Hospital, Australia;* [3]*Department of Obstetrics and Gynecology, The University of Melbourne, Australia*)

Preterm labour and delivery are major causes of perinatal morbidity and mortality. On the other hand, improving contractions when they fail in labour is also restricted necessitating caesarean section, with significant short and long term maternal consequences, as well as childhood issues for the offspring. Clinically safe and effective therapeutic options are limited for preterm labour tocolysis and dysfunctional labour augmentation. We aimed to better understand the mechanisms underpinning contraction in human myometrium before and during labour.

We studied myometrium from women undergoing caesarean delivery in the Royal Women's Hospital, Melbourne. Informed, written consent was obtained prior to surgery. Membrane potential or cytoplasmic calcium were recorded simultaneously with contraction in myometrial strips before and during labour. Freshly isolated smooth muscle from the same samples was studied with patch-clamp electrophysiology and ion channel protein expression was determined using Western blot.

We found ANO1 and $K_{IR}7.1$ channels in the myometrium before and during labour. Activity of ANO1 markedly contributes to the development of the long action potential plateau and contraction amplitude. Expression increased in labour facilitating stronger contractions. In contrast, $K_{IR}7.1$ terminates the plateau resulting in a smaller contraction. $K_{IR}7.1$ expression was reduced in labour, facilitating the increase in contraction amplitude required for successful vaginal delivery. (COI: No)

S78-5

Regulatory mechanisms underlying the contractility of intra-organ microvasculature

Hikaru Hashitani; Retsu Mitsui (*Department of Cell Physiology, Nagoya City University, Japan*)

Intra-organ microvasculature plays a fundamental role in regulating microcirculation. Spontaneous constrictions in arterioles or venules that are considered to facilitate the microcirculation appear to be driven by non-contractile capillary pericytes. Capillary pericytes generate spontaneous Ca^{2+} transients relying on the sarco-endoplasmic reticulum (SR/ER) Ca^{2+} release triggering Ca^{2+}-activated chloride channels (CaCCs) dependent depolarisations. CaCC-dependent depolarisations are effective enough to achieve the synchrony of spontaneous Ca^{2+} transients amongst capillary pericytes, while the subsequent activation of L-type voltage-dependent Ca^{2+} channels is required for the synchronous Ca^{2+} transients in arteriolar or venular mural cells. K^+ channels also play a critical role in maintaining the synchrony of spontaneous Ca^{2+} transients amongst mural cells by stabilizing their membrane to hyperpolarised levels. This prevents premature SR/ER Ca^{2+} release to ensure sufficient SR/ER Ca^{2+} refilling that is required for the subsequent regenerative Ca^{2+} release. Arterioles or venules predominantly receive sympathetic vasoconstrictor innervation, but also receive a peptidergic inhibitory innervation. In contrast, capillary and pre- and post-capillary microvasculatures lack a functional innervation. Thus, the contractility of the intra-organ microvasculature is determined by the integration of spontaneous and neural regulatory mechanisms in a microvascular segment specific manner. (COI: No)

Local Organizing Committee Symposium

Symposium79

Mechanomedicine

(Co-sponsored by Grant-in-Aid for Scientific Research (S) : Mechanomedicine)

(March 31, Sun., 10:30-12:00, Room I)

S79-1

Plasma membranes can act as mechanosensors in vascular endothelial cells

Kimiko Yamamoto[1]; Joji Ando[2] (*[1]The University of Tokyo, Japan; [2]Dokkyo Medical University, Japan*)

Vascular endothelial cells (ECs) play critical roles in regulating a variety of vascular functions, including maintenance of the vascular tone, blood coagulation and fibrinolysis, and provision of selective permeability to proteins. It has recently become apparent that ECs show alterations in their morphology, functions and gene expression profile in response to exposure to hemodynamic forces, namely, shear stress and stretch. These responses also play important roles in maintaining normal circulatory system functions and homeostasis, whereas their impairment leads to various vascular diseases, including hypertension, aneurysm and atherosclerosis. Plasma membranes of the ECs have recently been shown to respond differently to shear stress and stretch, by rapidly changing their lipid order, membrane fluidity, and cholesterol content. Artificial lipid-bilayer membranes also show similar changes of the lipid order in response to exposure to shear stress and stretch, indicating that these are physical phenomena rather than biological reactions. Such physical changes then activate the membrane receptors and cell responses specific to each type of force. These findings suggest that the plasma membranes of ECs act as mechanosensors, and in response to mechanical forces, they show alterations of their physical properties, with modification of the conformation and functions of the membrane proteins, which then trigger activation of the downstream signaling pathways. (COI: No)

S79-2

Wall stretch-induced anti-contractile signaling via smooth muscle expressed eNOS in pulmonary artery

Sung Joon Kim; Hae Jin Kim (*Department of Physiology, Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Korea*)

Despite the presence of mechanosensitive nonselective cation channels (NSCms) in pulmonary arterial smooth muscle cells (PASMC), PA do not show myogenic response, and large increase of pulmonary circulation is maintained with low perfusion pressure. We hypothesized that the wall stretch might induce anti-contractile signaling intrinsic to the PASMC. The tunica media of rat PA and PASMCs showed faint but discernable expression of eNOS. Wire myography results suggested that the muscular eNOS is activated by mechanical stretch when combined with nanomolar TXA_2. As for the signaling pathway, molecular biological analyses suggested ROS/Akt-dependent eNOS Ser^{1177}phosphorylation in PASMCs. Also, the effects of pharmacological inhibitor proposed a role of NSCms in the proximal level of the signaling cascade. In addition to the stretch/TXA2 effects, we also found that the characteristic transient contraction of PA by angiotensin II (AngII) is mediated by concomitant activation of the muscular eNOS (Ser^{1177}phosphorylation) via type 1 receptor (AT1)-Akt pathway in the endothelium-denuded PA. Furthermore, the inhibition of eNOS effectively rescued the long-lasting desensitization of AT1-mediated contractile responses in the rat PA. Collectively, we proposed that the auto-inhibitory role of the muscle eNOS in PAs play physiological roles of active relaxation under various vasoconstrictive conditions, preventing excessive contractile response of the low-pressure pulmonary circulation. (COI: No)

S79-3

Analysis of nanoscale vibrations in the inner ear by advanced vibrometries

Hiroshi Hibino[1,2]; Takeru Ota[1,2]; Samuel Choi[2,3]; Fumiaki Nin[1,2] (*[1]Department of Molecular Physiology, Niigata University School of Medicine, Japan; [2]AMED-CREST, AMED, Japan; [3]Department of Electrical and Electronics Engineering, Niigata University, Japan*)

Animals minutely analyze diverse sounds in the environment to obtain information necessary for their survival. Humans, whose audition ranges from 20 to 20,000 Hz, can distinguish frequencies that are only 0.2% apart, whereas they perceive millionfold differences in sound pressure level. These marked performances stem from sound-induced nanoscale vibrations in mechanoelectrical sensory epithelium inside the inner ear. This tissue is made up of three layers; hair-cell, supporting-cell, and extracellular-matrix layers. The characteristics of the epithelial motion and the underlying mechanisms remain uncertain. Recently we have developed two different vibrometries. These advanced instruments detected a few parameters of the epithelial vibrations, which are inaccessible to conventional methods. By combining a theoretical approach, we will discuss the physiological roles of the identified motions in hearing. (COI: No)

S79-4

Mechano-property of tendon/ligament and its application to regenerative medicine

Hiroshi Asahara[1,2] (*[1]Tokyo Medical and Dental University, Japan; [2]The Scripps Research Institute, Japan*)

Despite the unique and important role of tendons/ligaments in musculoskeletal function and disease, research in this area is not as advanced as in most other skeletal tissues. This is partly because critical and specific transcription factors had not been elucidated. In this regard, we and others identified Mohawk (Mkx) as a specific and critical transcription factor for tendon and ligament development. Mkx is expressed specifically in tendon and ligament cells during embryogenesis and Mkx-deficient mice have hypoplastic tendons throughout the body and deficient type I collagen production in tendon cells. To test the physiological function of Mkx in vivo, we generated Mkx knock out mice and rats, which was also utilized to collect enough amount of tenocytes. Wild-type (WT) mice demonstrated an increase in collagen fiber diameter and density in response to physical treadmill exercise, whereas in Mkx−/− mice, tendons failed to respond to the same mechanical stimulation, suggesting that Mkx is essential in mechanoresponsive tenogenesis through regulation of its downstream ECM genes such as type I collagens and proteoglycans such as fibromodulin both in vivo and in vitro. Mkx−/− rats showed systemic tendon hypoplasia similar to Mkx−/− mice. In addition, earlier heterotopic ossification of the Achilles tendon was observed in Mkx−/− rats. ChIP-seq of Mkx overexpressing tenorcytes revealed significant peaks in tenogenic-related genes, such as collagen type (Col)1a1 and Col3a1, and chondrogenic differentiation-related genes, such as SRY-box (Sox)5, Sox6, and Sox9, suggesting that Mkx has a dual role, including accelerating tendon differentiation and preventing chondrogenic/osteogenic differentiation. Our studies on Mkx have potential to advance current concepts on tendon/ligament tissues homeostasis and to generate engineered constructs for clinical applications. (COI: No)

Symposium80

Daily /adaptable Yin-Yang transitions in diverse physiological processes coordinated by multi-cellular Chrono-molecular signal

(March 31, Sun., 10:30-12:30, Room J)

S80-1

Cellular and molecular basis of chronotherapy for cancer

Masaaki Ikeda[1]; Megumi Kumagai[1]; Yasutsuna Sasaki[4]; Yoshihiro Nakajima[3]; Ken-Ichi Fujita[2] (*[1]Department of Physiology, Faculty of Medicine, Saitama Medical University, Japan; [2]Cancer Cell Biology, School of Pharmacy, Showa University, Japan; [3]Cellular Imaging Research Group, AIST Health Research Institute, Japan; [4]Department of Oncology, School of Medicine, Showa University, Japan*)

Chronotherapy is one of the promising ways to treat several diseases. The onset of the disease is known to concentrate in a limited time zone in a day and selection of the appropriate time zone for treatment will bring about the greatest result of treatment of the disease. Recently, several molecular targeted drugs for cancer therapy have been developed and can be administered orally, so limit of the administration time is reduced. Such an administration method will help to select the time of a day of treatment. In order to clarify the molecular mechanism of chronotherapy of such an anticancer drug, we established *Bmal1* knockout cells utilized by CRISPR/Cas9 system and investigated the influence on antiproliferation rate to cells regardless of presence or absence of *Bmal1* gene of anticancer drug. The multi-kinase inhibitors regorafenib and sorafenib significantly inhibited the proliferation of Bmal1 knockout cells. Synchronized cells to the rhythm of 24 hours by dexamethasone are more sensitive to gefitinib than cells not synchronized. These findings suggest that the sensitivity of cells to molecular targeted anticancer drugs is regulated by the circadian system. (COI: No)

S80-2

Initial protein events synchronizing cellular clocks to elicit environmental stress adaptation

Teruya Tamaru[1]; Genki Kawamura[2]; Hikari Yoshitane[3]; Yoshitaka Fukada[3]; Takeaki Ozawa[2]; Ken Takamatsu[1] (*[1]Department of Physiology, Toho University School of Medicine, Japan; [2]Department of Chemistry, School of Science, The University of Tokyo, Japan; [3]Department of Biological Sciences, School of Science, The University of Tokyo, Japan*)

Environmental stresses by global changes in the environments and disorder of human life style disturb temporally harmonized physiologies of the entire body, thereby cause various health problems and diseases, such as cancer and metabolic diseases. Impairment of stress adaptation / protection through cell-autonomous molecular circadian clocks (cellular clocks), with clock genes (*Bmal1, Clock, Cry1/2, Per1/2*), might be involved in the process. As the evidence, we previously demonstrated that coordinated interplay among several adaptive protection systems are triggered by stress-responsive cellular clock synchronization, to elicit adaptation against environmental stresses, including heat, reactive oxygen species and UV, potential pathogenic factors. Impaired response of this "circadian adaptation system" (CAS)eliminates the adaptive protection. To seek the common initial protein events against environmental cues/stresses to synchronize multi-cellular clocks to provoke adaptive responses, we focus on: 1) Interplays between clock and adaptive protection pathways through hierarchical interaction among BMAL1, HSF and p53 to coordinate heat-shock response and anti-tumor pathways for evoking protection systems. 2) Our newly identified structural basis in BMAL1 protein, indispensable for the synchronous cellular clock oscillation. 3) Multi-cellular nuclear BMAL1 synchronization immediately after clock-resetting treatment, maybe prerequisite for synchronous *Per1/2* induction. (COI: No)

S80-3

Dysregulation of Hepatic SREBP1c-CRY1 Axis Promotes Hyperglycemia in Obese Animals

Jae Bum Kim; Ye Young Kim; Hagoon Jang; Yong Keun Jeon (*Center for Adipose Tissue Remodeling, Institute of Molecular Biology and Genetics, School of Biological Sciences, Seoul National University, Korea*)

Glucose and lipid metabolisms are tightly regulated by hormones and circadian rhythm to main whole body energy homeostasis. SREBP1c is a key lipogenic transcription factor activated by insulin. Although SREBP1c appears to be involved in suppression of hepatic gluconeogenesis during postprandial periods, the molecular mechanism is not thoroughly understood. We have shown that hepatic CRY1 expression is increased by insulin-induced SREBP1c at specific circadian time points and CRY1 decreases hepatic gluconeogenesis through FOXO1 degradation. Both *SREBP1c*$^{-/-}$ and *CRY1*$^{-/-}$ mice exhibit higher blood glucose than wild type (WT) mice during pyruvate tolerance test, accompanied with enhanced expression of *PEPCK* and *G6Pase* genes. CRY1 promotes degradation of nuclear FOXO1 by augmenting its binding to the ubiquitin E3 ligase MDM2. Although SREBP1c fails to upregulate CRY1 expression in obese and diabetic *db/db* mice, overexpression of CRY1 attenuates hyperglycemia through reduction of hepatic FOXO1 protein and gluconeogenic gene expression. In this presentation, I will discuss our new data that reveals the molecular mechanisms of the downregulation of hepatic CRY1 proteins in obese and diabetic animal. Collectively, we have demonstrated that insulin-activated SREBP1c alleviates gluconeogenesis through CRY1-mediated FOXO1 degradation and that dysregulation of hepatic SREBP1c-CRY1 signaling could stimulate hyperglycemia in obese and diabetic animals. (COI: No)

S80-4

Mechanism of circadian regulation of memory in mice

Kimiko Shimizu; Erika Nakatsuji; Yodai Kobayashi; Yoshitaka Fukada (*Department of Biological Sciences, The University of Tokyo, Japan*)

Evidence from previous studies demonstrates that circadian rhythms affect memory formation. However, it is not yet clear if the internal clock regulates the efficiency of the memory formation, and there is no molecular-based evidence that connects the memory formation with circadian rhythms.

We performed novel object recognition task on mice over the circadian time. Long-term memory performance varied in a circadian manner and hence it appears to be controlled by the endogenous circadian clock. In fact, electrolytic lesion of the suprachiasmatic nucleus in the brain, a master circadian clock in mammals, disrupted the circadian rhythm of long-term memory formation. Forebrain-specific clock disruption in genetically engineered mice also abrogated the circadian oscillation of long-term memory formation. We focused on SCOP, a key molecule regulating hippocampus-dependent long-term memory for objects. The amounts of SCOP and its binding partner K-Ras in the hippocampal membrane rafts exhibit robust circadian changes, and SCOP knockdown in the hippocampal CA1 impairs long-term memory at night. Circadian changes in stimulus-dependent activation of ERK in the hippocampal neurons are dependent on the SCOP levels in the membrane rafts, while *Scop* knockout abrogates the ERK activation rhythm. We conclude that long-term memory formation is regulated by the circadian clock through SCOP dynamics in the membrane rafts of the hippocampal CA1. (COI: No)

S80-5

Good times, bad times …. Impact of the circadian clock on health and disease

Gijsbertus Van Der Horst (*Dept. of Molecular Genetics, Erasmus University Medical Center, The Netherlands*)

Like most organisms, we have developed an internal time keeping system that drives daily rhythms in metabolism, physiology and behavior, and allows us to optimally anticipate to the momentum of the day. At the basis of circadian timekeeping lies an intracellular molecular oscillator in which a set of clock genes cyclically regulate their own expression with an approximate (circa) 24-hour (dies) periodicity. As the circadian clock drives rhythmic expression of up to 10 % of the active genes (thereby conferring rhythmicity to a wide range of cellular processes such as, but certainly not limited to, energy metabolism, metabolic activation of drugs, detoxification, DNA repair and cell cycle control), it may not come as a surprise that disruption of the circadian system is associated with disease. Indeed, genetic disruption of the circadian system in rodent models by inactivation of clock genes has been found to increase tumor growth, accelerate aging, and disrupt metabolism. On the other hand, our 24/7 economy requires many people to work at "non-standard" times. Recently, epidemiological and experimental animal studies have revealed a relation between disturbance of our body clock by repeated shift-work and an increased risk for developing pathologies such as cancer, metabolic syndrome and cardiovascular disease. This presentation will address the biological/medical importance of the circadian clock, with special emphasis on the etiology, treatment and prevention of cancer. (COI: No)

Symposium81

Mechanisms of systemic beauty and health

(March 31, Sun., 10:30-12:30, Room K)

S81-1

How to use the natural products?: Inhibition of UV-induced melanogenesis by targeting ion channels

Joo Hyun Nam[1,2] (*[1]Department of Physiology, Dongguk University College of Medicine, Korea; [2]Channelopathy Research Center, Dongguk University College of Medicine, Korea*)

Ultraviolet (UV) radiation deeply penetrates the skin and causes inflammation and pigmentary changes. A recent study reported that the photopigment rhodopsin is expressed in human epidermal melanocytes (HEMs), which are involved in UV phototransduction. UV exposure rapidly induces Ca^{2+} mobilization via the G_q protein-coupled signaling pathway and generates early Ca^{2+}-dependent melanin synthesis. Therefore, controlling intracellular calcium concentration is an important strategy for preventing UV-induced skin pigmentation. Among the various calcium channels, the calcium release-activated calcium channel protein 1 (ORAI1) plays an important physiological role in UV-induced melanogenesis. ORAI1 channel-mediated increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) is an important step in melanogenesis, as it controls the active transport of L-phenylalanine and its turnover to L-tyrosine via calmodulin-dependent Ca^{2+}-ATPase. Therefore, the discovery that the inhibition compounds of UV-induced melanogenesis related ion channels, such as ORAI1, might prevent melanogenesis. Recently, increasing interest in various medicinal plants and their bioactive ingredients has led to increase attention to their safety and efficacy in the treatment of various skin diseases. In this presentation, I will discuss about our recent progress to find ion channel-modulating agents from natural sources and it's effect on UV-induced melanogenesis. (COI: No)

S81-2

PKCβII facilitates desmoglein internalization in *Rpgrip1l* mutant mice and pemphigus

Yeun Ja Choi[1]; Li Li[2]; Ning Yang[3]; Xuming Mao[4]; Kenneth R Shroyer[3]; Peter J Koch[5]; Yusuf A Hannun[6]; Richard A Clark[7]; Jiang Chen[3,7]

(*[1]Department of Biopharmaceutical Engineering, Dongguk University, Korea; [2]Department of Dermatology, Peking Union Medical College Hospital, China; [3]Department of Pathology, Stony Brook University, USA; [4]Department of Dermatology, University of Pennsylvania, USA; [5]Department of Dermatology and Center for Regenerative Medicine and Stem Cell Biology, University of Colorado USA; [6]Department of Medicine, Stony Brook University, USA; [7]Department of Dermatology, Stony Brook University, USA*)

Cilia-related proteins play important functions in skin by regulating primary cilia formation or function. Interestingly, many ciliary proteins are expressed in keratinocytes that are not ciliated, indicating cilia-independent functions in skin. Here we report that RPGRIP1L, a protein required for primary cilia formation during hair follicle morphogenesis, plays an unsuspected role in desmosomal junction formation. Disrupting the *RPGRIP1L* gene impaired desmosome morphology and function *in vivo* and *in vitro*, and resulted in intraepidermal blistering in mice. Moreover, *RPGRIP1L*-deficient cells exhibited diminished and mislocalized desmosomal proteins, which were associated with aberrant internalization of desmogleins. Desmosome phenotypes in *RPGRIP1L* mutants were consistently associated with upregulated protein kinase C beta II (PKCβII), the blocking of which could partially rescue these abnormalities. Because the intraepidermal blistering phenotypes of *Rpgrip1l* mutant mice resembled epidermal acantholysis in pemphigus, we examined pemphigus patient specimens and found that PKCβII is aberrantly upregulated. Blocking PKCβII with a small molecule inhibitor was able to rescue desmosomal phenotypes in keratinocytes induced by serum of pemphigus patients. Thus, data obtained from this study not only linked functions of a ciliary protein with keratinocyte adhesion, but also revealed the therapeutic potential of inhibiting PKCβII in ameliorating pemphigus. (COI: Properly Declared)

S81-3

Chiral amino acid analysis using 2D/3D-HPLC for the screening of functional molecules and biomarkers

Kenji Hamase (*Graduate School of Pharmaceutical Sciences, Kyushu University, Japan*)

Amino acids normally have L- and D-enantiomers due to the chiral alpha carbon, and the L-forms are predominant in living beings. The minor D-forms have long been believed non-natural or physiologically not significant especially in higher animals. However, along with the progress of analytical techniques, various D-forms were found in mammals including humans. The relationships between these D-amino acids and several diseases have also been clarified, and the D-enantiomers are now gathering attention as new bio-functional molecules and/or biomarkers. However, the amounts of these D-amino acids are trace, and highly sensitive/selective analytical methods are essential. In the present study, 2D/3D-HPLC methods have been designed and applied to the food and clinical analyses. As the 3D-HPLC approach, reversed-phase, anion-exchange and chiral separations are online connected to get extremely high selectivity, and the trace levels of D-amino acids could be determined without visible interference. Using the method, D-Asp, Ala and Glu are determined in fermented materials, and utilized to design functional products including beauty foods/beverages and cosmetics. As the clinical applications, the amounts of D-Ser, Asn, Ala and Pro are high in the plasma of patients with chronic kidney diseases, and these D-forms have significant diagnostic values. The multidimensional HPLC methods are useful for chiral amino acid metabolomics studies, and further investigations are ongoing. (COI: Properly Declared)

S81-4

Transport system of amino acids

Shushi Nagamori (*Nara Medical University, Japan*)

Amino acids are known as building blocks of proteins and substrates of metabolic and biosynthetic reactions. Furthermore, amino acids are also recognized as the signaling molecules to regulate cellular metabolism and cell growth. Include 20 proteinogenic amino acids, there are many amino acids in living organisms. To maintain homeostasis and signaling of amino acids, all known amino acid transporters exists over 50 molecules in human genome. These transporters act their own roles in each cell type of each organ or tissue. Generally, normal cells control nutrient uptake and metabolic activities to prevent aberrant cell proliferation. On the other hand, in cancer cells, nutrient transporters are constitutively activated to facilitate the nutrient uptake for robust cell growth. Several studies have reported the overexpression of some amino acid transporters in cancer. We have found that most of cultured cell lines has similar profiles of amino acids transporters, while each organ shows specific expression profiles of the transporters. Therefore, to understand systemic amino acid homeostasis and signaling, we are elucidating amino acid transport systems, i.e. profiles of amino acid transporters, in each organ and tissue. Although we are on the way to illustrate the whole picture of systemic amino acid homeostasis, the latest developments of technologies, such as proteomics or tissue engineering, are leading us to the goal. (COI: Properly Declared)

S81-5

Importance of receptor-activated Ca^{2+} influx in wound healing

Takuro Numaga-Tomita[1,2,3]; James W Putney, Jr[5]; Motohiro Nishida[1,2,3,4] (*[1]Department of Creative Research, Exploratory Research Center on Life and Living Systems: ExCELLS, National Institutes of Natural Sciences, Japan; [2]National Institute for Physiological Sciences (NIPS), National Institutes of Natural Sciences, Japan; [3]School of Life Sciences, SOKENDAI, Japan; [4]Graduate School of Pharmaceutical Sciences, Kyushu University, Japan; [5]National Institute of Environmental Health Sciences, National Institutes of Health, USA*)

Wound healing is an important but complicated process for our body to recover from injury. Calcium (Ca^{2+}) signaling plays a major role in the development and maintenance of the epidermis. We have revealed that Ca^{2+} influx initiated by cell surface receptor activation play critical roles in a plethora of cellular physiology. In epidermis, store-operated Ca^{2+} entry (SOCE), a major Ca^{2+} influx pathway in non-excitable cells, regulates both proliferation and differentiation. Those cellular responses are precisely controlled in epidermal wound healing. Aberration of the control resulted in ulcer and keloids. Based on the in vitro study of SOCE in keratinocyte physiology, we investigated the effect of SOCE deficiency on skin wound healing. To our surprise, defect of SOCE facilitates healing of epidermis. This effect was likely to be attributable to the reduced expression of proinflammatory cytokines in epidermis by the defect of SOCE. Cells participating to wound healing is not only keratinocytes but fibroblast and immune cells. The importance of other surface receptor coupled Ca^{2+} influx pathway in the whole process of wound healing is also discussed. (COI: No)

Symposium82

Amygdala Neuronal Circuits in Adaptive Behaviors

(March 31, Sun., 10:30-12:30, Room L)

S82-1

Neural Circuits Between the Central Amygdala and Basal Forebrain mediate Anxiety behaviours

Pankaj Sah; Ya-Jie Sun; Lei Qian; Li Xu (*Queensland Brain Institute, The University of Queensland, Australia*)

The amygdala, is a mid-temporal lobe structure that is critically involved in assigning emotional salience or value to events through associative learning. In particular it plays a central role in the analysis of fear and anxiety. The amygdala has extensive bidirectional connections with the basal forebrain (BF), a large structure in the ventral part of the brain. Input from the BF to the amygdala, is dominated by cholinergic afferents to the basolateral amygdala (BLA). However, the Central Amygdala (CeA), the amygdala's output region, with CeA also has extensive connections with the BF. Afferents from the lateral sector of the amygdala are known to target a region of the BF that has been called the sublenticular extended amygdala (SLEAc). I this talk will describe connections between the CeL and the SLEAc. We use a combination of viral injections, ex vivo slice electrophysiolgy and behavioural experiments and show the nature of these connections and how they may play a role in generation of anxiety like behaviours. (COI: No)

S82-2

Neuronal circuits underlying the regulation of aversive valence in mice

Ayako M Watabe (*Institute of Clinical Medicine and Research, Jikei University School of Medicine, Japan*)

Aversive and affective stimuli potently generate adaptive behaviors and emotional memory. The amygdala plays fundamental roles in aversive and affective information processing and adaptive behaviors. While the neural mechanisms underlying association of conditioned stimuli and unconditioned stimuli (US) has been intensively studied, the valence-related information of US *per se* is only partly understood. In particular, both flight and freezing behaviors can be elicited by exposing animals to an environmental threat, but little is known of neural circuits regulating this behavioral switch. To elucidate the neural mechanisms, we focused on the external lateral parabrachial nucleus (PB), which receives nociception-specific inputs and sends direct monosynaptic projections to the central amydala (CeA). Optogenetic activation of PB-CeA pathway induced aversive memory when paired with CS, suggesting this pathway may encode aversive valence. Furthermore, PB-CeA pathway activation triggered flight behavior. Since a proportion of PB-CeA fibers express calcitonin gene related peptide (CGRP), we next employed cell-type specific optogenetics to selectively activate CGRP-positive terminals. The CGRP-positive terminal activation induced aversive memory just like PB-CeA activation, but it triggered freezing behavior instead of impulsive escaping. These results suggest that the PB-CeC pathway may be composed of heterologous population to induce multiple forms of defensive behaviors. (COI: No)

S82-3

Brain circuits for triggering and reversing emotional memories

Joshua Johansen (*RIKEN Center for Brain Science, Japan*)

Aversive experiences are powerful triggers for memory formation and alter neural circuits to adaptively shape behavior. However, aversive memories need to be reduced when they are no longer appropriate to facilitate adaptive functioning. My lab studies the neural circuit and cell coding mechanisms which initiate learning and memory in response to aversive events and extinguish emotional responses when they are inappropriate. In this talk I will discuss recent work examining parallel neural circuits which trigger aversive associative learning and how feedback circuits regulate neural processing in these pathways to control the strength of fear memories. In addition, I will describe our recent discovery that distinct noradrenergic networks within the brainstem locus coeruleus either enhance fear memory formation or promote a switch from fear responding to behavioral flexibility, revealing a new framework for understanding noradrenaline circuit function. Together, these findings elucidate how neural circuit organization gives rise to neural coding and adaptive behavior and suggest novel strategies for the treatment of anxiety disorders associated with aberrant fear and extinction learning. (COI: No)

S82-4

Exploring molecular pathways involved in central amygdala-dependent control of emotional behaviors

Sayaka Takemoto-Kimura[1,2] ([1]*NeuroscienceI, RIEM, Nagoya University, Japan;* [2]*PRESTO-JST, Japan*)

Social and emotional behavior disabilities are exhibited in multiple psychiatric disorders. In addition to its well-known role as mediating fear and anxiety responses, the amygdala is a region in the brain that is crucial for social processing. Within the amygdala, the basolateral amygdala (BLA)-central nucleus of amygdala (CeA) circuit is known to mediate fear and anxiety responses. However, the involvement of this circuit in social behavior and its molecular basis remain to be elucidated. We found that the BLA is activated during social encounters, which indicates that this circuit is involved in social behavior regulation. To investigate the molecular basis of CeA-dependent social and emotional behaviors, we focused on a Ca^{2+}-dependent phosphorylation pathway, which is highly expressed in the CeA. We hypothesized thatthe Ca^{2+}-dependent signaling is essential in the amygdala-dependent emotional and social behaviors within the local circuit in the CeA. In accordance with this hypothesis, genetic and virus-mediated molecular manipulations of the kinase induced behavioral anomalies in emotional and social behavioral tasks. Furthermore, histological studies revealed the enrichment of the kinase in a subset of inhibitory neurons located in the CeA. Taken together, our study identify a novel amygdala Ca^{2+}-dependent signaling pathway that controls behavioral modifications triggered in response to external social environment. (COI: No)

Symposium83

Neurobiology of obesity and its metabolic comorbidities

(March 31, Sun., 10:30-12:30, Room M)

S83-1

Postprandial hormones regulate feeding and glucose metabolism via interacting with vagal afferents

Yusaku Iwasaki[1]; Toshihiko Yada[2,3] (*[1]Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan; [2]Center for Integrative Physiology, Kansai Electric Power Medical Research Institute, Japan; [3]System Physiology, Graduate School of Medicine, Kobe University, Japan*)

Hyperphagia is the leading cause of obesity and metabolic syndrome, however, few effective and safe medicines are available to treat feeding disorders and obesity. Peripheral metabolic status including nutrients, metabolites and hormones informs the hypothalamus via 1) penetrating through the blood-brain barrier and/or 2) acting on the vagal afferents, thereby regulating food intake. Vagal afferent nerves are visceral sensory nerves and sense peripheral signals from several organs to convey their information to the nucleus tractus solitarius of the medulla. However, the regulatory mechanism in the vagal afferents remains to be clarified.

We found that a subset of vagal afferent neurons (around 10%) was activated by meal-evoked satiety peptides such as posterior pituitary hormone oxytocin and intestinal glucagon-like peptide-1 (GLP-1). Recently, we demonstrated that a rare sugar D-allulose (Allu), which is a zero-calorie sweetener, induced GLP-1 release. Activation of a subset of vagal afferents by oxytocin or GLP-1 released by Allu ameliorates hyperphagia, obesity, glucose intolerance and diabetes. (COI: Properly Declared)

S83-2

Disruption of Steroid Receptor Coactivator-1 Signaling is Associated with Obesity

Yong Xu; Yongjie Yang; Liangru Zhu (*Department of Pediatrics, Baylor College of Medicine, USA*)

Steroid receptor coactivator-1 (SRC-1) is a coactivator which modulates the activity of nuclear hormone receptors and transcription factors. Here, we showed that in the hypothalamus, SRC-1 interacts with phosphorylated STAT3 to potentiate the ability of leptin to stimulate transcription of the anorectic peptide pro-opiomelanocortin (Pomc). In mice, targeted deletion of SRC-1 in Pomc neurons attenuated their depolarization by leptin, decreased Pomc expression and increased food intake leading to diet-induced obesity. In humans, we identified 15 rare heterozygous variants in SRC-1 in 2,548 severely obese individuals. These variants impaired leptin-mediated Pomc reporter activity in cells, whilst four rare variants found in 1,117 non-obese controls did not. We generated a knock-in mouse model of a human SRC-1 variant which exhibited increased food intake and weight gain, providing additional evidence that disruption of SRC-1- contributes to human obesity. Targeting this molecular interaction to modulate leptin sensitivity may be useful in the treatment of obesity-related metabolic disease. (COI: No)

S83-3

Withdrawn

S83-4

Gut hormone GIP drives hypothalamic pathogenesis of obesity via Epac-Rap1 signaling

Makoto Fukuda (*Baylor College of Medicine, USA*)

The hypothalamus has a critical role in the regulation of energy and glucose homeostasis. Over-nutrition triggers profound cellular and physiological changes of the hypothalamus diminishing hypothalamic responses to insulin and leptin, critical hormones maintaining normal blood glucose and body weight. This decline in hypothalamic responses has been proposed as crucial processes that underlie the pathophysiology of metabolic disease. Thus, identifying key factors that arise from excess caloric intake and regulate energy balance via signaling to central metabolic circuitry is a principal goal of obesity biology. Through brain explant, genetic, and pharmacological studies in mice, we identified the gut hormone gastric inhibitory polypeptide (GIP), whose levels are elevated during diet-induced obesity, as a neural mediator of obesity. Centrally administered GIP promoted obesity-associated pathogenesis, including reduced hypothalamic sensitivity to anorectic hormones and increased hypothalamic inflammation, via its receptor, GIPR. In contrast, *GIPR* deficiency protected against diet-induced neural leptin resistance. A centrally administered neutralizing GIPR antibody produced remarkable anti-obesity effects, including reduced adiposity and reversed hypothalamic pathogenic features in diet-induced obese mice. The small GTPase Rap1 mediates these effects in the brain. Altogether, our data suggest that GIP is a signal driving the neural pathogenesis of obesity. (COI: No)

S83-5

Neurohormonal mechanism for circadian feeding rhythm that prevents obesity

Toshihiko Yada[1,2]; Masanori Nakata[3] (*[1]Center for Integrative Physiology, Kansai Electric Power Medical Research Institute,Japan; [2]System Physiology, Graduate School of Medicine, Kobe University; [3]Physiology, Wakayama Prefectural Medical University*)

Humans eat at daytime and rest at nighttime. Inversely, rodents eat mostly at dark period (DP) and little at light period (LP). This circadian feeding rhythm maintains healthy physiologic conditions, and arrhythmic feeding induces obesity and diabetes. However, the mechanism for circadian feeding rhythm is little elucidated. This study sought to clarify the neurohormonal mechanisms underlying circadian feeding rhythm and its impairment.

In mice and rats, nesfatin-1/NUCB2 and oxytocin expression levels in the hypothalamic paraventricular nucleus (PVN) increase at LP and decrease at DP, exhibiting circadian rhythm in reverse phase to feeding rhythm (1,2). In high fat diet fed mice and Zucker fatty rats, the LP increases in nesfatin-1/NUCB2 and oxytocin are impaired in parallel with LP hyperphagia and obesity. The LP hyperphagia was ameliorated by icv injection of nesfatin-1. Furthermore, ip injection of FGF21, a hunger hormone, elevated expression and neuronal activity of nesfatin-1/NUCB2 in LP and corrected LP hyperphagia (3). These results indicate that the "peripheral FGF21 to PVN nesfatin-1/oxytocin axis" contributes to generate circadian feeding rhythm and to prevent obesity.

1. Nakata M, Yada T et al. Endocrinology 2016; 157:2322-.
3. Santoso P, Nakata M, Yada T et al. Sci Rep 2017; 7:45819-.
2. Sedbazar U, Nakata M, Yada T et al. Biochem Biophys Res Commun 2013; 434:434-
(COI: Properly Declared)

Symposium by the PSJ Committee on the Promotion of Gender Equality

Seeking Gender Equality in Science. A comparison of issues and initiatives in Japan and New Zealand

(March 29, Fri., 12:20-13:20, Room M)

MLS-1

Making room at the table: Gender equality initiatives at the Okinawa Institute of Science and Technology (OIST) Graduate University

Gail Tripp (*Okinawa Institute of Science and Technology Graduate University, Japan*)

Nationally and internationally women continue to be under-represented in academia, especially within the fields of science, technology, engineering and mathematics (STEM). Despite growing awareness of the private and public cost of such under-representation change comes slowly. In this talk I will discuss the efforts of the Okinawa Institute of Science and Technology Graduate University (OIST), to create a working environment that is attractive to, and supportive of, women at all ages and stages of their scientific and/or administrative careers. OIST, first accredited as a Japanese University in 2011, offers a five-year integrated PhD program taught in English. A number of the University's gender equality initiatives have resulted rapid positive change, other efforts have had less impact or continue to be "works in progress". While it is pleasing to acknowledge our successes, recognizing and addressing our "failures" is critical to OIST's ongoing commitment to achieving gender equality and, we hope, useful to other groups and institutions striving for equality. I will finish by identifying parallels and differences in the struggle for gender equality in academia and science in New Zealand and Japan. (COI: No)

MLS-2

Summary of the 4th Large-Scale Survey of Gender-Equality status in scientific professions

Tomoe Nakamura-Nishitani (*Department of Molecular Physiology, National Cerebral and Cardiovascular Center Research Institute, Japan*)

Since entering the 21st century, the issues of a declining birth rate, an aging population and unemployment have come to the forefront in many countries including Japan. To overcome these problems, all people including women, young and senior people should have equal opportunities to work. Although many efforts and actions have been made to improve the environment for the people involved in scientific fields so far, it is still difficult to say that Gender-Equality is fully achieved in the present situation. To identify the problems of this issue in research and engineering professions in Japan, we conducted a 4th-Large-Scale survey of actual conditions of Gender-Equality in these fields in 2017. The purpose of this talk is 1) to present what is the real situation and problems, 2) to identify the society's "mechanism" that have caused these problems, and 3) to summarize proposal and demands based on these data. The survey includes the real situation and differences between men and women scientists regarding 1) the ratio of regular and non-regular employment, 2) work-life balance, and 3) nursing care. The results indicated that the ratio of regular employment and salary are still lower in woman scientists compared to men scientists, and "work-life balance" is still difficult to continue their research for woman scientists, thus improving workplace environment and society support are absolutely necessary. This report should not simply end as a data, but we should use this to actually improve our work and family environments, even little by little. (COI: No)

Tutorial for Physiologists

Practical Approaches to Protein Structural Information

(March 31, Sun., 8:00-9:10, Room B)

no abstract

PSJ Awards

AP-1

Chronic stress causes excessive aggression by altering synaptic actin dynamics in the mPFC

Hirobumi Tada[1,2]; Takuya Takahashi[2] (*[1]Section of Neuroendocrinology, National Center for Geriatrics and Gerontology, Japan; [2]Department of Physiology, Yokohama City University*)

Behavioral and psychological symptoms of dementia (BPSD) are an integral part of dementia syndrome. In particular, BPSD such as chronic stress induced excessive aggression is known to be more stressful to caregivers than the cognitive and functional problems of the patients with dementia. Therefore, the effective treatment for excessive aggressive behavior is required. There is evidence that functional circuits in the medial prefrontal cortex (mPFC) regulate social cognitive functions including aggressive behaviors. Also, social isolation, one form of chronic stress environment, can lead to the development of excessive aggression. However, the underlying cellular and molecular mechanisms of the mPFC neural network involved in chronic stress environment induced aggression is largely unknown.

To clarify the molecular mechanism of mPFC neuronal network with excessive aggression, we examined aggressive behavior in rat model of chronic social isolation focusing on mPFC synaptic plasticity. We further investigated the relationship between synaptic actin dynamics and AMPARs delivery in spines of mPFC of chronic stressed animals. Here, we show that chronic stress environment changes spines in the mPFC by reducing actin dynamics, leading to the decrease of synaptic AMPA receptor delivery and altered social cognition and aggressive behavior. Our study provides molecular and cellular mechanisms underlying the influence of chronic stress environment on social cognition and aggression. (COI: Properly Declared)

AP-2

Characterization of the secondary auditory field in the mouse auditory cortex

Hiroaki Tsukano (*Department of Neurophysiology, Brain Research Institute, Niigata University, Japan*)

Tonotopy is an essential functional organization in the auditory cortex (ACx), and it is crucial to reveal how tonotopy is relayed to higher-order ACx such as the secondary auditory field (A2). The source of tonotopy reflected in the primary ACx (A1) is the incoming frequency-related topographical projections from the ventral division of the medial geniculate body (MGv). However, circuits that relay this functional organization to A2 have yet to be identified. In our recent tracing study conducted using mice, we discovered a new pathway that projects directly from the caudal part of MGv to A2, while the middle part of MGv projects to A1. Tonotopy was established in A2 even after primary fields including A1 were removed. These data suggest that tonotopy in A2 can be established solely by thalamic input. Moreover, the structural nature of differing thalamocortical connections was consistent with the functional organization of the target regions in ACx. Retrograde tracing revealed that the region of MGv input to a local area in A2 was broader than the region of MGv input to A1. Consistent with this anatomy, two-photon calcium imaging revealed that neuronal responses in the thalamocortical recipient layer of A2 showed wider bandwidth and greater heterogeneity of the best frequency distribution than those of A1. These findings demonstrate a new thalamocortical pathway that relays frequency information to A2 on the basis of the MGv compartmentalization. (COI: No)

AP-3

Cytoplasmic conformational changes of VSP detected by voltage clamp fluorescence spectroscopy

Akira Kawanabe; Tomoko Yonezawa; Yasushi Okamura (*Graduate School of Medicine, Osaka University, Japan*)

Voltage-sensing phosphatase (VSP) consists of the voltage sensor domain (VSD) and the cytoplasmic catalytic region (CCR) which acts as an enzyme that dephosphorylates $PI(4,5)P_2$ regulated by membrane potential change (Murata et al. 2005). The voltage-induced regulation mechanism of the CCR has not been fully understood. We previously reported the conformational changes of the CCR by voltage clamp fluorometry with environment-sensitive unnatural fluorescent amino acid (Anap). This powerful method shed light on the cytoplasmic conformational changes of membrane proteins in living cells, but the obtained information is so far limited because detailed molecular mechanisms of change of fluorescence intensity still remain unknown.

To gain more insights, we combined a spectrometer and inverted fluorescence microscope with electrophysiological instrument. This system can observe the absolute fluorescence spectra from selected region of a cell under voltage clamp condition. Using this method, we measured the fluorescence spectra of Anap incorporated at His-237 in the cytoplasmic region of *Ciona intesinalis*-VSP expressed in HEK293T. When we applied voltage step pulses from -80 to 100 mV in 20-mV increment to Ci-VSP H237Anap expressed cell, the fluorescence spectra of Anap showed the voltage-dependent changes, which may reflect the alteration of the local hydrophobic environment around incorporated Anap due to the conformational changes. (COI: No)

AP-4

Interaction of junctophilins and the CaV1.1 is essential for the skeletal muscle contraction

Tsutomu Nakada (*Department of Molecular pharmacology Shinshu University School of Medicine, Japan*)

Close physical association of $Ca_V1.1$ L-type calcium channels (LTCCs) at the sarcolemmal junctional membrane (JM) with ryanodine receptors (RyRs) of the sarcoplasmic reticulum (SR) is crucial for excitation–contraction coupling (ECC) in skeletal muscle. However, the molecular mechanism underlying the JM targeting of LTCCs is unexplored. Junctophilins (JPs) stabilize the JM by bridging the sarcolemmal and SR membranes. We examined the roles of JPs in localization and function of LTCCs. Knockdown of JP1 or 2 in cultured myotubes inhibited LTCC clustering at the JM and suppressed evoked Ca^{2+} transients without disrupting JM structure. Coimmunoprecipitation and glutathione S-transferase pull-down assays demonstrated that JPs physically interacted with 12 amino acid residues in the proximal C-terminus of the $Ca_V1.1$. A JP1 mutant lacking the C-terminus including the transmembrane domain (JP1ΔCT) interacted with the sarcolemmal/T-tubule membrane but not the SR membrane. Expression of this mutant in adult mouse muscles *in vivo* exerted a dominant-negative effect on endogenous JPs, impairing LTCC–RyR coupling at triads without disrupting JM morphology, and substantially reducing Ca^{2+} transients without affecting SR Ca^{2+} content. Moreover, the contractile force of the JP1ΔCT-expressed muscle was dramatically reduced compared with the control. Taken together, JPs recruit LTCCs to the JM through physical interaction and ensure robust ECC at triads in skeletal muscle. (COI: No)

AP-5

Physiological and pathophysiological significance of TRPC3-Nox2 coupling in the heart

Takuro Numaga-Tomita[1,2,3]; Tsukasa Shimauchi[4,5]; Naoyuki Kitajima[4]; Akiyuki Nishimura[2,4]; Motohiro Nishida[1,2,3,4] (*[1]Department of Creative Research, Exploratory Research Center on Life and Living Systems: ExCELLS, National Institutes of Natural Sciences, Japan; [2]National Institute for Physiological Sciences (NIPS), National Institutes of Natural Sciences; [3]School of life sciences, SOKENDAI; [4]Graduate School of Pharmaceutical Sciences, Kyushu University, 5Graduate School of Medical Sciences, Kyushu University*)

Chronic stresses induce pathological cardiac remodeling in which production of reactive oxygen species (ROS) plays a critical role. We have revealed that those ROS were produced by NADPH oxidase 2 (Nox2), despite low Nox2 expression levels in the normal heart. We demonstrate that transient receptor potential canonical 3 (TRPC3) Ca^{2+}-permeable channel acts as a positive regulator of Nox2 in both enzymatic activation and protein expression in cardiomyocytes during pathological remodeling. TRPC3 physically interacts with Nox2 through TRPC3 carboxyl-terminal regions, escaping Nox2 from proteasomal degradation, resulting in amplification of Ca^{2+}-dependent Nox2 activation. This TRPC3-Nox2 coupling mediates mechanical stress-induced cardiac fibrosis and a chemotherapy agent Doxorubicin-induced cardiac atrophy in mice. Inhibition of TRPC3-Nox2 coupling in cardiomyocytes could significantly suppressed Dox-induced cardiac atrophy. These results suggest that functional and physical coupling of TRPC3 and Nox2 mediates various stress-induced cardiac remodeling and inhibition of TRPC3-Nox2 coupling will be a promising therapeutic target for the treatment of pathological muscle remodeling. Furthermore, TRPC3 knockout heart showed more elastic property than wildtype heart, suggesting that TRPC3-Nox2 coupling regulates heart stiffness by two means of cardiomyocyte and extracellular matrix. (COI: No)

AP-6

Microglia permit climbing fiber pruning by promoting synaptic inhibition in the developing cerebellum

Hisako Nakayama (*Department of Physiology, School of Medicine, Tokyo Women's Medical University, 8-1, Kawada-cho, Sinjuku-ku, Tokyo, Japan*)

Circuit refinement during postnatal development is finely regulated by neuron–neuron interactions. Recent studies suggest participation of microglia in this process but it is unclear how microglia cooperatively act with neuronal mechanisms. The cerebellar cortex is an appropriate model system to study the mechanisms for constructing functional neuronal connections. In neonatal rodent, each Purkinje cell (PC) is innervated by multiple climbing fibers (CFs). Then, all but one CFs are eliminated by the end of the third postnatal week. Here we studied role of microglia in the development of cerebellar circuits including the establishment of mono-innervation of CF-PC connections. To examine roles of microglia, we ablated microglia by microglia-selective deletion of colony stimulating factor 1 receptor (Csf1r) by crossing floxed-Csf1r and Iba1-iCre mice (Csf1r-cKO). In Csf1r-cKO mice, refinement of CF-PC innervation after postnatal day 10 (P10)–P12 is severely impaired. However, there is no clear morphological evidence suggesting massive engulfment of CFs in microglia. In Csf1r-cKO mice, inhibitory synaptic transmission is impaired and CF elimination is restored by diazepam, which suggests that impairment of CF elimination is caused by a defect of GABAergic inhibition on PCs, a prerequisite for CF elimination. These results indicate that microglia primarily promote inhibitory synaptogenesis and secondarily facilitate the mechanism for CF elimination inherent in PCs. (COI: No)

AP-7

Inhibition of ghrelin-induced feeding in rats by treatment with a novel orexin receptor antagonist

Mariko So[1,2]; Hirofumi Hashimoto[2,4]; Reiko Saito[2,3]; Yukiyo Yamamoto[3]; Yasuhito Motojima[2]; Hiromichi Ueno[2]; Satomi Sonoda[2]; Mitsuhiro Yoshimura[2]; Takashi Maruyama[2]; Koichi Kusuhara[3]; Yoichi Ueta[2] (*[1]Department of Health and Nutritional Care, Faculty of Medical Science, University of East Asia, Shimonoseki 751-0807, Japan; [2]Department of Physiology, School of Medicine, University of Occupational and Environmental Health, 1-[1] Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan; [3]Department of Pediatrics, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan; [4]Department of Regulatory Physiology, Dokkyo Medical University, 880 Kitakobayashi, Mibu 321-0293, Japan.*)

Orexin-A and -B, and ghrelin are potent orexigenic peptides. The effects of ACT462206, a novel dual orexin receptor antagonist (DORA), on ghrelin-induced feeding were examined in adult male Wistar rats. Here, we examined the central effects of DORA on centrally administered ghrelin-induced feeding in conscious rats. Hyperphagia induced by the intracerebroventricular (icv) administration of ghrelin was significantly suppressed for at least 2 h by pretreatment with icv administration of DORA. A marked increase was observed in the number of neurons expressing Fos immunoreactivity in the paraventricular nucleus, arcuate nucleus and lateral hypothalamic area (LHA), 90 min after icv administration of ghrelin. Pretreatment with DORA significantly decreased the number of Fos-immunoreactive (IR) neurons, however, Fos immunoreactivity remained significantly increased. Double-immunostaining for Fos and orexin-A showed that many orexin-A-IR neurons in the LHA coexisted with Fos immunoreactivity after icv administration of ghrelin, however, their number of orexin-A-IR neurons with Fos in the LHA was significantly reduced by pretreatment with DORA. These results suggest that centrally administered ghrelin may activate the orexinergic and non-orexinergic pathways responsible for the regulation of feeding. (COI: No)

AP-8

Hypotonicity-induced cell swelling activates TRPA1

Fumitaka Fujita[1,2,3]; Kunitoshi Uchida[4]; Yasunori Takayama [1,5]; Yoshiro Suzuki [1,5]; Masayuki Takaishi[1,6]; Makoto Tominaga[1,5] (*[1]Division of Cell Signaling, National Institute for Physiological Sciences, Japan; [2]Basic Research Institute, Mandom Corp., Japan; [3]Laboratory of Advanced Cosmetic Science, Graduate School of Pharmaceutical Sciences, Osaka University, Japan; [4]Departments of Physiological Science and Molecular Biology and Morphological Biology, Fukuoka Dental College, Japan; [5]Thermal Biology Group, Exploratory Research Center on Life and Living Systems, Japan; [6]Product Assurance Division, Mandom Corp., Japan*)

Hypotonic solutions can cause painful sensations in nasal and ocular mucosa through molecular mechanisms that are not entirely understood. We clarified the ability of human TRPA1 (hTRPA1) to respond to physical stimulus, and evaluated the response of hTRPA1 to cell swelling under hypotonic conditions. Using a Ca2+-imaging method, we found that modulation of AITC-induced hTRPA1 activity occurred under hypotonic conditions. Moreover, cell swelling in hypotonic conditions evoked single-channel activation of hTRPA1 in a cell-attached mode when the patch pipette was attached after cell swelling under hypotonic conditions, but not before swelling. Single-channel currents activated by cell swelling were also inhibited by a known hTRPA1 blocker. Since pre-application of thapsigargin or pretreatment with the calcium chelator BAPTA did not affect the single-channel activation induced by cell swelling, changes in intracellular calcium concentrations are likely not related to hTRPA1 activation induced by physical stimuli. (COI: No)

AP-9

Epac activation inhibits IL-6-induced cardiac myocyte dysfunction

Huiling Jin[1]; Takayuki Fujita[1]; Meihua Jin[1,2]; Reiko Kurotani[1,3]; Yuko Hidaka[1]; Wenqian Cai[1]; Kenji Suita[1]; Rajesh Prajapati[1]; Chen Liang [1]; Yoshiki Ohnuki[4]; Yasumasa Mototani[4]; Masanari Umemura[1]; Utako Yokoyama[1]; Motohiko Sato[1,5]; Satoshi Okumura[1,4]; Yoshihiro Ishikawa[1] (*[1] Cardiovascular Research Institute, Yokohama City University Graduate School of Medicine, Japan; [2] Department of Cardiac Physiology, National Cerebral and Cardiovascular Center Research Institute, Japan; [3] Biochemical Engineering, Faculty of Engineering, Yamagata University, Japan; [4] Department of Physiology, Tsurumi University School of Dental Medicine, Japan; [5] Department of Physiology, Aichi Medical University, Japan*)

Pro-inflammatory cytokines are released in septic shock and impair cardiac function via the Jak-STAT pathway. It is well known that sympathetic and thus catecholamine signaling is activated thereafter to compensate for cardiac dysfunction. The mechanism of such compensation by catecholamine signaling has been traditionally understood to be cyclic AMP-dependent protein kinase (PKA)-mediated enforcement of cardiac contractility. We hypothesized that the exchange protein activated bycAMP (Epac), a newly identified target of cAMP signaling that functions independently of PKA, also plays a key role in this mechanism. In cultured cardiac myocytes, activation of Epac attenuated the inhibitory effect of interleukin-6 on the increase of intracellular Ca2+ concentration and contractility in response to isoproterenol, most likely through inhibition of the Jak-STAT pathway via SOCS3, with subsequent changes in inducible nitric oxide synthase expression. These findings suggest a new role of catecholamine signaling in compensating for cardiac dysfunction in heart failure. Epac and its downstream pathway may be a novel target for treating cardiac dysfunction in endotoxemia. (COI: No)

Poster Presentations

Day 2

（March 29, 13:20-14:10/14:10-15:00）

1P-001

Analysis of junctophilin2 knock out zebrafish

Souhei Sakata; Fumihito Ono (*Department of Physiology, Division of Life Sciences, Faculty of Medicine, Osaka Medical College, Japan*)

In muscle cells, junctional membrane complexes (JMC) between the plasma membrane and the endoplasmic / sarcoplasmic membrane provide a structural platform for the excitation-contraction coupling. Junctophilins (JPHs) are expressed in JMC and contribute to communications between channels on both membranes. Four JPH genes are encoded in the mouse genome; JPH1 and 2 are expressed in muscle cells and JPH3 and 4 are in neural cells. Previous reports in mice showed that the conditional cardiac knock down of JPH2 leads to heart failure and early mortality, while JPH1 knockouts die shortly after birth due to defective suckling. These data suggest that JPHs play essential roles in muscle contraction.

Zebrafish larva is transparent and develops ex-utero. This is an advantage for studying the early developmental stages, in contrast to the JPH KO mice whose analysis is hindered due to lethality in utero. In the zebrafish genome, there are two JPH1s (JPH1a and 1b), JPH2 and JPH3. We confirmed the JPH2 expression in skeletal muscles and the heart using in situ hybridization of larva, and generated JPH2 KO zebrafish line using the CRISPR / Cas9 system. Unlike mice, JPH2 KO zebrafish is not lethal. The heart rate and the morphology of larva were not significantly different from those of wild-type. Escape behaviors of KO larva were also similar to those of wild-type. Therefore JPHs in fish have distinct physiological significance compared to mammals. (COI: No)

1P-002

Evaluation of muscle contraction by electromyogram and sonography

Masafumi Katayama (*International university of health and welfare, Japan*)

[Background and purpose] Diagnostic methods leading to prevention of Sarcopenia are required. We investigated establish an evaluation of quadriceps femoris muscle contraction using electromyogram (EMG) and ultrasound (US) image.

[Methods] The surface EMG were recorded in rectus femoris muscle, vastus lateralis and vastus medialis during knee extension with load. We observed three muscular discharge ratio and they were evaluated for quantity of muscular discharge in search of the repand area by integration. By US image, we observed the length of the circumference and the cross sectional area on the transverse image and measured the pennation angle on the sagittal image.

[Results and conclusion] Environment of isometric contraction, amount of muscular discharge increased from weak to strong contraction. In the rectus femoris muscle, there was significant positive correlation between the imposed weight and the muscular discharge. Since the circumference was more stable than the cross sectional area, it was selected for evaluation. The pennation angle was more large in traction than resting. The circumference and the pennation angle tended to increase due to voluntary contraction. However, the value did not change when weight exceeded a certain quantity. By evaluating functional and morphologically using EMG and US image, there is a possibility that muscle weakness can be evaluated. (COI: No)

1P-003

Muscle representations in spinal motor circuitry in intact humans and an individual with SCI

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Electrical stimulation to the mammalian spinal cord have demonstrated that the proximal to distal limb muscles are gradually represented in a rostro-caudal configuration. However, we recently found that repetitive stimulation to the human lumbar spinal cord is capable of inducing walking-like leg movements that rostro-caudal muscle representation is hard to account for. To address this issue, we investigated muscle activation pattern elicited by single pulse transvertebral magnetic stimulation in intact humans and an individual with chronic thoracic spinal cord injury. Stimulation was percutaneously delivered to a 3 by 6 target grip over the intervertebral spaces from T11 to L5 to acquire the spinal motor map in bilateral hip, knee, ankle joint muscles. In intact individuals, we found that spinal muscle maps were segregated according to the vertebral level; lower leg and posterior thigh muscles were mainly represented at lower lumbar level (L3-L5), anterior thigh muscles for knee extension and hip flexion were mainly represented at high to middle lumbar level (L1-L4). We also observed similar pattern of muscle representations in an individual with spinal cord injury although posterior thigh muscles were represented at high to middle lumbar vertebrae. Our finding demonstrated that muscle representations exhibited by magnetic stimulation in human spinal motor circuitry. (COI: No)

1P-004

Generation of a transgenic zebrafish for monitoring murf1 expression

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Zebrafish is an excellent animal model for human diseases due to its high genetic homology to human, and easy genetic manipulation. To monitor the expression of murf1 gene, which is one of marker molecules of muscle atrophy, a transgenic zebrafish line was created with microinjection of murf1 promoter-EGFP cDNA construct using tol2 transposon system.

During early development in the transgenic fish (murf1:EGFP) line, EGFP signals were observed in skeletal muscle and heart from 1 day post-fertilization (dpf). RT-PCR analysis confirmed that the murf1 gene expression was corresponded with EGFP expression after 1 dpf. In the adult transgenic fish, murf1 expression corresponding with EGFP were mainly observed in skeletal muscle and heart. Treatment with dexamethasone solution at 4 dpf for 24 hours induced up-regulation of EGFP expression in murf1:EGFP zebrafish. These results indicated that the murf1 expression could be monitored using the murf1-EGFP-transgenic fish.

This transgenic fish line might be excellent tool to evaluate the expression of murf1 and is useful to therapeutic drug screening for muscle atrophy. (COI: No)

1P-005

Acetylcholinesterase inhibitor accelerates muscle differentiation in C2C12 myoblasts

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Injured skeletal muscle fibers are regenerated by proliferation and differentiation of intrinsic satellite cells, and chronic ischemia prevents the repairing process. We previously demonstrated that acetylcholinesterase inhibitor (AChEI) protected against skeletal muscle atrophy by promoting the proliferation of satellite cells in a mouse model of peripheral arterial disease. However, the effect of AChEI on the muscle differentiation remains unclear. In the present study, therefore, we investigated the expressions of myogenic regulatory factors in AChEI-treated murine myoblast cell line C2C12. We first measured the cytotoxicity of AChEI in C2C12 cells by WST-1 assay. AChEI at concentrations higher than 25 μM significantly decreased cell viability. Thus, we used AChEI at a concentration less than 25 μM for all further experiments. Then, we investigated the effect of AChEI on the expression of myogenic regulatory factors [MyoD, myogenin and myosin heavy chain (MyHC)] by qRT-PCR and western blot analysis. Although the expression of MyoD was comparable to that in the untreated control group, AChEI treatment significantly increased the expressions of myogenin and MyHC in a time- and a concentration-dependent manner. In addition, immunofluorescence microscopy showed that AChEI treatment enhanced the formation of MyHC-positive myotubes. These results indicate that AChEI accelerates the myoblast differentiation to myofibers in the muscle repairing process. (COI: No)

1P-006

Emerin deficiency exacerbates skeletal muscle pathology in *Lmna* $^{H222P/H222P}$ mutant mice

Eiji Wada; Megumi Kato; Kaori Yamashita; Yukiko K Hayashi (*Department of Pathophysiology, Tokyo Medical University, Japan*)

Nuclear envelopathy is caused by defects of nuclear membrane proteins, such as emerin and lamin A/C, of which cause Emery-Dreifuss muscular dystrophy (EDMD). EDMD is clinically characterized by slowly progressive muscle wasting and weakness, joint contractures, and cardiomyopathy with conduction defects. Several murine models of EDMD are generated; however, *Lmna*$^{H222P/H222P}$ mutant (H222P) mice show mild phenotype in skeletal muscle around 30 weeks of age when they start to die due to cardiac dysfunction, and Emerin-null (Emd) mice did not show obvious muscle phenotypes. Thus, the underlying molecular mechanism of muscle involvement due to nuclear abnormalities is still unclarified. In this study, we crossed H222P and Emd mice to generate double mutant (*Emd*$^{-/-}$/*Lmna*$^{H222P/H222P}$; EH) mice to characterize dystrophic changes and to elucidate interaction between emerin and lamin A/C in skeletal and cardiac muscles. In skeletal muscle, EH mice aggravated dystrophic changes and increased abnormal nuclei even at age of 12 weeks. Treadmill exercise test revealed that EH mice had significantly less muscle endurance capacity. On the other hand, there was no histological and functional difference in cardiac muscle from EH and H222P mice at 12 weeks of age, compared with control mice. Therefore, EH mouse is a preferable murine model to study skeletal muscle dysfunction in nuclear envelopathies and to understand the complex interactions and roles among nuclear proteins in different tissues. (COI: No)

1P-007

Cell surface flip-flop of phosphatidylserine is critical for PIEZO1-mediated myotube formation

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Myotube formation by fusion of myoblasts and subsequent elongation of the syncytia is essential for skeletal muscle formation. However, molecules that regulate myotube formation remain elusive. Here we identify PIEZO1, a mechanosensitive Ca^{2+} channel, as a key regulator of myotube formation. During myotube formation, phosphatidylserine, a phospholipid that resides in the inner leaflet of the plasma membrane, is transiently exposed to cell surface and promotes myoblast fusion. We show that cell surface phosphatidylserine inhibits PIEZO1 and that the inward translocation of phosphatidylserine, which is driven by the phospholipid flippase complex of ATP11A and CDC50A, is required for PIEZO1 activation. PIEZO1-mediated Ca^{2+} influx promotes RhoA/ROCK-mediated actomyosin assemblies at the lateral cortex of myotubes, thus preventing uncontrolled fusion of myotubes and leading to polarized elongation during myotube formation. Moreover, myoblasts-specific deletion of *Atp11a* gene causes morphological abnormalities in regenerating myofibres after cardiotoxin-induced injury. These results suggest that cell surface flip-flop of phosphatidylserine acts as a molecular switch for PIEZO1 activation that governs proper morphogenesis during myotube formation. (COI: No)

1P-008

Role of Ror-family receptor tyrosine kinases in the skeletal muscle

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The Ror-family of receptor tyrosine kinases, Ror1 and Ror2, play important roles in regulating the function of tissue stem cells, including neural progenitor cells, during embryonic development. However, it remains largely unknown whether Ror1 and Ror2 also regulate the function of tissue stem cells in the adult animals. Here, we show that *Ror1*, but not *Ror2*, is expressed highly in satellite cells (SCs), well documented residential tissue stem cells in the skeletal muscles. Analyses of SC-specific *Ror1* conditional knockout mice have revealed that Ror1 plays an important role in skeletal muscle regeneration by promoting proliferation of SCs. Interestingly, we also found that Ror2 is expressed highly in mesenchymal progenitor cells (MPCs) in the skeletal muscles. MPCs are known to be differentiated into adipocytes, contributing to an accumulation of intramuscular adipose tissue under some pathological conditions, although its underlying mechanism is still unclear. We therefore examined the role of Ror2 in regulating differentiation capacity of MPCs *in vitro*, and found that Ror2 is required for differentiation of MPCs into adipogenic lineage.These results indicate that Ror1 and Ror2 might play distinct roles in skeletal muscle regeneration after injury by regulating proliferation of SCs and differentiation of MPCs, respectively. It can be assumed that coordinated regulation of Ror1 and Ror2 might be required for muscle regeneration and homeostasis. (COI: Properly Declared)

1P-009

Bereitschaftspotential of the interference between attention distribution and finger movement timing

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Bereitschaftspotential (BP) identifies the slow rising negative electrocortical activity preceding motor acts, also known as readiness potential. Dual tasking is defined as performing two tasks concurrently. Humans commonly perform multiple tasks simultaneously (multitasking) in our daily life. This study aims to quantify the effects of dual-task complexity on the movement frequency that relies on an internal pacemaker and feedback and the accuracy of a visual number counting task using BP. Twenty right-handed, healthy volunteers participated in this study. They performed one single task and two dual tasks: simple and complex. The single task involved a self-paced tapping task in which the participants extended their right index finger at a 5-second interval. In the dual task, the participants performed the motor task and a visual number counting task simultaneously. The average time and coefficient of variation of the movement frequency in complex dual tasks were significantly more variable than those in the single task. Differences are more often detected for the channels over C3, C4, and Cz. The attention distribution lead to a significant changes in specific BP features of the motor task. These results suggest that attention distribution in dual-tasking situations plays an important role in movement execution and detection. (COI: No)

1P-010

Control of Keber's valve at rest, foot extension and retraction of the clam *Nodularia douglasiae*

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In order to analyse hydrostatic manipulation of the foot of the clam, flows of hemolymph in the cardiovascular system of *Nodularia douglasiae* were detected at rest, foot extension and retraction by magnetic resonance imaging at 20°C. At rest, in the beginning of systolic phase, flows in the anterior aorta and the pedal artery increased instantaneously. Flows in the pedal and visceral sinuses increased with a small delay. Then, these flows ceased at the end of systolic phase followed by inflow to the ventricle in diastolic phase. At the foot extension, flows of the pedal and visceral sinuses stopped within 3 heart-beats. Meanwhile, flows in the pedal artery continued longer than 3 heart-beats. At the foot retraction, flows of the pedal and visceral sinuses increased, and the initial flows were continuous flow, then, turned to pulsated flow. Based on these results, 1) at rest, compliance of the wall of foot is low enough to transfer pressure pulse from the anterior aorta to the visceral sinus. 2) Extension of the foot starts by relaxation of foot muscle. Then, the Keber's valve is closed so that the hemolymph filled in the foot haemocoel and increases volume of the foot. 3) Retraction of the foot starts by opening the Keber's valve followed by increase of outflow of the hemolymph. In conclusion, not only the Keber's valve, but also the compliance of the foot is essential for the circulation of the clam at rest, foot extension and retraction. (COI: No)

1P-011

Suppressive Activity of Chondroitin Sulfate on Nitric Oxide Production by Knee Synoviocytes In Vitro

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Background: Knee osteoarthritis (OA) is well known to be the degeneration of articular cartilage. Structure-modifying medications, such as nutraceuticals, including chondroitin sulfate (CS) and glucosamine hydrochloride, are said to be effective treatments for OA; however, the therapeutic mechanisms of these nutraceuticals are poorly understood. We examined the influence of CS on the production of nitric oxide (NO), a molecule associated with OA development, by synoviocytes obtained from an OA patient via an *in vitro* cell culture technique.
Method: Human fibroblast-like synoviocytes (1×10^5 cells/ml) were stimulated with 10.0 ng/ml IL-13 in the presence of various concentrations of CS after 48 h. The NO contents in culture supernatants were examined by the Griess method. We also examined the influence of CS on the signal transducer and activator of transcription factor 6 (STAT6) activation and inducible nitric oxide synthase (iNOS) mRNA expression in synoviocytes at 12 and 24 h after IL-13 stimulation.
Results: The addition of CS into cell culture suppressed NO production from synoviocytes induced by IL-13 stimulation through inhibition of STAT6 activation and iNOS mRNA expression. The minimum concentration of CS that caused a significant suppression of the NO production, STAT6 activation and iNOS mRNA expression was 7.5 μg/ml.
Conclusion: These results suggest that the ability of CS to suppress NO production from synoviocytes may contribute the clinical efficacy of CS on OA. (COI: No)

1P-012

Upregulation of osteclastogenic markers and impaired bone microstructure in hypertensive rats

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Hypertension and osteoporosis are the major health problem in the elderly worldwide. Interestingly, clinical studies have shown that hypertensive patients often experience significant bone loss, but the underlying mechanisms are largely unknown. In the present study, the spontaneously hypertensive rats (SHR) were used to study hypertensive bone changes and its cellular mechanisms. Female SHR rats at 18 weeks old were subjected to determination of volumetric bone mineral density (vBMD) and bone strength by using microcomputed tomography and three-point bending test, respectively. We found that cortical and trabecular vBMD as well as the cortical thickness and area were significantly decreased as compared to the age-matched normotensive rats. Furthermore, SHR exhibited the impairment of bone strength as indicated by decrease in ultimate load and stiffness. Then, the primary osteoblasts from normotensive rats and SHR were conducted to determine the mRNA expression levels of bone formation and resorption marker genes. A decrease in bone formation marker, ALP, was observed with the upregulated bone resorption markers, i.e., RANKL, MCSF and IL6. Our finding thus suggested that bone microstructure and bone strength were impaired in SHR, presumably by the uncoupling of bone remodeling process in which the osteoclast-mediated bone resorption was increased, while the osteoblast-mediated bone formation was decreased. This study was supported by Thailand Research Fund. (COI: No)

1P-013

Immature network function of the adult lumbosacral cord by loss of interferon regulatory factor 8

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Interferon regulatory factor 8 (IRF8) is a transcription factor expressed mainly in myeloid cells, such as macrophages and microglia (macrophage-like cells in the central nervous system [CNS]). Although the activities of IRF8 are complex, it provides innate resistance to infectious pathogens and also directs the development of myeloid cells. Simultaneously, microglia are likely to play important roles in forming the developing CNS and contributing to overall nervous system function.

We used the decerebrated and the hindlimb preparation and electrophysiologically investigated whether the impact arising from the absence of IRF8 influences the spinal network function of the adult IRF8$^{-/-}$ and wild type mice (P11 to 12 wks). Neuronal discharge was recorded from the phrenic and bilateral peripheral motor nerve. In both mice, discharge episodes consisting of locomotor-like and tonic discharge were induced by the modulated sympathetic tone and by the drug (serotonin, N-methyl-D,L-aspartate, and noradrenalin). Comparisons of the pattern of discharge episodes, induced by the modulated sympathetic tone and/or by the drug application, demonstrated that the network function of the lumbosacral cord in IRF8$^{-/-}$ mice was immature. Our results suggest the possibility that IRF8 plays a role in normal development of the spinal network function, possibly through the regulation of myeloid cells in the CNS.

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1P-014

Exercise is better than caloric restriction regarding improving fatigability in muscle of obese rats

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Purpose: To determine the effect of endurance exercise training (ET) and caloric restriction (CR) on skeletal muscle fatigability and mitochondrial function

Methods: Six female Wistar rats fed with a normal diet for 27 weeks were used as a control group. Twenty-four rats were fed with a high-fat diet (HFD) for a total of 27 weeks. At week 20, the HFD-fed rats were divided into 3 groups, which received no treatment, endurance ET 5 times/week and 60% of CR for 7 weeks. After in situ muscle contraction study was performed, rats were euthanized and vastus lateralis muscle was removed to measure skeletal muscle mitochondrial dynamics and function.

Results: We found that HFD-fed rats without therapy developed early fatigability; impaired skeletal muscle mitochondrial function, as indicated by increased ROS production, membrane depolarization and swelling; reduced mitochondrial dynamics as indicated by increased pDRP1/DRP1 ratio and decreased MFN2 expression ($p<0.05$ compared with ND groups, ANOVA test). ET significantly improved fatigability, corresponding with the improvement in PPAR delta expression in skeletal muscle. Both ET or CR significantly improved mitochondrial dynamics ($p<0.05$ compared with HFD sedentary group, ANOVA test), and had a trend to improve mitochondrial function of skeletal muscles.

Conclusion: ET was superior to CR regarding improving skeletal muscle fatigability and functions in obese-insulin resistant model. (COI: Properly Declared)

1P-015

Effects of Hypoxia on Skeletal Muscle Molecular Adaptations to Heavy Resistance Training

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We determined the effects of hypoxic resistance training (IHRT) on skeletal muscle molecular adaptations involved in muscle strength.

Eighteen participants trained over 7 weeks in either IHRT (FiO_2 0.143) or placebo (PLA, FiO_2 0.20). Vastus lateralis muscle biopsies were taken before and after the training programme.

Training increased Type II fibre CSA in both groups (mean±90% confidence limits (CL), effect size (ES); IHRT: 16.0±25.2%, ES 0.50; PLA: 22.0±31.8%, ES 0.42). Type I CSA only increased in PLA (16.1±23.3%, ES 0.48); however, the changes in Type I or II fibre CSA did not differ between groups. Training caused no change in total p70S6K in either group. Training caused a possibly trivial decrease in total mTOR in PLA, and a possibly small increase in IHRT, resulting in a greater increase in mTOR for IHRT compared to PLA (18.9±27.3%, ES 0.65). The content of sarcoplasmic reticulum (SR) associated proteins dihydropyridine receptor, SERCA1, and calsequestrin did not change in either group. SERCA2 increased in IHRT only (23.5±18.7%, ES 0.33), and this increase was greater compared to PLA (42.6±52.2%, ES 0.63). The force transfer protein dystrophin did not change in either group; however, alpha-actinin increased only in IHRT (47.8±67.5%, ES 0.67), and this was greater compared to PLA (63.1±79.5%, ES 1.10).

Greater strength increases following resistance training in hypoxia compared to normoxia may be due to enhanced SR calcium regulation and force transfer. (COI: No)

1P-016

Enriched environment attenuates hindlimb dysfunction in neonatal white matter injury model

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Neonatal white matter injury (WMI) caused by hypoxia-ischemia (H-I) in preterm infants is associated with permanent neurodevelopmental disabilities such as paralysis and cognitive dysfunction. We previously established a neonatal WMI model that received H-I (right common carotid artery occlusion and 6% hypoxia for 1 hour) at P3. It remains unclear whether enriched environment (EE) during the period of development can affect the recovery of disturbed function. Here, we assessed the efficacy and mechanism of EE focusing on hindlimb motor function in neonatal WMI rat model.

Male neonatal WMI model rats were divided to EE and standard environment (SE) conditions weaning from P25. Behavioral tests by hindlimb retraction, beam walk ability, horizontal ladder and rotarod were performed at P25, P35 and P70 followed by immunohistochemical investigations.

In EE group, better functional recovery in tests except for rotarod (hindlimb retraction, beam walk ability, and horizontal ladder) was shown at P35 as compared in SE group. Similar results were shown at P70 as well. It revealed that the thickness of the sensorimotor cortex is comparable to that of normal rat in EE group, although less thickness is shown in SE group.

These data suggest that EE during the period of development has a potency to improve deteriorated hindlimb motor function with apparent morphological changes in the sensorimotor cortex of neonatal WMI model rat. (COI: Properly Declared)

1P-017

Role of dopaminergic function in septum on exercise efficiency

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Exercise efficiency is important for sports performance. Although motor skill in exercise efficiency was subject to considerable research, it has not elucidated in light of energy cost in exercise efficiency. In this study, we aimed to clarify the mechanisms improvement in the exercise efficiency in a continuous trappy exercise.

Male C57BL/6J mice were habituated and practiced treadmill running (15 m/min, 0 degree incline) with intermittent obstacles on belt of treadmill (continuous trappy exercise like a hurdle race) for 8 days (15 min/day). Before and after exercise training, oxygen uptake (VO2) during obstacle-treadmill running was measured as energy cost-based exercise efficiency.

After obstacle exercise training, VO2 was decreased significantly with improvement of running behavior. Encompassing analysis revealed that c-fos expression after obstacle-treadmill running was increased in striatum and septum, and decreased in motor cortex by training. Most impressively, dopamine turnover (DOPAC/dopamine) in septum was significantly higher in mice after obstacle-treadmill training. The dopamine turnover was positively related to exercise efficiency. Furthermore, dopamine D1 receptor antagonist was directly administered into bilateral septum in mice significantly canceled the training effect.

Taken together, we conclude that dopamine turnover in septum is important for energy cost-based exercise efficiency. (COI: No)

1P-018

Enhanced muscle afferent responses to mechanical/chemical stimuli in type 2 diabetic rats in vitro

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The cardiovascular response to exercise is abnormally exaggerated in patients with type 2 diabetes (T2D). However, the mechanisms underlying the abnormal circulatory control in T2D remain to be elucidated. **Purpose:**To examine the impact of diabetes on neuronal responses to mechanical and chemical stimulation in skeletal muscle thin fiber afferents in vitro. It was hypothesized that the activity of mechanically and metabolically sensitive muscle afferent neurons is potentiated in T2D. **Methods:**Mechanically and chemically activated neurons (Group IV) were assessed by obtaining single-fiber recordings from rat muscle-nerve preparations in vitro. Rats were placed on a normal diet (control, n=5) or a high fat diet in combination with a low dose of streptozotocin (T2D, n=10) for 12-16 weeks. **Results:**T2D animals exhibited hyperglycemia after overnight fasting (104±5 vs. 161±10 mg/dL, $P<0.05$). Compared to control, the response magnitude to a ramp-shaped mechanical stimulation (196mN) was greater in T2D rats (20±7 vs. 40±8 spikes, $P=0.10$). Likewise, muscle afferent responses to capsaicin (1μM) were significantly augmented in diabetic animals compared to control (0.8±0.3 vs. 2.9±0.7 Hz, $P<0.05$). **Conclusion:**These findings suggest that the heightened cardiovascular response to exercise in T2D may be mediated by a potentiated action potential response to mechanical and chemical stimulation in muscle afferents. *Supported by Lawson & Rogers Lacy Research Fund in Cardiovascular Disease.* (COI: No)

1P-019

Sex difference in mitochondrial Ca^{2+} handling properties in mouse skeletal muscle

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An increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) after exercise seems to be greater in male than female, although the underlying mechanism(s) remains unclear. To address this mechanism, we investigated sex differences in mitochondrial Ca^{2+} handling properties in mouse skeletal muscle. Tibialis anterior muscles from male and female mice were exteriorized and fura 2-AM was loaded. Then, the distal tendon and body of the muscle were dissected free and the tendon was pinned as muscles were stretched to ~10% of resting length. The changes in $[Ca^{2+}]_i$ were measured during following treatment:1) cyclopiazonic acid (CPA) for 30 min (CPA treatment) and 2) CPA and 10 μM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) for 30 min. In either treatment, a graded concentration of CPA was applied every 10 min (i.e., 10 μM, 30 μM and 50 μM). Thirty min after induction of CPA treatment, the $[Ca^{2+}]_i$ was increased by 31.6 ± 2.0% and 13.5 ± 4.5% in male and female, respectively, and there was significant difference ($P < 0.01$) between sexes. In contrast, the increased $[Ca^{2+}]_i$ in female reached to similar level of that in male in the presence of FCCP. There were no differences in the content of proteins which were involved in Ca^{2+} handling of sarcoplasmic reticulum and mitochondria. These results suggest that i) mitochondrial Ca^{2+} uptake ability is greater in female than male and ii) this superior ability is not attribute to the change in protein expression level. (COI: No)

1P-020

Enhanced cerebro-cardiovascular responses before voluntary cycling in physically fit men

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[Purpose] We recently reported that countdown (CD) before voluntary exercise induced cerebral activation and pressor responses, followed by muscle vasodilation, which is likely advantageous for prompt oxygen delivery to contracting muscles. In the present study, we hypothesized that these responses would be associated with individual peak aerobic capacity (VO_{2peak}).

[Methods] Twenty-seven young men (VO_{2peak}, 25.2−61.4 ml/min/kg) performed voluntary cycling at 50%VO_{2peak}, while middle cerebral artery blood flow velocity (CBF; Doppler ultrasonography), heart rate (HR), blood pressure (BP; Finometer), oxygen consumption rate (VO_2), cardiac output (CO; cZ method) and total peripheral resistance (TPR) were measured. All subjects repeated 8 trials, intermitted by >5-min rest. In 4 trials randomly selected from the 8 trials, exercise onset was signalled by a 30-sec CD, whereas in the remaining 4 trials, exercise was started without CD.

[Results] During the CD, CBF, HR, CO and BP increased, TPR decreased, and VO_2 increased. When we determined individual areas under the curve (AUC) of the responses for each variable from their baselines during the 30-sec CD, the AUC of CBF and HR varied markedly among the subjects and were positively correlated with VO_{2peak} (both, $P<0.001$), whereas that of TPR was inversely correlated with VO_{2peak} ($P<0.001$).

[Conclusion] In young men, individual variations in cerebro-cardiovascular responses to CD before starting exercise were associated with VO_{2peak}. (COI: No)

1P-021

Unloading-induced sarcopenia in relation to mitochondrial disorder in skeletal muscle of old rats

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Purpose: Recent study with in vivo gene transfection has demonstrated that mitochondrial calcium uniporter (MCU) overexpression counteracts denervation-induced sarcopenia through activation of mitochondrial biogenesis and signaling pathways for hypertrophy (Cell report 10, 1269-79, 2015). Here, we tested the role of MCU in more physiological models of sarcopenia in old rats.

Methods: Old F344 female rats (2 years, n=21) were divided into control, unloading, and unloading + intermittent resistance-exercise (IRE) groups. Middle aged rats (1 year, n=7) were used as adult-control. Rats of unloading and unloading + IRE groups have their hindlimbs unloaded by tail-suspension during 3 weeks. 10-min IRE was performed in unloading + IRE group 3 times per day every 4 hour in dark period.

Results: Compared with adult-control, old control rats showed significantly smaller mass in fast-twitch planter-flexor muscles. Unloading seriously disrupted myofibrilar structure with a decrease in sarcomeric proteins and accumulation of abnormal mitochondria in type I fibers of soleus and lateral gastrocnemius muscles. Unloading-induced atrophy was more prominent in soleus, although IRE ameliorated atrophy in soleus as well as in fast-twitch planter-flexor muscles. Unloading decreased the protein expressions of MCU, PGC1, and LC3-II, and IRE increased the protein expressions of parkin, MCU, and LC3-II in soleus, but not in plantaris.

Conclusion: MCU specifically controls atrophy in unloading-condition. (COI: No)

1P-022

The effect of warm/cool stimulus to forearm/hand on brachial artery blood flow during leg exercise

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[Aim] brachial artery blood flow (BA-BF) increases during leg cycling exercise. This appears the key factor for endothelial adaptation of inactive upper limb beyond active lower limb. While BA supplies mainly skin blood flow (SBF) to both glabrous (palm; G) and non-glabrous (dorsal and forearm; NG) skins, it remains unclear which SBF is responsible to upstream BA-BF. The purpose of this study was, therefore, to elucidate the effect of G and/or NG SBFs modulated by warming/cooling on BA-BF responses during leg exercise. [Method] BA-BF and SBFs in forearm (f) and palm (p) were measured during 60 min of leg cycling exercise in seven healthy subjects. Between 20 and 50 min during exercise, either NG+G or G skins were warmed (43°C) or cooled (15°C) using by water bath. The study was approved by the Ethics Committee of the Prefectural University of Hiroshima (HH009). [Results] At 20 min during exercise, f-SBF and BA-BF significantly increased from resting baseline in all 4 trials. After that, when either NG+G or G skins were warmed during exercise, additional increases in f-SBF and a concomitant BA-BF were observed. Contrarily, when NG+G skins were cooled, p-SBF and BA-BF was slightly, but significantly decreased. [Conclusions] The result suggest that SBF response to NG skin plays a major role in increased BA-BF during leg exercise. (COI: No)

1P-023

Timing of nutrient intake after mild exercise: effects of gastrointestinal activity in humans

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[Purpose] We previously reported that nutrient ingestion immediately after strenuous exercise acutely delayed gastric emptying (GE) rate, which subsequently affected blood glucose (BG) and plasma branched-chain amino acids levels in blood circulation. However, no data exist on the GE rate after mild exercise. Therefore, we investigated the effect of the timing of carbohydrate supplementation after mild leg cycling exercise on GE rate, gastrointestinal blood flow (GBF) and BG. [Methods] Eight healthy young subjects performed the continuous leg cycling for 30 min. They ingested 300g of 15% glucose drink at the timing of either 5 min or 30 min after the cessation of exercise. As a control, same drink ingestion without exercise was performed. The blood flow of GBF and GE rate were assessed by ultrasonography. Capillary blood samples were collected via left index or middle finger skin pricks before exercise (baseline), immediately after exercise, just before and at 15, 30, 45 and 60 after ingestion of glucose drink. The study was approved by the Ethics Committee of the Prefectural University of Hiroshima (HH007). [Results] After ingestion of glucose drink, GE rate, GBF and BG were not significant differences among three trials. [Conclusions] The timing of nutrient ingestion after mild exercise might not impact the following gastrointestinal physiological responses. (COI: Properly Declared)

1P-024

Effects of continuous exercise with vocalization on the oxygen dissociation states in muscles

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[Purpose] We reported that vocalization during the continuous upper and lower body exercise at 80%VO_2peak tend to increase the value of $FetCO_2$ (=$PaCO_2$), and to change the peripheral circulation states. In this study, we investigated whether the high value of $FetCO_2$ caused by vocalization during the upper body exercise affects the oxygen dissociation states in exercising muscles. [Methods] 8 male subjects participated in this study. They performed the continuous upper body exercise using hand ergometer at 80%VO_2peak for 3-min under 2-conditions of "with vocalization (Voc)" or "without vocalization (non-Voc)". We measured respiratory variables ($FetCO_2$, etc) and tissue saturation index (TSI%) of triceps brachii (using near-infrared spectroscopy (NIRS)) during exercise for 1-min between the 2nd and 3rd min, and SpO_2 at just after exercise. [Results] The value of variation in $FetCO_2$ in Voc tended to increase compared to that in non-Voc. There was no difference between Voc and non-Voc in the value of SpO_2. The value of variation in TSI% in Voc tended to be suppressed decline compared to that in non-Voc. [Conclusions] The high value of $FetCO_2$ caused by vocalization during the upper body exercise tended to supply more O_2 to exercising muscles. Vocalization during exercise may control the oxygen dissociation states in exercising muscles. (COI: No)

1P-025

The salivary 11β-HSD2 activities is beneficial for continuous strength exercises in elderly people

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PURPOSE; Japan has the highest proportion of older adults and "Super-aged society" in the world. The Japanese Orthopedic Association proposed a concept called locomotive syndrome (LS) to identify middle-aged and older adults at high risk of requiring health care services because of problems with locomotion-associated lower muscle mass. The present study was designed to investigated the effect of salivary 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activities in the effective muscle strengthening exercise with increased muscle weight and basal metabolism to prevent LS by the measurement of salivary cortisone and salivary cortisol.
METHOD; The12 men and 14 women were assessed at baseline and 6 months. Body composition, physical strength and salivary cortisol and cortisone were analyzed. The exercise intervention program was performed by individual muscle endurance level.
RESULT; Body weight, muscle weight and basal metabolism were increased after exercise intervention. The 30-second sit-up test and 3-minute walking were increased, and the 10-time sit-to-stand was decreased significantly. This may be related to increase of leg and abdominal muscular strength. The exercise intervention program increased salivary 11β-HSD2 activities significantly.
CONCLUSION; These findings show that exercise associated with saliva cortisol as an index may be beneficial and should be considered in the overall management of middle-aged and older adults. (COI: Properly Declared)

1P-026

The differential dynamics of brachial artery and forearm skin blood flows during leg cycle exercise

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[Aims] While the elevated blood flow in brachial artery (BF-BA) during prolonged leg cycling exercise seemed to be induced by the increased forearm skin BF (SBF) for the thermoregulation, the relation of dynamic property of each other is still unclear. To clarify as to whether the BF-BA dynamics would be associated with its downstream f-SBF, we used the leg cycling exercise of sinusoidal (sine) fluctuating work rate (WR). [Methods] Nine healthy subjects performed leg ergometer exercise with a constant WR (mean of the sinusoidal WR) for 30-min followed by 16-min sine WR exercises of 4-min period fluctuated between 20 W to 60 % VO2max. During protocols, we continuously measured pulmonary gas exchange, heart rate, blood velocity and cross sectional area of BA, and SBF and sweating rate in forearm and palm. The measured and calculated variables: y(t) were fitted as $y(t) = M + A*\sin((2\pi/T)*t-\theta)$, where t: time, A/M: relative amplitude, T: period (=240 s), θ: phase. [Results] Almost variables were followed by the sine WR changes adequately, so that the phases (θ) of BF-BA and forearm and palm SBFs were compared. The responses of BF-BA and palm SBF showed an anti-phase (θ: approx. 180º) and was apparently disassociated to that of forearm SBF (θ: approx. 60º). [Conclusions] During sine WR leg cycling exercise, the dynamics of BF-BA was not reflected by the forearm BF, but seemed to be determined by the BF to palm and/or non-active skeletal muscles. (COI: No)

1P-027

Molecular hydrogen increases acetone excretion and changes lipid metabolism during exercise

Amane Hori; Ryota Masuda; Masatoshi Ichihara; Hisayoshi Ogata; Takaharu Kondo; Norio Hotta (*Chubu University, Japan*)

[Purpose] Many studies have reported that hydrogen (H_2) shows beneficial biological effects against many diseases involving inflammation, apoptosis, dysmetabolism, and oxidative stress; however, the number of studies on the effects of H_2 in humans during exercise is limited. In this study, we investigated the effects of H_2 inhalation on lipid metabolism in the human liver during exercise by measuring acetone excretion in the exhaled air. **[Methods]** Ten male subjects underwent 20-min submaximal (60% peak oxygen uptake) cycling after a 14-h fast. We calculated acetone excretion from the acetone concentration and minute ventilation during the test under H_2 gas inhalation (1% H_2, 21% O_2, 0% CO_2, and 78% N_2) and control gas inhalation (21% O_2, 0% CO_2, and 79% N_2). In addition, we tested the effects of H_2 gas inhalation on acetone excretion during the 35-min rest period (n=5). **[Results]** The exercise significantly ($P<0.01$) increased acetone excretion. In addition, H_2 gas significantly ($P<0.01$) augmented the exercise-induced increase in acetone excretion. However, itdid not increase acetone excretion during the rest period ($P=0.9$). **[Conclusions]** H_2 gas inhalation significantly increased expired acetone levels during exercise but not during rest. These results suggested that the interaction between H_2 inhalation and exercise may increase lipid metabolism in the liver during exercise. (COI: No)

1P-028

Combining Acute Exercise With Insulin Treatment increase Type 1 Diabetic Liver Antioxidant Capacity

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Purpose: To observe whether combining acute exercise with insulin treatment could improve antioxidant capacity of diabetic liver. **Method:** Study 1: Acute Exercise with Insulin Treatment to 18 weeks old SD Rats. Rats were divided into 6 groups: Control group (C); Diabetes group(S); Diabetes with insulin treatment(SI); Control with Acute Exercise(EC); Diabetes with Acute Exercise(ES). Study 2: Insulin Treatment to 22 weeks old SD Rats. Rats were divided into 3 groups: Control group(OC); Diabetes group (OS), Diabetes with insulin treatment(OSI). After one week of acclimatize, rats from STZ groups were injected with STZ. Then we measured fasting blood glucose by blood glucose meter. When blood glucose level reached 300 mg/dL or above, started injected insulin(0.25 IU/ml) twice daily. After 14 days of feeding, pentobarbital(40 mg/kg) was injected to test body composition by DXA. Liver and perirenal fat was took in sacrifice to have TBARS and Glutathione analysis. **Result:** Diabetes in the old rats cause rapid decline on body weight($p<0.01$), insulin treatment slow down the loss of BW($p<0.01$). For rats with acute exercise, the antioxidant capacity of glutathione was increased($p<0.05$). Insulin treatment with acute exercise can increase antioxidant capacity and decrease oxidative stress of liver($p<0.05$). **Conclusion:** Insulin treatment controlled diabetic weight loss. Acute exercise with insulin treatment contributed to increase antioxidant capacity and decrease oxidative stress.

(COI: Properly Declared)

1P-029

Longitudinal changes of trunk skeletal muscle characteristics in Japanese elderly males and females

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[Purpose] This study aimed to evaluate the 1-year change of the quantity and quality in trunk skeletal muscle in Japanese elderly. [Methods] Community-dwelling 26 males and 21 females (72.2±5.2 years, body mass index: 22.4±2.4 kg/m2) participated in this study. They came to our laboratory twice with 1-year interval. At each time, their height, body weight, waist circumference, body fat, and physical functional scores: hand grip strength, times of sit-up, supine-up, sit-to-stand, and 5-m maximal walk and distance of 6 minutes' walk were recorded. The thickness of rectus abdominis and lumber multifidus muscles (index of skeletal muscle quantity), and subcutaneous fat above those two muscles, and echo intensities of each two muscles (index of muscle quality) were measured by ultrasonography. [Results] After 1-year, although the body weight did not significantly change, abdominal subcutaneous fat thickness significantly increased in both sex (9.3% for males and 9.1% for females). In females, skeletal muscle thickness of the rectus abdominis and the lumbar multifidus muscles significantly decreased (-6.5% and -5.7%), and the echo intensity of the lumbar multifidus muscle significantly increased (14.7%). There was no significant change in other parameters in both sexes. [Conclusions] In the case of elder females, there is a possibility that trunk skeletal muscle quantity and quality might significantly change even if the morphological data did not significantly change. (COI: No)

1P-030

Relationship between occlusal balance and agility in Japanese elite female junior badminton players

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The aim of this study was to examine the relationship between occlusal balance and motor ability in Japanese elite female junior badminton players. Participants were 35 female badminton players nominated as junior Japan representative candidates by the Nippon Badminton Association. They were divided into two groups according to stability of occlusal balance: 16 participants (mean age: 12.4±1.2 years) with stable occlusal balance (stable group) and 19 participants (12.3±1.4 years) with unstable occlusal balance (unstable group). Occlusal state were analyzed using a pressure-sensitive film and an analysis apparatus. The following sports tests were conducted according to the rules of the Nippon Badminton Association: sit-ups, side steps, jump rope (double jump 1 min), and sprint (50-m sprint). Differences in occlusal contact area, occlusal force, and sports test results between the stable and unstable groups were analyzed. Occlusal contact area and occlusal force were significantly higher in the stable group than in the unstable group. The stable group showed superior results in the side steps and jump rope tests compared with the unstable group. No significant differences were observed between the two groups in the sit-ups or sprint tests. By examining the relationship between occlusal balance and motor ability in Japanese elite female junior badminton players, we found that occlusal balance is associated with agility and stabilization of the trunk. (COI: No)

1P-031

Estimation of maximal oxygen uptake from oxygen uptake efficiency slope by leg or arm ergometer

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Purpose
We tried to investigate 1) the influence of exercise modes, i.e., treadmill, bicycle, and arm cranking, on the measurements of oxygen uptake efficiency slope (OUES), and 2) the validity of the estimated maximal oxygen uptake (VO_{2max}) from the OUES measure by leg cycling or arm cranking exercise.
Methods
Twenty-two healthy young male volunteers (mean age: 21.3 y.o.) participated in the three types of incremental exercise testing until exhaustion. VO_{2max} was obtained from treadmill exercise testing. Criteria for maximal exercise were: 1) plateau in VO_2, or 2) the achievement of gas exchange ratio > 1.15 and heart rate exceeding 90% of age-predicted maximal values.
Results
Peak oxygen uptake (VO_{2peak}) and the OUES showed a close linear relationship: VO_{2peak} = 0.7649•OUES + 499 (r = 0.959), with the average errors of estimated VO_{2peak} of 168 ml/min (s.d. = 141), or 7.3 % (s.d. = 7.9), irrespective of the exercise modes. Estimated VO_{2max} from the OUES in leg cycling and arm cranking exercise testing were: VO_{2max} = 0.6322•OUES (leg cycling) + 144 (r = 0.814), or VO_{2max} = 0.8302•OUES (arm cranking) + 1857 (r = 0.760), respectively. The use of submaximal OUES (using the gas exchange data of 80% of exercise time) did not greatly affect these results.
Conclusion
These results provide us with further clinical application of OUES, especially in subjects who cannot run on the treadmill due to orthopedic or neurologic conditions even if they cannot reach maximal exercise. (COI: No)

1P-032

Effect of low-volume high-intensity interval exercise on post-exercise inhibitory control

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[Purpose] We previously reported that post-exercise inhibitory control (IC) improvements are associated with increased amount of exercise volume. Yet, a standard high-intensity interval exercise (HIIE) can maintain post-exercise IC improvements compared to volume-matched moderate-intensity continuous exercise (MCE); in other words, particularly exercise intensity may play an important role in post-exercise IC. We hypothesized that even though the volume of exercise is reduced, post-exercise IC improvements would be maintained by low-volume HIIE (LV-HIIE) as much as MCE. To address this hypothesis, we aimed to examine the effect of LV-HIIE on post-exercise IC compared to MCE.
[Methods] Fifteen healthy men performed cycling exercise with either LV-HIIE or MCE. The LV-HIIE was consisted of ten 1-min bouts at 90% of $VO_{2\ peak}$ with 1-min active recovery at 30% of $VO_{2\ peak}$, which was performed for a total 20 min. The MCE was performed for 40 min at 60% of $VO_{2\ peak}$. To evaluate IC, the Stroop test was administered before exercise, immediately after exercise, and every 10 min during the 30-min post-exercise recovery period.
[Results] The post-exercise improvements of IC were observed in both LV-HIIE and MCE. The levels of post-exercise IC improvements did not differ significantly between the two conditions.
[Conclusion] Given that LV-HIIE can similarly improve post-exercise IC compared to MCE, despite exercise duration and volume are half, the LV-HIIE may be effective in improving IC. (COI: No)

1P-033

Atrioventricular nodal function during dynamic exercise in elite endurance athletes

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Purpose: When HR exceeds 90–100 beats/min during dynamic exercise, the atrioventricular (AV) nodal mechanism will function to cancel fluctuation in PP interval within one beat and keep RR interval constant. It still remains unknown how endurance training modifies AV nodal function during exercise, we compared the responses in PP, PR, RR intervals to dynamic exercise between sedentary and endurance trained subjects.
Methods: Nine sedentary and seven professional long-distance runners participated in this study. Subjects performed cycle ergometer exercise, and increased HR from the baseline at different 4 levels (100, 120, 140, 160 beats/min). The negative slope of the relationship between PP interval and the subsequent changes in PR interval, which considered as sensitivity of the AV nodal function, was plotted against the average level of PP interval. Then, by fitting a sigmoidal curve, the lower and higher plateau levels were estimated.
***Results*:** The lower threshold level was significantly lower in endurance athletes than sedentary subjects (90 vs. 100 beats/min). The higher plateau level was also significantly lower in endurance athletes than sedentary subjects (110 vs. 125 beats/min).
***Conclusions*:** Endurance training modifies AV nodal function during exercise, which starts to operate and fully functions at lower HR level. (COI: No)

1P-035

The influence of aerobics dance exercise on energy intake, appetite, and mood in young women

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[Purpose] The purpose of study was to examine the influence of aerobics dance exercise (ADEX) on appetite and energy intake.
[Methods] With the use of a crossover design, 11 young women completed two 1-h experimental conditions: sedentary (SED) and ADEX followed by an ad libitum lunch. Heart rate (HR) was assessed throughout each 1-hour experimental condition. Mood affect were measured by using the Profile of Mood States 2nd Edition (POMS2) at two time points during only exercise day. Energy intake (EI) was determined by measuring food intake at lunch. Physiological stress was assessed using amylase activity in salivary (AAS). Appetite sensations were assessed using visual analog scales at three time points during the testing day.
[Results] Average and maximum HR during the ADEX condition were significantly higher than during the SED conditions. Total, positive, and negative mood disturbance scores were not statistically different between pre and post ADEX. EI at lunch was not statistically different between after the SED and ADEX. AAS and appetite sensations were similar between conditions at all time points. Changes of each mood disturbance score in POMS2 and change of amylase activity were not statistically correlated with EI in ADEX day.
[Conclusions] We revealed that such ADEX did not affect EI, appetite and mood in young women. Moreover, our study suggested that change of mood by ADEX did not relate change of EI. (COI: No)

1P-036

Shortening velocity of knee extensor in frog *in vivo*

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The present study was investigated to know how the shortening velocity (SV) of knee extensor was influenced by the contraction of other lower-limb muscles. We measured the force-velocity relationship (F-V), *in vivo*, in whole muscle preparations in knee extensor (TFM) of the frog, *Rana catesbeiana* (N = 5).
Frogs were anesthetized by injecting urethane intraperitonially. Their isotonic SV and the isometric forces were measured at various steps of load. SVs and loads (forces) were expressed by the normalized values by the bone length of thigh (BL) and the maximum isometric force (Po) respectively. In the first preparations (TC), all the muscles of thigh were contracted by stimulating sciatic nerve. Next, the connecting tissues between muscles of thigh was cut and separated partially into TFM and other limb muscles after the recording in TC (TCS).
On the F-V in TC, the SV at lighter load of 0-0.6Po (LL) followed the Hill's equation but at heavier load of 0.6-1.0Po (HL) it did not follow. SV decreased rapidly above 0.6Po and reached 0 at 0.84Po. The curve of SV in TCS was nearly the same as in TC at LL and reached 0 at 0.91Po at HL. The maximum SV (5.8±0.4 BL/s) in TC was appreciably faster than that (5.3±0.4 BL/s) in TCS (P = 0.051).
These results indicate that the mechanical kinetics of muscle is influenced by the contraction of neighboring ones and that the F-V in HL range does not follow the Hill's equation, thereby stabilizing and protecting knee joint at HL. (COI: No)

1P-037

CO_2-water bath promotes a recovery from the muscle fatigue induced by high intensity exercise

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In response to the CO_2-water bath, the reduction of sympathetic nerve activity may imply the facilitation of muscle fatigue recovery. To investigate whether the whole body bath with CO_2-water (CO_2≥1000 ppm) influences recovery of the muscle fatigue after high intensity exercise. Healthy male subjects (n=6, 18-21yrs, 171.3±6.7cm, 73.6±13.0kg) participated in this study. Core temperature (CoreT) and ECG were recorded continuously throughout the experiment. The subjects performed 30-s maximal pedaling exercise, and took bath in tap- or CO_2-water at 35 °C for 10 minute after exercise. Subjective thermal sensation (TS) in the body bath was also recorded. Vastus lateralis (VL) dominant muscle hardness was evaluated using the elastography. Evaluation of blood lactate (BLa) and visual analog scale of muscle (VAS) were performed at pre- and immediately after-exercise, and at 10 min after exercise. The strain ratio (SR) between the VL and a reference material was calculated. TS in the CO_2-water was significantly higher than in the tap-water (tap-water vs. CO_2-water, -0.17±0.76 vs. 1.17±0.41, $p<0.01$). At 10 min in recovery, in the CO_2-water compared with the tap-water, SR significantly decreased quicker (0.49±0.25 vs. 0.91±0.25, $p<0.01$). However, there was no significant difference in CoreT, BLa and VAS between these two water kinds. The present results suggested that CO_2-water bath may contribute to rapid recovery from the muscular hardness induced by high intensity exercise. (COI: No)

1P-038

How does voluntary exercise frequency affect cardiac function in dilated cardiomyopathy model mice?

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Dilated cardiomyopathy (DCM) is one of major causes of heart failure (HF). The effects of exercise on patients with inherited DCM have not been established because DCM is associated with high risk of worsening HF and sudden death (SD). A knock-in mouse model of human inherited DCM, TNNT2 ΔK210, shows similar characteristics to DCM patients and is useful model for evaluating therapeutic effects. We aimed to examine how the influence on cardiac function differs depending on amount of voluntary exercise using this model mouse. Homozygous ΔK210 (DCM) mice showed enlarged heart and frequent SD with $t_{1/2}$ of ~70 days. DCM mice were divided into 3 groups based on the frequency of voluntary exercise: no exercise control (no-ex), every 2 days (2D) and daily exercise (ED). The 2D and ED groups started running at 1 month of age. At the 2 months of age, mice were sacrificed after an investigation with echocardiography, and their heart, lung, lower extremity muscles (soleus, plantaris and gastrocnemius) and body weights were measured. Gene expressions of HF- and arrhythmia-related genes in myocardium were quantified by qPCR analysis. The weights of soleus muscles were significantly and similarly increased in 2D and ED groups. The ejection fraction(EF) was significantly improved in ED group (36.9±9.0%) compared with 2D (24.7±6.4%) and no-ex groups(21.2±7.3%). We further discuss the relationship between exercise intensity and electrical remodeling. (COI: No)

1P-039

Effect of lower body positive pressure and walking on fluid turnover in human legs

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We have developed a rehabilitation device with a treadmill in a lower body positive pressure (LBPP) chamber that can unload the lower extremities of patients. The application of LBPP possibly prevents development of edema in the legs because the positive pressure may reduce fluid filtration through a decrease in the transmural pressure gradient in the capillaries. We hypothesized that LBPP and walking exercise exert additive effects for improving gravity-induced edema in the legs of upright humans. To test this hypothesis, we first examined changes in the circumferences of the thigh, calf and ankle after three procedures (WALK, 10 minutes walking without LBPP; LBPP, 10 minutes standing still with 15 mmHg LBPP; and LBPP+WALK, 10 minutes walking with 15 mmHg LBPP). Secondly, fluid retention of the calf was examined using bioelectrical impedance analysis after 10 minutes walking with 15 mmHg LBPP. While the patients stood still before the test, the circumferences increased gradually in each group and reached a plateau level after 30 minutes and impedance of the calf decreased. After LBPP+WALK, the circumference decreased significantly in all three regions . The reduction in the circumference of the thigh was greater after LBPP+WALK than after WALK or LBPP. These results suggest that LBPP and walking exercise have an additive effect on the protection from edema in human legs. (COI: No)

1P-040

Changes in weight bearing index (WBI) before and after skyrunning in Mt. Fuji

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BACKGROUNDS&AIM: According to the ISF, Skyrunning (SR) is defined as an activity that is an extreme form of mountain running in the area above 2,000 meters where the running course includes the slope with the inclination of more than 30%. For skyrunners, we examined the effects of 1,800 m in altitude difference on lower limb muscle strength.

METHODS: Ten healthy 10 adults (age: 33.9±6.5 yrs, VO2max: 64.7±10.2 ml / kg·min) were subjected to lower limb muscle force measurements around SR@ Mt. Fuji from altitude 1500 m to altitude 3300 m. Lower limb muscular strength was calculated for weight bearing index(WBI).

RESULTS: The mean of WBI before SR was 1.12±0.17 on the right leg and 1.11±0.23 on the left leg, and statistically no left / right difference was observed (p> 0.05). The WBI after SR was 1.04±0.18 on the right leg, which was not statistically different from the pre-SR value (NS), but was significantly lower than the pre-SR value by 0.95±0.14 on the left leg (p <0.03). The elapsed time of SR took: 2:18:55±0:30:02 in climbing, 0:38:15±0:13:36 on the descending. The total time was 2:57:10±0:41:21.

CONCLUSIONS: It was shown that lower limb muscular strength after SR with altitude difference of 1,800 m decreased by 10% on average on WBI. In this study, we can figure out detailed change of lower limb muscle strength which could not be grasped by simple test, and these results are expected to lead to conditioning and reconditioning in SR race and training. (COI: No)

1P-041

Electrophysiological analyses of multi-ion channel blockers in hiPSC-CMs sheets with MEA system

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[Purpose] We examined electrophysiological indices of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) sheets in order to quantitatively estimate Na^+, K^+ and Ca^{2+} channel blocking actions of bepridil and amiodarone using microelectrode array (MEA) system in comparison with that of E-4031. [Methods] We analyzed the field potential duration, effective refractory period, current threshold and conduction property using a programmed electrical stimulation protocol to obtain the post repolarization refractoriness and coefficient *a* of the relationship between the pacing cycle length and field potential duration. [Results & Conclusions] Electropharmacological profile of each drug was successfully characterized; namely, 1) Na^+ channel blocking kinetics was estimated by the changes in the current threshold and conduction property, 2) drug-induced inhibition of human ether-à-go-go-related gene (hERG) K^+ channel was reflected in the relationship between pacing cycle length and field potential duration, 3) the relative contribution of these drugs to Na^+ and K^+ channel blockade was represented by the post repolarization refractoriness, and 4) L-type Ca^{2+} channel blocking action was more obvious in the field potential waveform of the hiPSC-CMs sheets than that expected in the electrocardiogram in humans, which will help to predict whether the net balance of Ca^{2+} and K^+ channel blockade of a drug is proarrhythmic or antiarrhythmic. (COI: No)

1P-042

A CMOS camera depicted the excitation spread during arrhythmia in an isolated rat atrial preparation

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Using a complementary metal-oxide semiconductor image sensor camera (CMOS) and a voltage-sensitive absorption dye (NK2761), we visualized the excitation spread during experimental tachyarrhythmia (tachycardia-like excitation, TE) in an isolated rat atrial preparation. We recorded optical action potentials simultaneously from 256 contiguous sites in the preparation using a 16 X 16 -element photodiode array (PDA) and mapped the excitation spread pattern during TE. In order to improve the spatial resolution of the optical imaging and to directly visualize the spatiotemporal pattern of the excitation spread, we tried to use a CMOS camera (ORCA flash4.0, Hamamatsu Photonics Ltd., Hamamatsu, Japan, or Zyla 5.5 10-tap, Andor Technology Ltd., Belfast, UK) as the photodetector. We could record the optical action potential with a spatial resolution of 1000 X 1000 (ORCA) or 1392 X 1040 (Zyla) and a temporal resolution of about 100 frame/second. Using digital image processing, we succeeded in visualizing the excitation spread during TE and made video clips of the excitation spread. In these movies, we demonstrated the circus movement of the excitatory wave (i.e. micro re-entry) and the abnormal automatism during TE. This method could be a new tool for the study of basic pathoelectoropysiology of atrial fibrillation. This study was approved by the Animal Care and Use Committee, University of the Ryukyus, and was conducted in accordance with its recommendations. (COI: No)

1P-043

Potential link between Ca^{2+}-activated cation TRPM4 channels and I_{st} in mouse cardiac pacemaker cells

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We have recently reported that L-type $Ca_V1.3$ Ca^{2+} channels are involved in the generation of a nifedipine-sensitive Na^+ current, previously described as the sustained inward current, I_{st}, in heart pacemaker cells. This introduces a new concept that $Ca_V1.3$ channels serve a dual role in the pacemaker potential as a source of Ca^{2+} entry and as a persistent inward Na^+ current. However, currently available recombinant $Ca_V1.3$ channels are highly selective for $Ca^{2+}$and it remains a challenge to elucidate the molecular mechanism allowing $Ca_V1.3$ channels to generate a Na^+current. Here we show that I_{st} is inhibited by 9-phenathrol and flufenamic acid, both are known to block TRPM4 Ca^{2+}-activated cation channels. In addition, reduction in I_{st} was observed either by intracellular BAPTA loadings or by srcoplasmic reticulum block with ryanodine and thapsigargin. These results suggest that I_{st} is a Ca^{2+}-activated Na^+ current through TRPM4 channels. Combined measurements of membrane currents and intracellular Ca^{2+} showed that I_{st} activation was accompanied by a sustained elevation of intracellular Ca^{2+}. However, intracellular Ca^{2+} dynamics was largely dependent on Ca^{2+} influx through L-type Ca^{2+}channels, while I_{st} did not decrease by lowering external Ca^{2+}. Our data, although not conclusive, support the hypothesis that functional coupling of $Ca_V1.3$ and TRPM4 channels via intracellular Ca^{2+}mediates I_{st} in cardiac pacemaker cells. We await the results of ongoing study using TRPM4 knockout mice. (COI: No)

1P-044

Functional role of delayed rectifier K^+ current in the automaticity of pulmonary vein cardiomyocytes

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Pulmonary veins contain a myocardial layer and its electrical activity is considered to underlie their arrhythmogenicity in the heart. Here, we examined the functional role of delayed rectifier K^+ current (I_{Ks}), which is one of the contributors to cardiac repolarization in the spontaneous action potentials of pulmonary vein cardiomyocytes (PVC). The spontaneous action potential and membrane currents were recorded from PVC using perforated patch-clamp mode and conventional whole-cell patch-clamp mode. The PVC exhibited spontaneous action potentials in the normal Tyrode solution with an average firing rate of 166±5/min (n=6). Bath application of the selective I_{Ks} blocker HMR-1556 decreased the firing rate. β-adrenergic stimulate isoproterenol (100 nM) increased firing rate of the action potentials. Concomitant addition of HMR-1556 markedly reduced the firing rate compared with control condition. We also found that I_{Ks} was strongly enhanced by both β_1- and β_2-adrenoceptor (AR) stimulation with a negative voltage shift in the current activation. Both β_1-AR and β_2-AR mediated enhancements of I_{Ks} were markedly attenuated by the treatments with adenylyl cyclase inhibitor SQ22536 or protein kinase A (PKA) inhibitor H89. Our results suggest that in guinea pig PVC the functional role of I_{Ks} in spontaneous automaticity increases during sympathetic excitation, and the effect of β-adrenergic stimulations on I_{Ks} activation is mediated through a cAMP-PKA pathway.

(COI: No)

1P-045

Pacemaking ion channel remodelling underlies chronic exercise-induced atrioventricular block

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There is a higher incidence of various bradyarrhythmias including bradycardia and atrioventricular (AV) block in veteran endurance athletes. We previously reported molecular remodelling of cardiac ion channels, induced by altered expression of transcription factors and microRNAs in the sinoatrial node, as a key cause of exercise-induced bradycardia. However, the mechanisms underlying AV block in athletes has not yet been investigated.

We instituted a protocol of long-term swimming (60 min/day, 5 days/week for 5 months) in 10-week-old male mice and assessed the electrophysiology and molecular profile of the AV node (AVN). Trained mice showed prolonged PR intervals during ECG recordings and an increased Wenckebach cycle length and AVN effective refractory period under *in vivo* programmed electrical stimulation. These alterations were concomitant with widespread downregulation of ion channels in the trained AVN. Notably, the pacemaker HCN4 channel and the L-type Ca^{2+} channel subunit $Ca_V1.2$ were decreased at mRNA and protein levels resulting in a corresponding decrease in I_f and $I_{Ca,L}$ current density in the AVN of trained mice.

These results demonstrate that chronic endurance exercise causes AVN dysfunction characterized by significant transcriptional remodelling, corresponding to reduced current density of key ionic currents involved in AVN impulse generation and conduction. We conclude that AVN electrical remodelling is a key mechanism underlying heart block in athletes. (COI: No)

1P-046

Cardiac Iron Overload: Impacts on Cellular Electrophysiology and Calcium Handling

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Purpose: Iron (Fe) overload cardiomyopathy is the leading cause of death in hemochromatotic patients, yet the mechanistic insight is still incomplete. We investigated alterations of action potentials (APs) and intracellular Ca^{2+} ($[Ca^{2+}]_i$) in Fe-loaded left ventricular cardiomyocytes (LVCMs), as well as functional impacts of Fe overload on single-cell contraction and whole-heart arrhythmias.

Methods: Isolated mouse LVCMs were superfused with Fe^{3+}/8-hydroxyquinoline (8-HQ) complex (5-100 μM). $[Ca^{2+}]_i$ was evaluated by Fluo-4 fluorescence assay. APs, L-type Ca^{2+} current ($I_{Ca,L}$), and total outward K^+ current (I_K) were recorded by the patch-clamp technique. Cell contraction was measured by a video-based edge detection system. Arrhythmias were evaluated in Langendorff-perfused hearts under S_1-S_2 stimulation protocol.

Results: Fe treatment prolonged AP durations (APD) at 90% repolarization (46.8±2.8 vs. 203.6±63.4 ms, $p<0.05$), induced early and delayed afterdepolarizations (EAD: 0.0±0.0 vs. 45.0±15.0% and DAD: 4.3±1.4 vs. 27.0±7.0%, $p<0.05$) in LVCMs. It also decreased peak $I_{Ca,L}$ (16.5±1.7 vs.11.4±1.3 pA/pF, $p<0.01$), decreased contractility (4.8±0.5 vs.3.5±0.4% $p<0.01$) and altered Ca^{2+} transient patterns. Arrhythmia incidence was increased in Fe^{3+}/8-HQ-perfused hearts. The mechanism for increased APD is under further study.

Conclusions: In LVCMs, Fe overload induced arrhythmogenic AP prolongation and afterdepolarizations, aberrant $[Ca^{2+}]_i$ dynamics, and impaired contractility. (COI: No)

1P-047

Species difference of the hyperpolarized-activated current in pulmonary vein cardiomyocytes

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Ectopic activity of pulmonary vein (PV) cardiomyocytes is thought to be responsible for initiation and maintenance of atrial fibrillation. It is well known that PV cardiomyocytes have different electrophysiological properties from those of working cardiomyocytes, thereby leading to easily generate spontaneous activity. It has been reported that hyperpolarization-activated cation current (Ih) which promotes slow diastolic depolarization in normal pacemaker cells of sinoatrial node, exists in PV cardiomyocytes. On the other hand, a Cl- current with a similar slow time course of activation (ICl,h) has been recorded in rat PV cardiomyocytes. Also, a K+ current was identified as a hyperpolarization-activated current in dog PV cardiomyocytes.

In present study, we examined ionic nature of hyperpolarization-activated current of PV cardiomyocytes in various species including rats, guinea-pigs and rabbits. The results showed that guinea-pig and rat PV cardiomyocytes possessed sizeable amplitudes of hyperpolarization activation current, among which the current in guinea-pig was suppressed by Cs, a blocker of Ih, whereas in rats the current was not suppressed by Cs, but by Cd, a blocker of ICl,h. The current density of the hyperpolarized-activated current of rabbit PV cardiomyocytes was significantly smaller than those of other species. It is suggested that the ion channel that carries the pacemaker current of PV cardiomyocytes differ depending on the animal species. (COI: No)

1P-048

The mitochondrial Na^+-Ca^{2+} exchanger is involved in automaticity of murine sinoatrial nodal cells

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It has been widely accepted that the automaticity of sinoatrial nodal cells (SNCs) attributes to the activities of ion channels at the plasma membrane. However, after the discovery of spontaneous local calcium release (LCR) from the sarcoplasmic reticulum in rabbit SNCs, there is growing evidence that LCR regulates the pacemaker activity. Previously, we have reported that the mitochondrial Na^+-Ca^{2+} exchanger, NCXm, regulates automaticity of HL-1 cardiomyocytes. While, the functional role of NCXm in generating LCR and automaticity in SNCs has not been clearly demonstrated. **Purpose:** Here we investigated if NCXm contributes to the generation of LCR and pacemaker activity of murine SNCs. **Methods:** Ca^{2+} imaging was performed on isolated murine SNCs, and an NCXm antagonist, CGP-37157 (1 μM), was applied to a bath solution. **Results:** LCRs were detected between rhythmic firing of Ca^{2+} transients in almost all SNCs, and there was a positive correlation between LCR period (time from the prior Ca^{2+} transient peak to the LCR occurrence) and Ca^{2+} transient cycle length (CL). Application of CGP-37157 significantly reduced the LCR amplitude and prolonged the LCR period as well as the CL. On the other hand, beta-adrenergic stimulation by isoprenaline significantly increased LCR amplitude and shortened the LCR period, reducing CL. CGP-37157 attenuated the positive chronotropic effects of isoprenaline. **Conclusions:** NCXm is involved in the generation of LCR and automaticity in murine SNCs. (COI: No)

1P-049

Low T-tubule density is related with vulnerability of sympathetic atrial arrhythmia

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We studied whether low T-tubule density or disorganized T-tubules determines vulnerability of sympathetic arrhythmia of the atria. Rat atrial myocytes were isolated separately from each chamber (LA and RA). The T-tubule density, electrophysiological property, arrhythmic incidence to sympathetic stimulation, and the effect of detubulation were compared between LA and RA myocytes. RA myocytes were smaller than the LA, and they have poorly developed T-tubules. As a result of highly expressed Ito current, RA myocytes showed much shorter action potential than LA myocytes. Compare to the LA, RA myocytes have a higher L-type Ca2+ channel (LTCC) density and showed a higher LTCC increase to isoproterenol stimulation. The RA myocytes with sparse T-tubules showed a higher incidence of sympathetic arrhythmias than LA myocytes. Detubulation of LA myocytes caused shortening of action potential and increase of sympathetic arrhythmias. These suggest that atrial myocytes with under-developed T-tubules or disorganized T-tubules are vulnerable to Ca2+ overload and sympathetic arrhythmias. This study was performed in accordance with the guidelines and approval of the IACUC of Sungkyunkwan Univesity (SKKUIACUC2018-04-08-3). There is no actual or potential conflict of interest in relation to this presentation. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D1A1B03934748). (COI: No)

1P-050

Effect of Myocyte Mechanical Properties on Transmural Distribution of Stress and Energy Consumption

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Force velocity relation (FVR) and force length relation (FLR) are well known mechanical properties of the ventricular myocyte. Regarding FVR, muscle contraction force decreases according to the increase in muscle shortening velocity. FLR is positive correlation between sarcomere length (SL) and muscle contraction force. The left ventricular (LV) wall is reported to have transmural distribution that the end diastolic (ED) SL and the sarcomere shortening ratio (SR) changes according to the transmural location. We can analyze the effect of FVR and FLR on the LV transmural distribution of stress and the energy consumption by using the mathematical model. In this study, we analyzed the effect of FVR and FLR on the transmural distribution of stress and energy consumption by using the 10-layer LV model, under various ED SL, and SR distributions. When the endocardial ED SL is shorter than that of epicardium, the stress and energy consumption of endocardium become lower than that of epicardium by the effect of FLR. When the endocardial SR is larger than that of epicardium, the stress and energy consumption of endocardium become lower than that of epicardium by the effect of FVR. If we assume simple geometrical characteristics in LV wall, the SR is larger for endocardium than epicardium. Thus, if the ED SL of endocardium is larger than that of epicardium, then the transmural distribution of stress and energy consumption tends to become homogeneous. (COI: No)

1P-051

D-galactose worsens cardiac function via aggravating mitochondrial dysfunction in obese rats

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Purpose: Chronic administration of D-galactose has been shown to induce left ventricular (LV) dysfunction that is similar to signs of aging. Although mitochondria play an important role in maintaining normal LV function, the effects of D-galactose on cardiac and mitochondrial function in obese condition have never been investigated. We hypothesized that D-galactose aggravates LV dysfunction in obese rats by deteriorating cardiac mitochondrial function.

Methods: Twenty male Wistar rats were fed with high-fat diet (HFD) for 12 weeks. Then, they received subcutaneous injection of either 0.9% NSS or 150 mg/kg/d of D-galactose for 4 or 8 weeks. LV and cardiac mitochondrial function were determined at the end of weeks 4 and 8.

Results: At week 4, both HFD rats treated with vehicle (HFV4) and HFD rats treated with D-galactose (HFD4) showed significant impaired LV and cardiac mitochondrial function as indicated by increased end diastolic pressure, decreased stroke volume, and increased cardiac mitochondrial ROS levels, swelling and membrane depolarization, compared to their baseline. However, there was no significant difference in all parameters between HFV4 and HFD4. Interestingly, at week 8, HFD rats treated with D-galactose (HFD8) had worse impairment of LV and cardiac mitochondrial function than HFD rats treated with vehicle.

Conclusion: Aging further impaired LV function via deteriorating cardiac mitochondrial function in subjects with obese insulin-resistant conditions. (COI: No)

1P-052

Drug Effect Estimation System that Uses Cardiac Action Potential Waveforms

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Drug effect estimation system was developed to reduce cost for research and development of new drugs by estimating the drug effects to specific ventricular myocyte ion channels in silico, which is necessary to evaluate drug safety. The system estimates the effects of drugs on ion channels by using action potential waveforms (APWs) of ventricular myocytes. This system searches for APWs similar to measured APWs from pregenerated simulation APWs database (APWs-DB) whose APWs are generated from the various combinations of the channel permeabilities in the guinea-pig ventricular myocyte model, and estimates the effects of drugs on ion channels from the permeability values used to generate the corresponding APW. Input APWs is measured from guinea-pig ventricular myocytes before and after drug administration. In the estimation process, the permeabilities are determined by the stochastic analysis, since the effects of ion channels for APW are difficult to distinguish each other. We used two methods to evaluate the similarities of APWs; 1) differences in membrane potential during plateau and repolarizing phase and 2) temporal differences in repolarizing phase are used to evaluate the similarity. Estimation results on the animal experimental APW using I_{Kr} blocker (E-4031), showed that I_{Kr} channel was blocked about 50%, while other channel blockades were not clearly estimated. (COI: No)

1P-053

Acute Overstretch Causes Abrupt Inner Mitochondrial Collapsing of Rat Papillary Muscles

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Purpose: We investigated the functional and morphological changes in cardiac papillary muscle by acute overstretch with isometrical contraction.**Methods:**We used male SD-rats and dissected papillary muscles from the right ventricle. A papillary muscle preparation was stretched to Lmax. We overstretched it within 2 seconds up to 110% of Lmax (110%OS) or 120% of Lmax (120%OS), and stimulated it (1Hz, 36°C) with tension measurement, compared with Lmax group (non overstretched; nOS). We also analyzed the changes in sarcomere length and morphological changes in organelles by an electronic microscope. **Results**:The active tension was immediately decreased after overstretch to 67.8±3.8% (n=6) in 110%OS, and to 17.3±4.0% (n=5) in 120%OS, whereas passive tension was abruptly increased by step-wised OS. The sarcomere length was stretched to 2.42±0.01μm in 110%OS, and to 2.51±0.01μm in 120%OS, whereas the structure remained organized well. In contrast, in 110%OS, inner mitochondrial density was lower than that in nOS, indicating swelled mitochondria. In 120%OS, inner mitochondrial empty space with vacuolation was found in the large area of myocardium, indicating that inner mitochondria cristae was susceptible to mechanical stress-induced deterioration.

Conclusions: Acute overstretch of rat papillary muscles caused inner mitochondrial collapsing with preserved the sarcomere structure. It could account for the mechanisms on pathogenesis of acute volume-overloaded heart failure. (COI: No)

1P-054

PCSK9 Inhibitor Attenuates Cardiac and Mitochondrial Dysfunction in Obese-Insulin Resistant Rats

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Purpose: Obese-insulin resistance is one of the major risk factors for cardiovascular diseases due to its impact on the impairment of metabolic function, cardiac contractile function and cardiac mitochondrial function. PCSK9 inhibitors, a newer class of cholesterol-lowering drugs have been shown to effectively reduce cholesterol levels. However, the effects of PCSK9 inhibitor on cardiac function and cardiac mitochondrial function in obese-insulin resistant rats have not been investigated.

Methods: Twelve female Wistar rats were divided to receive either normal diet (ND) or high fat diet (HFD) for 12 weeks. Then, HFD rats were divided into 2 subgroups to receive either vehicle (HFV) or PCSK9 inhibitor (HFP) (4 mg/kg/day; s.c.) for an additional 3 weeks. Left ventricular (LV) function and mitochondrial function were determined.

Results: HFV and HFP rats developed obese-insulin resistance as indicated by impaired glucose tolerance test. HFV rats had markedly reduced %LVEF [63±1 vs 73±1] and impaired cardiac mitochondrial function, compared to ND rats. However, PCSK9 inhibitor effectively reduced these impairments by increasing %LVEF [68±1 vs 63±1] and attenuated cardiac mitochondrial dysfunction as indicated by decreased mitochondrial ROS production [568±51 vs 674±47], compared to HFV rats.

Conclusion: PCSK9 inhibitor attenuated LV dysfunction via improving cardiac mitochondrial function in obese-insulin resistant rats. (COI: No)

1P-055

Evaluating the Role of Individual Types of Ca^{2+} Channels in the Sinoatrial Node Pacemaker Cell Model

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It is now well established that three types of voltage gated calcium channels are expressed in the mammalian cardiac pacemaker cells in the sinoatrial node (SAN), such as the T-type (Cav3.1) and the two L-type (Cav1.2, Cav1.3) channels. The gating mechanisms are quite variable and thereby, different roles of these channels are expected in the pacemaker mechanisms. Furthermore, the Ca^{2+}-mediated inactivation of the L-type Ca^{2+} channels (LCCs) should be revised according to the tight coupling of LCC-ryanodine receptors as suggested in our human ventricular cell model (Himeno et al., 2015). The object of the present study is to reexamine the pacemaker mechanisms by incorporating mathematical models of Cav1.2 and Cav1.3 coupled with the Ca^{2+}-releasing unit (CaRU) in our mathematical model of SAN cells. We used the experimental data of Mesirca et al (2015) to optimize the parameters of the voltage-dependent activation. The Ca^{2+}-mediated inactivation kinetics were totally revised according to the local Ca^{2+} transient in the CaRU models of Hinch (2014). The new channel models of the three types of Ca^{2+} channels and pacemaker potentials in our preliminary SAN model will be presented. (COI: No)

1P-056

Experimental Autoimmune Myocarditis (EAM) Model in Nonhuman Primates

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The ultimate treatment for cardiomyopathies of all etiologies is cardiac transplantation. Recently, autoimmune myocarditis has been reported as one of the etiologies of dilated cardiomyopathy. For development of new treatment and/or early diagnostic methods, elucidation of the causes and mechanisms of the disease is vital. In this study, we aimed to establish a primate model of experimental autoimmune myocarditis (EAM) using five cynomolgus monkeys. For this, monkeys were given an intradermal injection of plain myosin mixed with IFAon the medial aspect of the thighto stimulate an immune reaction. One monkey, to serve as a control, was injected with OVA. Cardio-specific examinations, including echocardiography and other evaluations were performed pre-injection and at several weeks after the injection. We confirmed the symptoms of myocarditis and progress of the medical condition after second injection. Lastly, we conducted clinicopathological examinations of all the monkeys, which revealed that the immunized monkeys showed notable increases in cardiac hormone levels, prolonged QTc interval, ventricular dilation. Pathologically, there was cardiac fibrosis and cardiomyocyte deficiency. These findings mimicked human myocarditis and cardiomyopathy. In conclusion, we established a nonhuman primate model of EAM. Several pathological changes were observed, as with human EAM, suggesting that this model of EAM in nonhuman primates could be a useful model for the human disease. (COI: Properly Declared)

1P-057

Physiological role of TRPC6 upregulation in hyperglycemia-exposed mice hearts

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<Purpose> Receptor-activated Ca^{2+}-permeable cation channels (RACCs) have been attracted attention as novel pharmacological targets of heart failure. Transient receptor potential canonical (TRPC) 3/6 are molecular entities of RACCs and reportedly upregulated in pathologically remodeling heart. We have recently reported that increased TRPC3 positively regulates reactive oxygen species (ROS) production with NADPH oxidase 2 (Nox2). In contrast, a relation between TRPC6 and heart failure is unclear. In this study, we analyzed the role of TRPC6 upregulation in hyperglycemia-induced heart failure, focusing on crosstalk with TRPC3 and Nox2.

<Methods/Results> TRPC6 was upregulated in streptozotocin (STZ)-treated mice hearts and neonatal rat cardiomyocytes (NRCMs) treated with high glucose while Nox2 was downregulated. After STZ treatment, only TRPC6-deficient mice showed decreased cardiac function and increased oxidative stress. TRPC6-silenced NRCMs treated with high glucose showed high levels of ROS. In TRPC3/TRPC6/Nox2-expressing cells, TRPC6 inhibited increase of Nox2 protein expression by TRPC3 in its channel activity-independent manner.

<Conclusions> Upregulation of TRPC6 in hearts exposed to hyperglycemia inhibited formation of TRPC3-Nox2 complex and suppressed Nox2-dependent ROS production, suggesting that TRPC6 upregulation contributes to adaptation for hyperglycemic stress in the heart. (COI: No)

1P-058

IL-6 may have protective roles in Lmna-related cardiomyopathy

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Purpose: Mutations in *LMNA* which encodes A-type lamins can cause several human diseases including fatal cardiomyopathy with conduction defects. Interleukin-6 (IL-6) is a multi-functional cytokine and known to promote fibrosis in heart. In addition, the IL-6 receptor antibody (MR16-1) was reported to suppress inflammation after myocardial infarction, and improved cardiac remodeling in mice. In this study, we investigated the roles of IL-6 in Lmna-related cardiomyopathy and the therapeutic effects of MR16-1. **Methods**: We used Lmna p.H222P knock-in mice (H222P) and C57BL/6J mice as control (WT). We performed qRT-PCR to check gene expression, ELISA to measure IL-6 levels in serum and heart, and histological analyses of Masson's trichrome staining. **Results**: mRNA of IL-6 was significantly increased in H222P heart, but its protein levels was not different from WT. After MR16-1 treatment, mRNA of IL-6 in H222P heart was decreased, whereas mRNA of IL-6 receptorα, ANP, collagenIα1, and TGFβ2 was increased. IL-6 protein levels in heart were maintained constantly, and there was no notable histological changes. **Conclusions**: These results may suggest protective roles of IL-6 in Lmna-related cardiomyopathy, although short time treatment could not show prominent histological improvement. (COI: No)

1P-059

Sonic hedgehog signaling regulates the mammalian cardiac regenerative response

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Certain organisms, including zebrafish, are capable of complete cardiac regeneration in response to injury. This response has also been observed in newborn mice, although in this case, the regenerative capacity is lost at approximately one week of age. The mechanisms regulating this short temporal window of cardiac regeneration in mice are not well understood.

Here, we show that sonic hedgehog (Shh) signaling modulates the neonatal mouse regenerative response. In particular, we demonstrate that following apical resection of the heart on postnatal day 1, mice activate Shh ligand expression and downstream signaling. This response is largely absent when surgery is performed on non-regenerative, postnatal day 7 pups. Furthermore, an enhanced cardiac regeneration response was detected in ptch heterozygous mice which have a genetically-based constitutive increase in Shh signaling. We further show that Shh ligand is produced in the myocardium by non-myocytes and appears to regulate cardiomyocyte proliferation, as well as the recruitment of monocytes/macrophages to the regenerating area. Finally, we demonstrate that a small molecule activator of Shh signaling promotes heart regeneration, whereas an inhibitor of Shh signaling impairs the regenerative response. Together, these results implicate Shh signaling as a regulator of mammalian heart regeneration and suggest that modulating this pathway may lead to new potential therapies for cardiovascular diseases. (COI: No)

1P-060

Analysis of Diabetic Cardiomyopathy with type 2 Diabetes Mellitus in Nonhuman Primate

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Cardiovascular diseases and the subsequent cardiomyopathies have diverse etiologies and mechanisms and require variable treatment strategies. Diabetes mellitus is one of the many causes of cardiomyopathy. The mechanism of diabetic cardiomyopathy (DCM) is unknown. The present study aimed to determine the symptoms and clinicopathological signs of cardiovascular disease in cynomolgus monkeys with spontaneous type 2 DM by blood test. And cardiac biomarkers (atrial and brain natriuretic peptides, ANP and BNP), electrocardiography (ECG), echocardiography and chest X-ray were evaluated for evidence of severe heart failure. We performed also histopathological and immunohistochemical analyses for the phyisiological evidence of DCM. In echocardiography, that aimed, indicated depression of cardiac function, and cardiac biomarkers were clearly increased. Cardiomyocytes showed steatosis and fibrosis with excessive deposition of amyloid polypeptide (amylin). These results were concordant with the most recent research on DCM and indicated the novel pathological mechanisms of DCM. This nonhuman primate model faithfully mimicked the pathophysiology of human DCM. This model will be useful for development of new therapies and diagnostic procedures for DCM. (COI: Properly Declared)

1P-061

Role of Cardiac Hormones in a Nonhuman Primate Model of Cardiac Disease

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Nonhuman primates are commonly used as experimental animals because of their biological resemblance to humans. In patients with cardiac diseases, levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) tend to increase due to cardiac damage. Therefore, the levels of ANP and BNP are used as indicators in the diagnosis of human heart failure. However, there are no reports of reference values for ANP and BNP with heart disease in nonhuman primates. In this study, we recorded the age, sex, and weight of 162 cynomolgus monkeys. We then performed evaluations to assess ANP, BNP, electrocardiography and echocardiography, and accordingly divided the monkeys into two groups: healthy monkeys and those with spontaneous cardiac disease (i.e. those who showed symptoms of valvular disease and heart failure due to dilated cardiomyopathy). Statistical analysis was performed using IBM SPSS to compare the relationship between ANP and BNP and the factors of age, sex and weight. There were no significant relationships between factors such as age, sex, and weight and ANP and BNP. On the other hand, both ANP and BNP were significantly different between the two groups. Similar to human beings, ANP and BNP levels tend to increase with cardiac disease in monkeys. Based on these results, we conclude that ANP and BNP are important leading indicators of cardiac disease in nonhuman primates, and that this nonhuman primate cardiac disease model is useful for research in cardiology. (COI: Properly Declared)

1P-062

Activation of SIRT1 Attenuates Cardiac fibrosis via preventing Endothelial-to-Mesenchymal Transition

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Purpose: To determine whether SIRT1 activated by resveratrol (RSV) prevent EndMT and discuss the possible mechanism.**Methods**: Cardiac fibrosis was induced in C57BL/6 mice by subcutaneous injection of Isoproterenol (ISO). The H5V cell line was treated by TGF-β1-induced EndMT and pretreated with or without SRT2104, RSV and/or EX527. Lentiviruses-mediated overexpression of SIRT1 was used to further evidence that SIRT1 plays a key role in EndMT. All animal experimental protocols were approved by the Animal Care and Use Committee of Laboratory Animal Centre of Wenzhou Medical University, and were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).**Results**: Activation of SIRT1 ameliorated the compensatory cardiac fibrosis and prevented EndMT. Meanwhile, the expression of TGF-βR1, P-Smad2/3, P-Akt and P-Erk1/2 also decreased, whereas EX527 (a specific inhibitor of SIRT1) significantly inhibited these effects. In H5V cells, both activation of SIRT1 by RSV/SRT2104 and overexpression of SIRT1 by lentivirus transduction reduced TGF-β1-induced EndMT. Furthermore, the expression of TGF-βR1, P-Smad2/3, P-Akt and P-Erk1/2 also decreased.**Conclusions**: Activation of SIRT1 attenuates cardiac fibrosis via preventing EndMT *in vivo* and *in vitro*. Various pathways participate in this progress, including TGF-β/Smad pathway, Akt and Erk1/2 pathway. (COI: No)

1P-063

Insulin signaling deficiency is responsible for diastolic dysfunction of diabetic cardiomyopathy

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Purpose: Left ventricular (LV) diastolic dysfunction is observed in the early stage of diabetic cardiomyopathy (DMCM). The defective Ca^{2+}signaling is one of the candidate mechanisms of diastolic dysfunction. The aim of this study was to elucidate the mechanism of Ca^{2+} signaling dysfunction underlying DMCM.

Methods: Diabetes mellitus (DM) was induced in mice by streptozotocin injection (*i.p.*). The mice 4 weeks after injection were investigated.

Results: Echocardiograph of DM mice showed that diastolic function was impaired without reduction of ejection fraction, thus mimicking the early stage of DMCM. In the isolated ventricular myocytes from DM mice, the Ca^{2+} transient decay rate was slower than that from control. In the ventricles of DM mice, the phosphorylated phospholamban (p-PLN) level was lower than that of control. However, neither the myocardial responses to bAR stimulation or the expression levels of bARs were altered in DM mice. To elucidate whether insulin signaling is responsible for diastolic function, we examined the effects of insulin. In the insulin-treated mice, the p-PLN level and the relaxation rate of the isolated ventricular myocardium were recovered to the control level. Furthermore, insulin/PKG signaling was required for the maintenance of basal p-PLN level in the primary cultured neonatal mouse ventricular myocytes.

Conclusion: The reduction of p-PLN level caused by insulin deficiency is responsible for LV diastolic dysfunction in the early stage of DMCM. (COI: No)

1P-064

Vitamin B1 pretreatment prevents cardiac mitochondrial morphology from ischemia/reperfusion injury

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Vitamin B1 (VitB1) deficiency was recognized as a cause of Beriberi (Kakke; a neurological disease and heart failure). We previously investigated that the pretreatment of thiamine pyrophosphate (TPP), an active form of VitB1, preserved cardiac contraction after ischemia/reperfusion (I/R). To investigate the mechanism of preserved cardiac function by TPP after I/R injury, we performed further biochemical analysis in this study. Male Sprague-Dawley rats (around 10 weeks old) were used. After 5 min perfusion of Tyrode's solution with or without 300 μM TPP in the Langendorff system, the hearts were treated with 40 min global ischemia. In metabolome analysis, levels of ATP and ADP in TPP-treated heart were increased (n=4 each, $p<0.05$). We also performed electron-microscopic (EM) study and analyzed morphological changes in organelles including myofilaments and mitochondria. In TPP-treated heart, size of mitochondria was bigger than that in untreated heart (counted over 600 mitochondria from 4 hearts each, $p<0.05$). Moreover, in TPP-treated heart, 1) small sized mitochondria (less than 0.04 μm^2) was decreased, 2) medium sized (from 0.04 to 0.06 μm^2) was identical and 3) large sized mitochondria (over than 0.06 μm^2) was increased in comparison with untreated heart. Taken together, pretreatment of VitB1 preserves the ATP level of heart muscle in ischemic condition possibly via maintaining mitochondrial morphology by activating fusion and inhibiting fission. (COI: No)

1P-065

Regulation of Orai1 in Angiotensin II-Induced Cardiac Hypertrophy

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Heart failure initiates with pathological cardiac hypertrophy which is a response of heart to increased workload. The cardiac hypertrophy is characterized as increased cardiomyocytes size, develops during the process of disorders such as hypertension and myocardial infarction. Calcium, as a second messenger, plays vital roles in mediating a wide range of cardiovascular diseases. Few studies reported store operated Ca2+ entry (SOCE) is associated with cardiac hypertrophy yet. Here, we hypothesized Orai1-mediated SOCE is responsible for the Angiotensin II-induced cardiac hypertrophy. Our data showed after the subcutaneous implantation of Ang II osmotic pump in C57BL6 mice, the heart size significantly increased. However, this effect could be abolished by knocking down Orai1 using AAV-Orai1-shRNA. A real-time PCR and western blots results showed the hypertrophic marker genes ANF, BNP, ß-MHC and cTnT upregulated in Ang II treatment group, while they remained nearly unchanged after the treatment of AAV-Orai1-shRNA, indicating Orai1 could rescue the Ang II perfusion induced cardiac hypertrophy *in vivo*. Moreover, Masson's Trichrome staining convinced type I collagen levels in heart increased after Angiotensin II perfusion while it is attenuated after blocking Orai1, showing the progress of cardiac fibrosis during Orai1-mediated cardiac hypertrophy. Taken together, these findings suggest Orai1 as a novel regulator involved in Angiotensin II induced cardiac hypertrophy *in vivo*. (COI: No)

1P-066

Plasma Proteomic Analysis of Acute Myocardial Infarction in Young Adults

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INTRODUCTION - Although advancing age is a well-established risk factor for acute myocardial infarction (AMI), the incidence of AMI in young adults is upsettingly increasing. Previous proteomic studies of AMI mainly focused on elderly AMI, thus a molecular study of AMI in young adults remain limited. Therefore, this study aims to analyse protein expression of AMI in young adults compared to control subjects. **METHOD -**Ten AMI patients aged 18 to 45 years, and ten age, gender and race-matched control were recruited. Proteins in pooled plasma samples from each group were separated using two-dimensional electrophoresis (2-DE). The protein spots were analysed using the PD Quest analysis software. Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) was used to identify the protein spots that were found to have been expressed differently between the two groups. **RESULTS -** Apolipoprotein AI (Apo AI), Apolipoprotein AIV (Apo AIV) and Haptoglobin were found to be differently expressed in young AMI. These proteins were expressed higher in young AMI patients compared to control subjects ($p< 0.05$). **CONCLUSION -** The up regulation of Apo AI, Apo AIV and Haptoglobin in young AMI patients may suggest a significant role of these proteins in response to the inflammatory process associated with the recent cardiac event. This discovery would be the preliminary step towards the improvement of diagnosis and management of AMI in young adults. (COI: No)

1P-067

Angiotensin-(1-5)-mediated cardioprotection via AT2R-PI3K-Akt-eNOS pathway

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We investigated the effect of Ang-(1-5) on myocardial ischemia-reperfusion (I/R) injury. Before ischemia, Sprague-Dawley rats received Ang-(1-5) (1 mg/kg/day) for 3 day. Anesthetized rats were subjected to 45 min of ischemia by ligation of left anterior descending coronary artery followed by reperfusion and rats were sacrificed 1 day after reperfusion. Pretreatment with Ang-(1-5) attenuated I/R-induced increases in plasma creatine kinase (CPK) and lactate dehydrogenase (LDH) concentrations, and infarct size. I/R caused increases in oxidative stress markers in ventricles, which were attenuated by pretreatment with Ang-(1-5). I/R also caused increases in Bax, caspase-3 and caspase-9 protein levels, and a decrease in Bcl-2 protein level in ventricles, which were attenuated by pretreatment with Ang-(1-5). Co-treatment with MasR antagonist or inhibitors of downstream signaling pathway including phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and endothelial nitric oxide synthase (eNOS) attenuated Ang-(1-5)-induced changes in CPK and LDH levels, infarct size, and apoptosis-related proteins. Therefore, these results suggest that Ang-(1-5) has cardioprotective effect against I/R injury by inhibiting apoptosis via AT2R and PI3K-Akt-eNOS pathway.

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1P-068

Palmitic Acid Contributes to the Development of Ca^{2+} Oscillations in Adult Rat Cardiomyocyte

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Purpose: It is known that palmitic acid (PA) induces a dose-dependent Ca^{2+} increase and nuclear condensation in the death of myocytes. However, how PA produces Ca^{2+} still remains to be investigated. The Ca^{2+} oscillation frequency is considered critical for some frequency-dependent cellular responses in signal transduction. In this study, we attempted to determine the mechanism of how PA affects intracellular Ca^{2+}, in particular the role of Ca^{2+} oscillations in cardiomyocytes.
Methods: Rat cardiomyocytes were treated with Fura-2 to detect the changes in $[Ca^{2+}]_i$. DiBAC probe was used to measure the membrane potential.
Results: According to our data, it was detected that the perfusion of PA caused dose-dependent Ca^{2+} oscillations. However, this phenomenon stopped from occurring with the elimination of $[Ca^{2+}]_{ex}$ and the treatment of nifedipine, an L-type Ca^{2+} channel blocker, or CPA, a specific inhibitor of SERCA pump in cardiomyocytes. PA was found to induce Ca^{2+} influx, which then caused depolarization of the membrane. After the membrane depolarized, L-type Ca^{2+} channels opened, resulting in the increase of $[Ca^{2+}]_i$, which would be withdrawn back into ER/SR by SERCA.
Conclusion: In conclusion, extracellular Ca^{2+}, L-type Ca^{2+} channels and SERCA pumps were indicated to play important roles in the process of PA-induced Ca^{2+} oscillations in rat cardiomyocytes. (COI: No)

1P-069

Insights into signaling mechanism of ANP receptor by x-ray crystallography

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A cardiac hormone, atrial natriuretic peptide (ANP) plays a major role in the regulation of blood pressure and volume regulation. ANP receptor, a single span transmembrane receptor carrying intrinsic guanylate cyclase activity, mediate the action of ANP. The ANP receptor is a member of GCase-coupled receptors that share a similar overall molecular configuration and, presumably, a common signal transduction mechanism. However, the mechanism of signal transduction by the ANP receptor as well as GCase-coupled receptor remains largely unknown. Here, we have solved crystal structures of extracellular ANP-binging domain in complex with three ligands (ANP, ANP lacking its C-terminus region and Dendroaspis natriuretic peptide (DNP), isolated from the venom of the green Mamba snake Dendroaspis angusticeps). High resolution structure allows us to build the bound ligands precisely, including water molecules. Plausible mechanisms of ligand recognition and transmembrane signal transduction mechanism, and insight from the structures will be discussed. (COI: No)

1P-070(AP-5)

Physiological and pathophysiological significance of TRPC3-Nox2 coupling in the heart

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Chronic stresses induce pathological cardiac remodeling in which production of reactive oxygen species (ROS) plays a critical role. We have revealed that those ROS were produced by NADPH oxidase 2 (Nox2), despite low Nox2 expression levels in the normal heart. We demonstrate that transient receptor potential canonical 3 (TRPC3) Ca^{2+}-permeable channel acts as a positive regulator of Nox2 in both enzymatic activation and protein expression in cardiomyocytes during pathological remodeling. TRPC3 physically interacts with Nox2 through TRPC3 carboxyl-terminal regions, escaping Nox2 from proteasomal degradation, resulting in amplification of Ca^{2+}-dependent Nox2 activation. This TRPC3-Nox2 coupling mediates mechanical stress-induced cardiac fibrosis and a chemotherapy agent Doxorubicin-induced cardiac atrophy in mice. Inhibition of TRPC3-Nox2 coupling in cardiomyocytes could significantly suppressed Dox-induced cardiac atrophy. These results suggest that functional and physical coupling of TRPC3 and Nox2 mediates various stress-induced cardiac remodeling and inhibition of TRPC3-Nox2 coupling will be a promising therapeutic target for the treatment of pathological muscle remodeling. Furthermore, TRPC3 knockout heart showed more elastic property than wildtype heart, suggesting that TRPC3-Nox2 coupling regulates heart stiffness by two means of cardiomyocyte and extracellular matrix. (COI: No)

1P-071

Nuclear connectin novex-3 is essential for proliferation of hypoxic fetal cardiomyocytes

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Mammalian cardiomyocytes lose their proliferative capacity shortly after birth, which is a major obstacle for therapeutic heart regeneration in adults. We and others have recently shown the importance of hypoxic in utero environments for active fetal cardiomyocyte proliferation. Here we report the unexpected expression of novex-3, the short splice variant of the giant sarcomeric protein connectin (titin), in the cardiomyocyte nucleus specifically during the hypoxic fetal stage in mice. This nuclear localization appeared to be regulated by the N-terminal region of novex-3, which contains the nuclear localization signal. Importantly, the nuclear expression of novex-3 in hypoxic fetal cardiomyocytes was repressed at the postnatal stage following the onset of breathing and the resulting elevation of oxygen tension, whereas the sarcomeric expression remained unchanged. Novex-3 knockdown in fetal cardiomyocytes repressed cell cycle-promoting genes and proliferation, whereas its overexpression enhanced proliferation. Mechanical analysis by atomic force microscopy and microneedle-based tensile tests demonstrated that novex-3 in hypoxic fetal cardiomyocytes contributes to the elasticity/compliance of the nucleus at interphase and facilitates proliferation, by promoting phosphorylation-induced disassembly of multimer structures of nuclear lamins. We propose that novex-3 has a previously unrecognized role in promoting cardiomyocyte proliferation specifically at the hypoxic fetal stage. (COI: No)

1P-072

Effect of autonomic nervous system on early and late repolarization intervals in children

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Introduction
The QT interval which reflect myocardial repolarization may be divided into the QRS width and the JT interval.JT interval can further be divided into the early repolarization (J point to T peak interval: JTp) and late repolarization (T peak to end interval: Tp-e). To clarify the characteristics of repolarization subintervals and the dependency on heart rate and autonomic nervous activity.
Methods
300 infants and children (0-7 years of age) without heart disease were included. ECGs were recorded by the CM5 lead using a Biopac biological polygraph recording device.We measured RR, JTp and Tp-e intervals. The relationships between RR interval and JTp, Tp-e intervals were evaluated as heart rate dependency. In addition, the frequency domain analysis was used for theassessment of heart rate variability (HRV). Low frequency (LF) and high frequency (HF) were measured and LF/HF and HF/(LF+HF) were calculated. The relationships between HRV parameters and JTp, Tp-e intervalswere evaluated.
Results
Positive correlation was observed between RR interval and JTp ($r=0.792$, $p<0.001$), Tp-e ($r=0.396$, $p<0.001$). The correlation was observed between LF/HF ($r=-0.475$, $p<0.001$), HF/(LF+HF) ($r=0.419$, $p<0.001$) and JTp. On the other hand, the weak correlation was found between LF/HF ($r=-0.294$, $p<0.001$), HF/(LF+HF) ($r=0.251$, $p<0.001$) and Tp-e.
Conclusion
The Tp-e interval has lower dependence on RR interval, and lower correlation with autonomic nervous activity than the JTp interval. (COI: No)

1P-073

Pilocarpine but not Ach permeate the mouse footpads and induce perspiration, sedation and arrhythmia

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Purpose: Footpad injection of pilocarpine has been only one practical method to induce perspiration on mouse footpads under anesthesia. Therefore, we examined whether pilocarpine permeate the footpads of unanesthetized mice and induce perspiration, which would help the noninvasive ECG detection by a new multi-dry-electrode plate ECG (MDEP)-sensor system.
Methods: ECGs of freely-behaving C57BL/6J mice were individually recorded by the MDEP-sensor system for 1 h following free walking in a cage on the paper soaked with pilocarpine-containing ophthalmic solution or acetylcholine solution for 10 min. Their activities were also recorded by a camera and a DVD recorder. The obtained ECGs were compared with that of no treatment (no-drug) group.
Results: In contrast to the fragmented ECGs in no-drug and acetylcholine groups, the mice of pilocarpine group showed long lasting ECGs. Also, pilocarpine may have permeated the vascular walls and influenced the central nervous system because mice exhibited sedation and arrhythmia, which were not observed in no-drug group.
Conclusion: Pilocarpine but not acetylcholine likely permeate the footpads of mice and induce perspiration that improves the ECG signal quality of the MDEP-sensor system. Moreover, the present method also induced sedation and arrhythmia. Thus, the present protocol may provide a new noninvasive tool to investigate cardiac diseases in freely-behaving mice without the need for device-implantation surgery. (COI: No)

1P-074

Expression change of cytokine in principal organ during cardiopulmonary bypass

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[Background] Multiple organ failure, such as acute lung injury, acute kidney injury and inflammatory cardiac injury after cardiopulmonary bypass (CPB) still is a major problem in patients undergoing cardiovascular surgery. Our current study showed that CPB lead to cytokine release and organ damage in the rat CPB model.
[Purpose] This study aimed to investigate cytokine expression of each organ during cardiopulmonary bypass.
[Method] CPB system consisted of a membranous oxygenator, tubing line and a roller pump. Priming volume of this system is only 7 ml. The left common carotid artery was cannulated with the arterial return cannula. The venous uptake cannula was advanced through the right external jugular vein into the right atrium. CPB flow was initiated and maintained at 70 ml/kg/min. Male SD rats (450-500g) were divided into three groups: Control (n = 3), SHAM (received surgical preparation only without CPB) group (n = 4) and CPB (120min) group (n = 3). Three heart, lung, kidney and liver samples from a rat and examined gene expression of Monocyte Chemotactic Protein (MCP)-1 and Interleukin (IL)-6 by real-time PCR.
[Result] Gene expression of MCP-1 and IL-6 in hearts and lungs of CPB group significantly increased compared with control and SHAM groups. Suspicions are raised about the effects of unphysiological blood flow distribution during CPB.
[Conclusion] Expression of MCP-1 and IL-6 in heart and lung during CPB increased. (COI: Properly Declared)

1P-075

Irregular division of the nucleus without cytokinesis in cardiac progenitor cells of mouse heart

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The adult mammalian heart comprises several kinds of cardiac stem or progenitor cells. Atypically-shaped cardiomyocytes (ACMs) are stem cells antigen-1 (Sca-1)-negative cardiac progenitor cells derived from mouse myocardium that spontaneously develop into beating cardiomyocytes. These cells do not appreciably proliferate but mostly possess multiple nuclei. In the present study, we examined the characteristics of the nuclei in ACMs with high-resolution microscope. We observed peculiar morphology of the nuclei, such as irregular shapes, clusterization and DNA bridges, presumably caused by the unusual division of the nuclei. The immunostaining analyses revealed that Lamin B, components of the nuclear lamina, was expressed even in clusterized nuclei, and the cell cycle markers Cyclin D1 and YAP protein were substantially expressed in quite a lot of cells. However, the cytokinesis was not observed during the long-term culture (>60 days). The results suggest the possibility that the abnormal division of nucleus makes one of the obstacles to progress cell division in these cardiac progenitor cells. (COI: No)

1P-076

Usefulness of anti-arrhythmic drug therapy targeting cardiac adenylyl cyclase

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Sympathetic nervous system plays an important role in maintaining cardiac function against acute harmful stresses on the heart. However, chronic sympathetic activation is known to be a cause of clinically important arrhythmias including atrial fibrillation (AF) and ventricular arrhythmia. Although the usefulness of β-adrenergic receptor blockade therapy is widely accepted, its multiple critical side effects often prevent its initiation or continuation. We have established a long-lasting AF model induced by adrenergic activation in mice. Vidarabine, an anti-herpes drug which we identified as an inhibitor of cardiac adenylyl cyclase (AC), shortened AF duration and reduced the incidence of sympathetic activation-induced ventricular arrhythmias. Consistent with the physiological data, vidarabine inhibited adrenergic receptor stimulation-induced arrhythmogenic events in atrial and ventricular myocytes. Vidarabine also inhibits reactive oxygen species (ROS) production induced by sympathetic activation in cardiac myocytes. The pivotal role of vidarabine's inhibitory effect on ROS production with regard to its anti-arrhythmic property has also been implied in animal studies. Importantly, indices of cardiac function including ejection fraction and heart rate were not affected by a dosage of vidarabine sufficient to exert an anti-arrhythmic effect. These results suggest that vidarabine or other cardiac AC-targeted therapy is useful for the prevention and/or treatment of arrhythmias. (COI: No)

1P-077

Stress intensity exhibited by E-PASS score and development of atrial fibrillation

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Background : Stress is a risk factor for atrial fibrillation (AF), but it is unknown how much stress intensity in humans contributes to the development of AF. **Objective :** In order to verify the relationship between occurrence of AF and stress intensity, we decided to investigate the relationship between incidence of de novo AF after non-cardiac surgery and surgical invasive stress. **Method and Result :** Surgical invasive stress of 943 patients (64±12y) who underwent gastrointestinal surgery at our hospital in 2013 were obtained by E–PASS(Estimation of Physiologic Ability and Surgical Stress) score derived from presence of severe heart disease, severe lung disease, diabetes, age, performance status index, American Society of Anesthesiologists physiological status classification, blood loss volume, operation time and extent of skin incision (Haga Y. SurgToday1999:29:219-25). The occurrence of AF was found in 23 cases on 3(2-8) postoperative day, and its frequency increased with increasing E-PASS score ($p<0.001$). In logistic regression analysis which included occurrence of post-operative AF as a dependent variable, and conventionally known risk factors for AF, E-PASS score, post-operative peak CRP and surgical region as independent variables. E-PASS score (OR16.2, 95%CI4.4-60.3) and surgical region /Esophagectomy (OR4.6, 95%CI1.4-14.5) were detected as occurrence factor of AF. **Conclusion :** Even higher surgical invasiveness stress can be existed for factor of development of AF. (COI: No)

1P-078

Initiation of the heartbeat in rat embryonic heart precedes sarcomere formation

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Backgrounds: We have demonstrated that the heartbeat in rat embryo initiates at embryonic day 9.99-10.13 (E9.99-10.13). Although regular sarcomere structure is exclusively unique morphological feature on striated muscles including cardiomyocytes, it still remains unclear whether sarcomere formation is essential for the initiation of the heartbeat or not.
Methods and Results: The cardiac crescents or heart tubes were isolated from embryos in pregnant Wister rats and served for transmission electron microscopy (TEM) observation. To investigate the relationship between sarcomere formation and initiation of the heartbeat, the embryo was divided into two groups by the hearts without (pre-) or with (post-) heartbeats. To our surprise, TEM images revealed that sarcomere structure, which is defined as the segment between two neighboring Z-Lines, was not found in both pre-heartbeat and post-heartbeat groups. Although apparent Z-lines were observed in embryonic heart at E11.00, clear M-Band was still not observed. At E14.00, regular sarcomere formation was observed; however, liner arrays of myofibril were not completed in the embryonic heart of this developmental stage. Finally, linear arrays of myofibrils with regular sarcomere formation was found in embryonic heart at E18.00 just before the birth.
Conclusions: The findings suggest that initiation of the heartbeat in rat embryonic heart does not require regular sarcomere structure. (COI: No)

1P-079

Contribution of the rostroventral midbrain to movement-related cardiovascular activation

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Sympathetic and cardiovascular adjustment occurs just before or at the onset of movement not only in conscious animals but also in unanesthetized decorticate animals, suggesting that the brain stem is responsible for the movement-related cardiovascular regulation. It is, however, unknown which of brain regions plays a role in the movement-related cardiovascular regulation in the decerebrate animals. Fifteen rats were divided into three decerebrate preparations: the rostral midbrain, the caudal midbrain, and the rostroventral midbrain preparations. Heart rate (HR), arterial blood pressure (AP), and tibial nerve activity (as an index of motor activity) were examined for 2 hours after decerebration. In the rostral and rostroventral midbrain preparations, HR and AP increased in association with spontaneous tibial nerve discharges, whereas spontaneous motor activity and concomitant cardiovascular responses did not occur in the caudal midbrain preparation. The frequency of spontaneous motor activity was much greater ($P < 0.05$) in the rostral and rostroventral midbrain preparations than that in the caudal midbrain preparation. Thus it is suggested that the rostroventral midbrain plays a role in evoking spontaneous motor activity and concomitant cardiovascular response in the decerebrate rat preparation and may contribute to feedforward cardiovascular regulation in association with exercise. (COI: No)

1P-080

Mechanism of augmentation of hydrogen sulfide-induced ANP secretion in hypoxic condition

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Hypoxia is a common disorder which is induced by lacking oxygen supply or insufficient blood distribution. In hypoxic condition, ATP sensitive potassium (KATP) channel is overexpressed as a compensatory mechanism. Recently, we have verified that sodium hydrosulfide (NaHS) augments high stretch induced-atrial natriuretic peptide (ANP) secretion partially via KATP channel. However, whether NaSH affects ANP secretion during hypoxia remains unclear. The aim of the present study is to investigate the effect of NaHS on ANP secretion during hypoxia and to define its signaling pathway. Isolated beating rat atria were perfused with buffer exposed to different O2 tension (100% O2, normoxia; 50% O2, hypoxia; 10% O2, anoxia). The ANP secretion increased negatively correlated with O2 tension. In hypoxic condition, the expression of PPAR-γ protein but not HIF1α protein was increased. NaHS (50μM) augmented ANP secretion in hypoxic condition which was blocked by the pretreatment with KATP channel blocker (glibenclamide), HIF1α inhibitor (2-methoxyestradiol), and PPAR-γ inhibitor (GW9662). Interestingly, NaHS-induced ANP secretion was augmented KATP channel activator (pinacidil). However, NaHS did not show any significant effect in normoxia condition. These results suggest that NaHS stimulates hypoxia-induced ANP secretion partly through KATP channel, PPAR-γ, and HIF1α pathway.

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1P-081

In vitro generation of goblet cell hyperplasia model using iPS cells and cigarette smoking solution

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[Background] Habitual cigarette smoking leads to destruction of lung tissue due to airway obstruction and excessive mucus production by the goblet cell hyperplasia. However, the mechanism of goblet cell differentiation and mucus production by cigarette smoking is not fully clarified.The aim of this study is to generate goblet cell hyperplasia model in vitro by the use of mouse iPS cell-derived airway epithelium and cigarette smoke solution and to elucidate the mechanism of goblet cell hyperplasia.
[Methods] We have generated airway epithelium via embryoid bodies formed from iPS cells by culturing based on serum-free conditions supplemented with Activin and bFGF, and by the air-liquid interface method. Moreover, in order to generate goblet cell hyperplasia model,these cells were treated with cigarette smoking solution and evaluated by gene expression and histological examination.
[Results] Gene expression and histological examination indicated that airway epithelium generated from iPS cells expressed airway epithelium markers and formed pseudostratified ciliated columnar epithelium like mouse tracheal epithelium. Furthermore, these cells treated with cigarette smoking solution expressed goblet cell marker and were Alcian Blue-positive cells.
[Conclusions] These results demonstrate that airway epithelium generated from iPS cells had native characteristics and these cells treated with cigarette smoking solution showed characteristics like goblet cell hyperplasia. (COI: No)

1P-082

Pulmonary Hypertension Downregulated Mitochondria Associated Membrane Tethering Proteins In Rat

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Introduction and Objectives: Pulmonary hypertension (PH) is a lethal vascular disease. The outcome of the patients still remains poor. Recently, mitochondria associated membrane (MAM) has been revealed to play crucial roles to maintain mitochondria function. However, it largely remains unclear whether MAM tethering proteins contribute to disrupted MAM and then PH. Methods: We used two different rat models of PH: monocrotaline(MCT)-induced pulmonary artery hypertension (PAH) in male Sprague-Dawley rats and left atrium stenosis-induced PH (LAS-PH) in male Sprague-Dawley rats. Rats were analyzed with echocardiography and subsequently excised to examine PDZD8 and other proteins. Results: The analysis showed that MCT treatment caused PAH. In MCT-injected PAH rats, the expression of PDZD8 mRNA was significantly downregulated in the lung of MCT group and LAS-PH group. In addition, the expression of mitofusin-2 (MFN2) that plays an important role in MCT and MAM formation was significantly reduced at transcriptional level in lung. Furthermore, PDZD8 and MFN2 proteins were significantly reduced in the lung of MCT group. Conclusions: Both PDZD8 and MFN2 are reported as the endoplasmic reticulum mitochondria tethers, whereas MFN2 affects mitochondrial fusion but PDZD8 does not. We found that PDZD8 and MFN2 were significantly downregulated in the lung of two different rat PH models, suggesting that impaired MAM formation is involved in pathogenesis of PH. (COI: No)

1P-083

NF- κB-mediated upregulation of miR-335-3p contributes to the induction of hypoxic PAH in mice

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Aim: To investigate the role of NF-κB/miR-335-3p signaling in chronic normobaric hypoxia (CNH)-induced pulmonary arterial hypertension (PAH) in mice, as well as the potential mechanism. **Methods and Results:**Adult male C57BL/6 mice were exposed to CNH for 5 weeks to establish hypoxic PAH model. High-Throughput Sequencing and qRT-PCR results showed that miR-335-3p was obviously increased in PAH mice lung tissues. Blockade of miR-335-3p attenuated proliferation of PASMCs, alleviated pulmonary vascular remodeling, and exhibited preventive and therapeutic effects in CNH-induced PAH. CNH exposure facilitated NF-κB nucleus translocation and NF-κB blockade diminished PAH. Moreover, NF-κB knockout mice were resistant to PAH. Luciferase reporter assay indicated that NF-κB was a transcriptional regulator upstream of miR-335-3p, and pyrrolidine dithiocarbamate treatment reversed CNH-induced increase in miR-335-3p expression. Finally, the receptor of apelin (APJ) was identified as a direct targeting gene downstream of miR-335-3p, and gain-of-function of APJ by apelin-13 treatment ameliorated the hypoxic pulmonary vascular remodeling and diminished CNH-induced PAH. **Conclusions:**Our results implicate that NF-κB-mediated transcriptional up-regulation of miR-335-3p contribute to the inhibition of APJ and induction of hypoxic PAH, miR-335-3p be a potential therapeutic target for hypoxic PAH.
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1P-084

The role of vascular smooth muscle NCX1 in the pathogenesis of pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is a severe and progressive disease that causes right heart failure. Recent studies suggested that the hypercontraction and excessive proliferation of the pulmonary artery may be involved in the pathogenesis of PAH, though their molecular mechanisms remain unclear. In this study, we focused on the pathophysiological role of Na^+/Ca^{2+} exchanger type 1 (NCX1) in hypoxia-induced PAH model. We found that the expression of NCX1, as well as HIF-1α, was markedly increased in pulmonary arteries of hypoxia-induced PAH model mice. Therefore, we created hypoxia-induced PAH models using vascular smooth muscle (VSM)-specific NCX1-transgenic (vsmN1-Tg) and conditional knockout mice (vsmN1-KO). In vsmN1-Tg mice, hypoxia-induced right ventricular systolic pressure elevation and pulmonary vessel muscularization were significantly higher than those in wild-type mice. In contrast, these pathological parameters induced by hypoxia were significantly suppressed in vsmN1-KO mice and wild-type mice treated with NCX1 inhibitors. In hypoxia-exposed human pulmonary VSM cells, NCX1 inhibitors diminished endothelin-1-induced Ca^{2+} signaling. Intriguingly, we confirmed that NCX1 protein was highly expressed in the plexiform lesion of Sugen/Hypoxia rat, a human PAH-like pathological model. These results suggest that VSM NCX1 is involved in the pathogenesis of hypoxia-induced PAH. NCX1 inhibitors might be useful therapeutically for PAH. (COI: No)

1P-085

Inflammatory effects of menthol versus non-menthol cigarette smoke on the mouse lungs

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Menthol cigarette smoking is associated with more severe lung inflammation than non-menthol cigarette smoking in smokers, but the mechanisms remain unclear. Menthol is a ligand of transient receptor potential melastatin-8 (TRPM8). In this study, mice were exposed to non-menthol cigarette smoke (Non-M-CS) or menthol cigarette smoke (M-CS) for 7 days. Compared to Non-M-CS exposure, M-CS exposure up-regulated the epithelial and lung expression of TRPM8, induced more vigorous activation of epithelial and lung mitogen-activated protein kinases (MAPKs), and caused more severe lung inflammation. The MAPK activation was evidenced by increased expression of phosphor-ERK and phosphor-JNK. The lung inflammation was evidenced by pathohistological findings and increases in inflammatory indices. Importantly, treatment with a TRPM8 antagonist greatly suppressed the MAPK activation and lung inflammation induced by both Non-M-CS and M-CS. However, levels of biomarkers of acute CS exposure (20 minutes), including carboxyhemoglobin and cotinine (a nicotine metabolite) in blood plasma as well as superoxide and hydrogen peroxide in bronchoalveolar lavage fluid did not show significant differences between mice with Non-M-CS and M-CS exposure. We concluded that M-CS could induce greater TRPM8-mediated activation of MAPKs and lung inflammation than Non-M-CS could in mice. The augmented effects of M-CS may be due to an additional stimulation of epithelial and lung TRPM8 by menthol in M-CS. (COI: No)

1P-086

Nerve growth factor contributes laryngeal airway hyperreactivity in rats with intermittent hypoxia

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Obstructive sleep apnea, manifested by intermittent hypoxia (IH), is associated with laryngeal airway hyperreactivity (LAH) and airway inflammation. Nerve growth factor (NGF), a major type of neurotrophins, is expressed in capsaicin-sensitive afferent fibers and airway structural cells, and may potentially contribute to pathophysiology of several airway diseases. We investigated whether IH augment reflex apneic responses to laryngeal provocations of chemical stimulants (capsaicin, phenylbiguanide, and a,b-methylene-ATP) and the role of NGF in the development of LAH. Conscious male rats were exposed to IH or room air (RA) for 14 consecutive days. On day 15, the reflex apneic responses to laryngeal provocations of chemical stimulants in IH rats were significantly greater than those in RA rats. The augmented apneic responses were abolished by bilateral sectioning of superior laryngeal nerves or perineural capsaicin treatment of superior laryngeal nerves. The potentiating effect of IH on reflex apneic responses to laryngeal provocations was largely attenuated by daily treatment with anti-NGF antibody, suggesting the involvement of NGF. After 14 days of IH exposure, elevated the levels of protein expression of NGF in the larynx of rats were also found. Our results suggest that IH exposure sensitizes superior capsaicin-sensitive laryngeal afferents, leading to exaggerated apneic reflex responses; this sensitizing effect is mediated at least in part through upregulation of NGF. (COI: No)

1P-088

Successful cigarette smoke extract-induced emphysema model defined by histology and inflammation

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Purpose: To characterize a mouse model of cigarette smoke extract (CSE)-induced lung inflammation and emphysema in regard to histological outcome and inflammatory profiles.

Methods: Twenty-eight C57BL/6 mice were divided into control and CSE (n=14 each) groups. CSE was freshly prepared by sucking the smoke from 4 lit cigarettes through the glass tube submerging in 4 mL of phosphate buffered saline (PBS). Optical density was monitored for the amount of dissolved particles. PBS or CSE was administered in each group via intraperitoneal injection (I.P.) on day 1, 12, and 23, respectively. On day 29, bronchoalveolar lavage fluid (BALF) was collected for differential cell count and tumor necrosis factor alpha (TNFα) quantification with ELISA. Lungs were inflated at a transpulmonary pressure of 25 cmH_2O for morphometry.

Results: CSE significantly increased total BALF cells ($13.5\pm1.3x10^4$ vs. $7.6\pm1.1x10^4$ cells [mean±SEM], $p<0.01$), macrophages ($12.5\pm1.1x10^4$ vs. $7.6\pm1.1x10^4$, $p<0.01$), neutrophils (5,229±1,026 vs. 161±101, $p<0.0001$), eosinophils (690.8±209.2 vs. 19.0±13.6, $p<0.01$), and TNFα level (31.0±2.6 vs. 23.0±2.7 pg/mL, $p<0.05$) compared with control group. Mice induced by CSE had an increase in mean alveolar linear intercept by 23.45% (26.99±1.16 vs. 21.86±0.59 μm, $p<0.01$), indicating airspace enlargement.

Conclusion: I.P. CSE can be used to induce mouse lung inflammation and enlarged airspace for evaluation of emphysema as well as protective and therapeutic strategies. (COI: No)

1P-089

Functional Role of TRPC5 in Platelets

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Platelets play an essential role in thrombosis and hemostasis. They may be abnormal either quantitatively (too many or too few) or qualitatively (the right number but they don't work correctly). There are many platelet-related diseases, such as ischemic cardiovascular and cerebrovascular disorders, thrombocytopenia etc, which are lifethreatening.Previously, we identified a novel function of TRPC5 channels in promoting blood coagulation. However, which processes TRPC5 participates in and the underlying mechanism are not understood. In the beginning, we found that various physiological procoagulant agents, such as thrombin and collagen activate TRPC5 to induce cytosolic Ca^{2+}rise in platelets. Subsequently, those agents may result in surface exposure of phosphatidylserine and consequent platelet aggregation and blood coagulation through activating TRPC5. In the present proposal, we will utilize a variety of different technologies, including assay for cell surface exposure of phosphatidylserine by flow cytometry, assay for platelet activation/aggregation by flow cytometry/96-well microplate reader, assay for platelet adhesion/spreading and *in vivo* thrombus formation, to validate the functional role of TRPC5 and elucidate the underlying molecular mechanism. We expect this study to uncover novel mechanism for thrombus formation, which may help us to develop novel therapies for ischemic cardiovascular and cerebrovascular disorders. (COI: No)

1P-090

Decreased expression of KATP channel in human umbilical smooth muscle during gestational diabetes

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We investigated the alterations of ATP-sensitive K^+ (K_{ATP}) channels in human umbilical arterial smooth muscle cells during gestational diabetes mellitus (GDM). The amplitude of the K_{ATP} current induced by application of the K_{ATP} channel opener pinacidil (10 μM) was reduced in the GDM group than in the control group. Pinacidil-induced vasorelaxation was also predominant in the normal group compared with the GDM group. Reverse transcription polymerase chain reaction and Western blot analysis suggested that the expression of K_{ATP} channel subunits such as Kir6.1, Kir6.2, and SUR2B, were decreased in the GDM group relative to the normal group. The application of forskolin and adenosine, which activate protein kinase A (PKA) and thereby K_{ATP} channels, elicited K_{ATP} current in both the normal and GDM groups. However, the current amplitudes were not different between the normal and GDM groups. In addition, the expression levels of PKA subunits were not altered between the two groups. These results suggest that the reduction of K_{ATP} current and K_{ATP} channel-induced vasorelaxation are due to the decreased expression of K_{ATP} channels, not to the impairment of K_{ATP}-related signaling pathways. (COI: No)

1P-091

Vildagliptin induces vasodilation via SERCA pump and Kv channel activation in aortic smooth muscle

Mi Seon Seo; Won Sun Park (*Department of Physiology, Kangwon National University School of Medicine*)

This study investigated vildagliptin-induced vasodilation and its related mechanisms using phenylephrine induced precontracted rabbit aortic rings. Vildagliptin induced vasodilation in a concentration-dependent manner. Pretreatment with the large-conductance Ca^{2+}-activated K^+ channel blocker paxilline, ATP-sensitive K^+ channel blocker glibenclamide and inwardly rectifying K^+ channel blocker Ba^{2+} did not affect the vasodilatory effects of vildagliptin. However, application of the voltage-dependent K+ (Kv) channel inhibitor 4-aminopyridine significantly reduced the vasodilatory effects of vildagliptin. In addition, application of either of two sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitors, thapsigargin or cyclopiazonic acid, effectively inhibited the vasodilatory effects of vildagliptin. These vasodilatory effects were not affected by pretreatment with adenylyl cyclase, protein kinase A (PKA), guanylyl cyclase, or protein kinase G (PKG) inhibitors, or by removal of the endothelium. From these results, we concluded that vildagliptin induced vasodilation via activation of Kv channels and the SERCA pump. However, other K^+ channels, PKA/PKG-related signaling cascades associated with vascular dilation, and the endothelium were not involved in vildagliptin-induced vasodilation. (COI: No)

1P-092

Withdrawn

1P-093

Roles of K^+ channels in synchronising spontaneous Ca^{2+} transients in mural cells of rectal arteriole

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[Background] The mural cells (vascular smooth muscle cells and pericytes) of rectal arterioles exhibit synchronous spontaneous Ca^{2+} transients that may cause spontaneous constrictions of upstream arteriole to facilitate blood flow. Here, we examined the roles of K^+ channels in maintaining the synchrony of spontaneous Ca^{2+} transients. **[Methods]** Cal-520 intracellular Ca^{2+} imaging was performed in the rat rectal submucosa. **[Results]** In the mural cells of submucosal arterioles, synchronous Ca^{2+} transients were converted into asynchronous Ca^{2+} fluctuations by the voltage-dependent K^+ (K_v7) channel blocker XE 991 (10 μM) or the inward rectifier K^+ (K_{ir}) channel blocker Ba^{2+} (50 μM) or by increasing $[K^+]_o$ from 5.9 mM to 29.7 mM. A small increase in $[K^+]_o$ from 5.9 mM to 10.7 mM inhibited the spontaneous Ca^{2+} transient amplitude presumably by increasing K_{ir} conductance. In XE 991-treated preparations, the ATP-sensitive K^+ channel opener levcromakalim restored the intercellular synchrony. Ca^{2+}-activated K^+ channel blockers had no effect on the spontaneous activity. **[Conclusion]** In the rectal arterioles, K_v7 and K_{ir} channels appear to stabilise the membrane of mural cells to prevent asynchronous Ca^{2+} oscillation. This mechanism ensures sarco-endoplasmic reticulum Ca^{2+} store refilling that is required for the subsequent regenerative Ca^{2+} release to develop synchronous spontaneous Ca^{2+} transients. (COI: No)

1P-094

Periodic assessment of (ET-1) and Nitric Oxide (NO) in hypertensive disorders of pregnancy (HDP)

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INTRODUCTION
Hypertensive Disorders of Pregnancy (HDP) is an independent risk factor of cardiovascular (CVS) disease. Endothelin-1 (ET-1) a potent vasoconstrictor, has been identified as a pivotal mediator in both essential hypertension and HDP. Disturbances in Nitric Oxide (NO) bioavailability found in endothelial dysfunction may increase susceptibility to cardiovascular diseases.
METHODOLOGY
Thirty six pregnant women at 30-36 weeks period of gestation from the following categories (i) pregnancy induced hypertension (PIH) (ii) chronic hypertension during pregnancy (CH) and (iii) normal pregnant women (Control). Blood pressure indices measurements and sample collection was done at antepartum (30-36 weeks), post partum (8 weeks and 12 weeks). Endothelin-1 and serum NO were measured using the Human ET-1 (Endothelin-1) and NO ELISA Kit.
RESULTS
All blood pressure indices were significantly higher in HDP patients compared to control during antenatal and post partum periods. Serum ET-1 was significantly higher in patients with HDP compared to control during antenatal until 3 months post partum. This was accompanied by significantly lower levels of serum NO in HDP patients.
CONCLUSION
Persistently high levels of ET-1 and low levels of NO up to 3 months post partum in patients with history of HDP indicate presence of persistent endothelial dysfunction despite BP normalisation in PIH patients. Long term NO/ET-1 imbalance may account for the increased CVS disease risk. (COI: No)

1P-095

L-Cysteine's carotid flow responses mapped in pre-sympathetic areas of the rat ventral medulla

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Thiol-containing amino acid L-cysteine (L-cys) produces changes in arterial pressure (AP) via activation of ionotropic excitatory amino acid receptors (iEAAr) when microinjected into the rat ventrolateral medulla (VLM) areas which are involved in pre-sympathetic neuronal regulation. The present study mapped common carotid blood flow (CAF) responses to L-cys microinjections in the VLM. In anesthetized rats, a window was opened above the ventral medulla after removal of the insertion parts of the sternohyoid muscles (SM) in the ventral neck to obtain a wider operating space, then right CAF and AP via left femoral artery were monitored, calculating CAR (AP/CAF). L-Cys microinjections decreased CAR in the caudal VLM depressor sites with a tendency to keep flow constant or autoregulation. Then, CAR was increased at posterior part of the rostral (R) VLM pressor sites but decreased at its anterior part, showing apparent distinct responding areas. However, L-cys injections increased CAR in all RVLM pressor sites in rats which had the SM intact. In superior cervical sympathectomized rats, CAF was highly dependent on AP changes or lost autoregulation in all VLM responding sites. Results indicate that L-cys stimulation of the VLM areas sympathetically evokes changes in CAR parallel to those in AP. Anterior part of the RVLM may be involved in the cervical SM vascular bed regulation. Head and cervical sympathetic vascular regulation appears to involve iEAAr in the VLM areas. (COI: Properly Declared)

1P-096

Role of c-Abl/YAP^{Y357} in integrin α5 activation in endothelial atherogenic responses

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Objective-In this study, we aimed to investigate a possible crosstalk in integrin α5 and YAP pathways in endothelial cells (ECs) in relation to atherogenesis.
Methods and Results-We report here that integrin α5 heterozygous deficiency blocked YAP nuclear translocation and that YAP overexpression in ECs blunted the anti-atheroprone effect of an integrin-α5 blocking peptide in ApoE$^{-/-}$ mice. OSS led to integrin α5 activation and strong, continuous nuclear translocation of YAP in ECs, as well as increased phosphorylation of YAP at $Y^{357}$and a decreasing trend of that at S^{127} and S^{397}. Blocking integrin α5 inhibited the OSS-induced YAP tyrosine phosphorylation at Y^{357} and nuclear translocation, but had minimal effect on the serine phosphorylation of YAP at S^{127} and S^{397}. Mechanistic studies showed that both Src and c-Abl inhibitors abolished the OSS-induced YAP tyrosine phosphorylation, with the effect of c-Abl inhibitor being more profound. Furthermore, the phosphorylation of c-Abl and YAP^{Y357} was significantly increased in ECs in atherosclerotic vessels of mice induced by partial ligation and Western diet and in human plaques *vs.* normal vessels. Bosutinib, a Src and c-Abl dual inhibitor, markedly reduced the level of YAP^{Y357} and the development of atherosclerosis in ApoE$^{-/-}$ mice.
Conclusions-The activation of integrin α5 contributes to OSS-induced EC activation and atherosclerosis via a c-Abl/ YAP^{Y357} pathway, providing a potential therapeutic strategy for atherogenesis. (COI: No)

1P-097

Different effects of α and β_1 blockers on Beta in the elastic and muscular arteries in rabbits

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Purpose: The regulatory system of arterial stiffness of elastic artery and muscle artery during administration of antihypertensive agents was not well understood. The theory of cardio-ankle vascular index (CAVI) was applied to the entire aorta (elastic artery) (AoBeta), and to the iliac and femoral arteries (muscular artery) (ifBeta). The effects of non-selective α blocker (phentolamine; PHN, 50 μg/kg/min) and β_1 blocker (atenolol; ATN, 10 mg/kg/min) on both indices were studied in rabbits.
Methods: Rabbits aged 10-12 months were anesthetized with pentobarbital sodium (30 mg/kg, iv). Change in pulse waves at the ascending aorta (AA), distal end of the abdominal aorta (dAbd) and distal end of the left femoral artery (dFemo) were simultaneously recorded when PHN and ATN were infused. Beta was determined as $\mathrm{Beta}=2\rho/\Delta P\times\ln(Ps/Pd)\times PWV^2$ (ρ: blood density, Ps, Pd and ΔP: systolic, diastolic and pulse pressures). AoBeta, ifBeta and afBeta were calculated by PWV in the aorta, from dAbd to dFemo and from AA to dFemo.
Results: AoBeta increased significantly while ifBeta decreased in response to PHE. AoBeta, ifBeta and afBeta did not correlate with BP when ATN was infused, whereas each PWV correlated with BP during infusion of both agents.
Conclusions: Using CAVI theory, it was demonstrated that proper arterial stiffness of the elastic and muscular arteries was not uniformly changed by infusion of PHN and ATN. The mechanism of contradictory changes was under investigation. (COI: Properly Declared)

1P-098

Angiopoietin-2 is released after vascular leak onset during anaphylaxis in un- and anesthetized rats

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Purpose: Angiopoietin (Angpt)-2, a permeability-increasing endothelial growth factor, is released in response to secretagogues such as histamine and leukotrienes and is involved in vascular leakage of sepsis and acute lung injury. However, roles of Angpt-2 in the hyperpermeability during systemic anaphylaxis are not known. Thus, we determined the plasma Angpt-2 levels and vascular permeability of various organs during anaphylactic hypotension in anesthetized and awake ovalbumin-sensitized rats.
Methods: In both anesthetized and awake rats, mean arterial blood pressure (MBP) was continuously measured for 60 min after antigen, and hematocrit (Hct) and Angpt-2 levels in the plasma were assessed at baseline and at 3, 10, 20 and 60 min. They were measured further at 2, 10 and 24 hr in awake rats. Separately, vascular permeability to albumin was measured using the Evans blue dye method at 20 min in anesthetized rats.
Results: At 10 min after antigen, MBP and Hct decreased and increased, respectively, significantly and maximally in sensitized rats. Vascular permeability in the trachea, bronchus, skeletal muscle and mesentery increased at 20 min. Plasma Angpt-2 levels did not increase significantly until 1 hr in both anesthetized and awake sensitized rats. In awake rats, the Angpt-2 levels reached the peak at 10 hr and returned to baseline at 24 hr.
Conclusions: Angpt-2 is released after occurrence of anaphylaxis-induced vascular leak in anesthetized and unanesthetized rats. (COI: No)

1P-099

Apolipoprotein C3-rich LDL induces endothelial dysfunction and vascular cells senescence *in vivo*

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Purpose: Dyslipids are associated with the age-related cardiovascular disease. Apolipoprotein CIII plays a causal role in atherosclerotic lesion development independent of its deleterious effects on lipid metabolism. We examine the effect of Apolipoprotein CIII-rich LDL (AC3RL) on endothelial senescence and its underlying molecular mechanisms.
Methods: AC3RL was isolated from plasma LDL of patients with ischemic stroke and healthy subjects with the affinity-purified method.
Results: Human aortic endothelial cells exposed to AC3RL induced the phosphorylation of H2AX, FBXO31 increase, and p53 activation, thereby cellular senescence. Pharmacologic or genetic manipulation of the FBXO31/MDM2 and p53 arrested AC3RL-induced endothelial cells senescence. Syrian hamsters fed a high-fat diet (HFD) for 16 weeks had a higher level of plasma AC3RL than did a normal chow diet (control group) ($p<0.01$; n=8 per group). Senescence-associated β-galactosidase (SA-β-gal) staining and gamma-H2AX indicative of premature senescence were markedly increased in the thoracic aortic tissue of hamsters in the HFD groups but not control group. The phenomenon of premature senescence was blocked in presence of sesamol (a natural anti-oxidant) in hamsters fed with HFD.
Conclusions: AC3RL may trigger DNA damage to induce vascular endothelial cell senescence and atherosclerosis. The novel prevents or therapeutic target of cardiovascular disease may be provided from AC3RL-induced endothelial cell senescence. (COI: Properly Declared)

1P-100

The Role of KLF1 in Mediating Immune Response

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Krüppel-like factor 1 (Klf1) was a transcription factor, specific expressed in erythroid cells. However, mRNA expression of Klf1 was found in certain populations of leukocyte in recent year. We generated a mouse which loss the sumoylated site of Klf1 (Kin), to investigate the biologic function of Klf1 sumoylation. We surprisingly observed the populations of certain leukocytes were altered in peripheral blood of Kin mice. Therefore, we hypothesized Kin mice reveal some specific immune characteristic.
Significantly high survival rate and moderate symptoms was observed in Kin mice after intervention. Earlier neutrophil migration and lower pro-inflammatory cytokine releasing were observed in Kin mice. Conversely, cytokines of late inflammatory stage were ascent during 6 to 18 hours in Kin mice, but not in WT mice. In addition, less bacteria colonies and higher macrophage phagocytic activity were seen in Kin mice. We found some difference of gene expression which involved in cell migration, cell rolling and pro-inflammatory response though microarray profile.
Kin mice improve the survival due to a shorter time window of immune response and higher phagocytic activity. Klf1 may regulate downstream gene which involved in immune process though the sumoylated site. Klf1 could be a new target for inflammatory related disease. Through this model, we could establish a panel of anti-inflammatory drug development in future. (COI: No)

1P-101

YAP promotes angiogenesis via STAT3 in endothelial cells

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Rationale: Angiogenesis is a complex process regulating endothelial cell (EC) functions. Emerging lines of evidence support that Yes-associated protein (YAP) plays an important role in regulating the angiogenic activity of ECs.
Objective: To specify the effect of EC YAP on angiogenesis and its underlying mechanisms.
Method and Results: In ECs, vascular endothelial growth factor reduced YAP phosphorylation time- and dose-dependently and increased its nuclear accumulation. Using Tie2Cre-mediated YAP transgenic (Tie2Cre-YAP^{Tg}) mice, we found that YAP promoted angiogenesis in the postnatal retina and tumor tissues. Mass spectrometry revealed signal transducer and activator of transcription 3 (STAT3) as a potential binding partner of YAP in ECs. Western blot and immunoprecipitation assays indicated that binding with YAP prolonged interleukin 6–induced STAT3 nuclear accumulation by blocking chromosomal maintenance 1-mediated STAT3 nuclear export without affecting its phosphorylation. Moreover, angiopoietin-2 (Ang2) expression induced by STAT3 was enhanced by YAP overexpression in ECs. Finally, a selective STAT3 inhibitor or Ang2 blockage partly attenuated retinal angiogenesis in Tie2Cre-YAP^{Tg} mice.
Conclusions: YAP binding sustained STAT3 in the nucleus to enhance the latter's transcriptional activity and promote angiogenesis via regulation of Ang2. (COI: No)

1P-102

Inhibition of PRC2 Protects against Restenosis via Suppressing Tri-methylation of H3K27 in SMCs

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Objectives Vascular restenosis is the most common complication involved in angioplasty surgery. Various epigenetic modifications are involved in restenosis. Here, we aimed to explore the effects of histone epigenetics on restenosis.
Approaches and results Human aortic vascular smooth muscle cells (HAVSMCs) were challenged with PDGF-BB. Total histones were extracted using acid extraction method coupled with HPLC/MS. We found PDGF-BB treatment remarkably increased H3K27me3 and its catalase EZH2. For study in vivo, rats were subjected to wire-guided common carotid injury (CWI). EZH2 and H3K27me3 peaked at day 3 post CWI and waned at day 7 and day 14 post CWI. H3K27me3 inhibition by EZH2 inhibitor UNC1999 significantly attenuated proliferation and migration of HAVSMCs treated with PDGF-BB. Consistently, neointimal formation was attenuated significantly by oral and perivascular administration of UNC1999. Mechanistically, increased H3K27me3 inhibited transcription of P16^{INK4A} and thus promoted VSMCs proliferation.
Conclusions Vascular injury elevates EZH2 and downstream targets H3K27me3 within neointimal formation model. As a result, p16^{INK4A} expression in smooth muscle cells is suppressed followed by robust proliferation which promotes restenosis. (COI: Properly Declared)

1P-103

Gaseous components of cigarette smoke upregulate prostaglandin E2 receptor EP4 in aortic aneurysm

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[Purpose] Abdominal aortic aneurysm (AAA) progressively enlarges, and once it ruptures, mortality rate is 28% even if patients undergo aortic aneurysm repair surgery. Cigarette smoking is a risk factor for AAA. However, the molecular mechanism of smoking-induced AAA is largely unknown. We previously demonstrated that prostaglandin E2 receptor EP4 was upregulated in human AAA smooth muscle layer and EP4 stimulation exacerbated AAA in mouse models. The aim of this study was to investigate the association of smoking with EP4 signaling in AAA. **[Methods]** Human aorta smooth muscle cells (SMCs) were stimulated with cigarette smoke extract (CSE) and acrolein which is a major constituent of CSE. Expression level of EP4 mRNA was quantified by RT-PCR. We generated mice with vascular SMC-specific overexpression of EP4 using the Cre-loxP system (EP4$^{loxP-/-}$/SM22-Cre mice: EP4-Tg and EP4$^{-/-}$/SM22-Cre mice: Non-Tg), and administered angiotensin II to EP4-Tg and Non-Tg mice. **[Results]** Acrolein stimulation (10 μmol/l) for 24 h increased EP4 mRNA (1.9 ± 0.2-fold, n = 6-7, $p < 0.001$) in human aorta SMCs. CSE stimulation (0.1 mg/ml) for 24 h increased EP4 mRNA by 2.5-fold, as well. EP4-Tg mice administered with angiotensin II exhibited AAA, and 75 % of EP4-Tg mice died of AAA rupture within two weeks (n = 8). However, none of Non-Tg mice exhibited AAA. **[Conclusions]** These data suggest that gaseous components of cigarette smoke increased EP4 expression in vascular SMCs, which may cause AAA. (COI: Properly Declared)

1P-104

Central command increases oxygenation of the non-contracting arm muscles during fine hand movement

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Our laboratory has demonstrated that central command increases the oxygenation of the non-contracting arm muscles during one-armed cranking exercise. The present study examined whether central command may increase the oxygenation of the non-contracting arm muscles during fine hand movement without the systemic cardiovascular responses. Thirteen males and seven females participated in this study. We used a two-ball rotation task requiring fine motor control of the hand. The subjects rotated the two balls as quickly and precisely as possible on the palm side of the right hand. The relative changes in oxygenated-hemoglobin concentration (Oxy-Hb) of the non-contracting arm muscles (the anterior deltoid, triceps brachii, biceps brachii, and extensor carpi radialis) and the systemic cardiovascular responses were measured during voluntary two-ball rotation and mental imagery of the task for 1 min. Two-ball rotation movement increased Oxy-Hb of the non-contracting proximal and distal arm muscles without changing systemic hemodynamics. The increased Oxy-Hb of the non-contracting arm muscles was attenuated by intravenous injection of atropine (10-15 μg/kg). Since mental imagery of the two-ball rotation increased the Oxy-Hb of the arm muscles, it is likely that central command increases muscular oxygenation of both proximal and distal muscles in the non-contracting arm during fine hand movement, probably via the sympathetic cholinergic vasodilator system. (COI: No)

1P-105

Cold stimulation for the tympanic membrane decreases heart rate

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Cold face stimulation induces decrease in heart rate (HR) and increase in arterial pressure (AP) Similar to the procedure of cold face stimulation, caloric test has been used in the field of Otolaryngology. However, changes in AP and HR during caloric test is unknown. In the present study, we investigate changes in AP and HR during air caloric test using cold air. For 24 healthy subjects, cold airflow of 15 °C was applied to left and bilateral tympanic membrane, the Tragus, and airflow of 37 °C was applied to left tympanic membrane. In 15 subjects, cold airflow for left and bilateral tympanic membrane did not change AP (No-AP-Down), but HR immediately and significantly decreased. In 9 subjects, AP decreased during cold air stimulation (AP-Down), but HR significantly decreased similar to that in No-AP-Down group. Thus, cold air flow for the tympanic membrane decreases HR regardless of AP change. Cold air stimulation for the Tragus did not change AP and HR for both groups. Thus, decrease in HR during the tympanic membrane stimulation might be different from that during cold face stimulation or the reflex via the trigeminal nerves. Air stimulation of 37 degrees C did not change AP and HR, neither. Thus, the decrease in HR during cold stimulation is not induced by mechanical stimulation but cold or caloric stimulation for the tympanic membrane. Tympanic membrane might sense cold temperature, and directly or reflexively decreases HR regardless of AP change. (COI: No)

1P-106

mGluR2/3 Agonist Suppresses Hypertension Development in SHR

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Signals of baroreceptor afferents are transmitted by glutamate in nucleus tractus solitarius (NTS), which is a crucial site for regulating blood pressure. Group II metabotropic glutamate receptors (=mGluR2/3) in NTS synapses can modulate baroreflex signal transmission, and thus malfunction of mGluR2/3 might set blood pressure unproperly, resulting hypertension. We hypothesized that continuous mGluR2/3 signaling could weaken or stop hypertension development. Using 6 week-old spontaneously hypertensive rats (SHRs), mGluR2/3 agonist (LY379268; 0.4, 1.3 & 4.0 μg/day) was continuously applied to the dorsal medulla oblongata via a foramen magnum catheter with implantable mini-osmotic pump until 12 week-old. Tail-cuff method was used to record blood pressure from conscious animals. The systolic blood pressure (SBP) of sham control group reached to 200 mmHg at 13 week-old, while experimental groups' showed less than 165 mmHg. All agonist doses showed almost equal effect on SBP, indicating no dose dependency on reaching to maximum response with 0.4 μg/day of LY379268. Even after finishing LY379268 application, SBP still remained around 165 mmHg in experimental groups, suggesting that continuous mGluR2/3 signaling in dorsal medulla oblongata could change the set point of blood pressure lower for the rest of life. In conclusion, stimulating dorsal medulla oblongata's mGluR2/3 do suppress hypertension development, which may be a pharmacological target of preventing hypertensive onset. (COI: No)

1P-107

Standard-dose gentamicin does not increase a risk of patent ductus

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Background: The ductus arteriosus (DA) is an essential artery that bypasses the main pulmonary artery and the descending aorta during a fetal period. DA closure sometimes delays in neonates with infection, although the DA immediately closes after birth. Clinically, GM is widely used for early-onset infection in neonates. Recently, Vucovich et al reported that standard-dose gentamicin (GM) caused patency of DA in mice. However, it has been insufficiently investigated the effect of GM in *in vivo* experiment afterward. We then reevaluated the *in vivo* effect of standard-dose GM on patency of the rat DA.
Methods: 1) To evaluate the effect of GM on prolongation of DA patency, GM was intraperitoneally injected immediately after birth. 2) To evaluate the effect of GM on reopening of the DA, GM was intraperitoneally injected 30 min after birth. Then, the inner diameter of the DA was measured 30 min after administration of GM by a rapid whole-body freezing method.
Results: Standard-dose GM (5 μg/g) did not prolong the patency of DA nor reopen DA when compared to control neonates. However, when the dose of GM was increased to 100 μg/g, DA closure was significantly delayed in rat neonates. Furthermore, high-dose GM (100 μg/g) tended to reopen the DA in rat neonates, although the dilative effect did not reach a statistical significance.
Conclusion: Standard-dose GM does not increase the risk of PDA in rat neonates.
(COI: No)

1P-108

Role of TRPV4 on the spontaneous electrical properties of guinea pig mesenteric lymphatic vessel

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This study designed to investigate the effect of TRPV4 in the electrical activity of the lymphatic vessels. The guinea pig mesenteric lymphatic vessels were isolated and pinned on the silicone sheet and perfused by 37 C Krebs solution. The diameter change was measured with the edge tracking system. 2 tungsten wires were inserted into the lymphatic vessel lumen and inhibit the spontaneous vasomotion to record the membrane potential of the lymphatic vessel using the conventional glass microelectrode method. The resting membrane potential of the lymphatic vessels were -45 mV, and spontaneous action potentials were observed. Application of GSK initially hyperpolarized and decreased the frequency followed by the increase of the frequency. In presence of LNA, GSK increased the frequency and did not hyperpolarize the vessel or decrease the frequency of the action potential. On the endothelium denuded vessels, the TRPV4 agonist also expressed the activating effect on the spontaneous electrical activity. These results suggest that the activation of TRPV4 on the smooth muscle of the lymphatic vessel is increasing the frequency of the action potential to enhance the peristalsis. (COI: Properly Declared)

1P-109

Physiological evidence that mesenteric lymph has been called as white blood

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The mesenteric lymph was named "white blood" by Hippocrates in ancient Greece. However, detailed mechanisms of the development of a white color in the mesenteric lymph are not fully elucidated. Therefore, we conducted rat and rabbit in vivo experiments to investigate the effects of intragastric administration of distilled water on the jejunal-originated lymph flow, and the concentrations and total flux of cells, albumin, long-chain fatty acids through mesenteric lymph vessels. The intragastric administration of distilled water (3 ml) caused significant increases in rat mesenteric lymph flow and in the total flux of cells, albumin, long-chain fatty acids through the lymph vessels. The white color of the lymph collected over a period of 1 h after the intragastric administration of water, was more intense. The intravenously injected Evans blue dye rapidly leaked into rabbit mesenteric lymph vessel and chylocyst. In conclusion, the absorbed water in the jejunum is transported through mesenteric lymph vessels. The leaked albumin in the jejunal microcirculation may play key roles of the transport of consumed water and the reservoir and transporter of long-chain fatty acids. Thus, these findings may be related to the physiological evidence that mesenteric lymph has been called as white blood (COI: No)

1P-110

Mutual interaction of orexin-A and glucagon-like peptide-1 on reflex swallowing in anesthetized rats

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We previously reported that orexin-A and glucagon-like peptide-1 (GLP-1) independently suppress swallowing reflex. Orexin-A has an appetite enhancing effect. Oppositely, GLP-1 has an anorectic effect. It seems to be inconsistent that both appetite-enhancing peptide and anorectic peptide have a same effect. It is however possible that appetite-enhancing peptide and anorectic peptide interact antagonistically. In the present study, we examined the relationship between orexin-A and GLP-1 on the regulation of reflex swallowing in anaesthetized rats. Swallowing was induced by repeated electrical stimulation of the central cut end of the super laryngeal nerve and was identified by the electromyogram lead penetrated the mylohyoid muscle through bipolar electrodes. Pre-administration of orexin-A diminished the suppressive effect of GLP-1 on the reflex swallowing, and pre-administration of orexin-1 receptor antagonist (SB334867) enhanced the suppressive effect of GLP-1. Pre-administration of GLP-1 diminished the suppressive effect of orexin-A on the reflex swallowing, and pre-administration of GLP-1 receptor antagonist (exendin 5-39) enhached the suppressive effect of orexin-A. These results suggested that GLP-1 and orexin-A mutually inhibit the suppressive effect on swallowing reflex. The experimental protocols were approved by the Okayama University Animal Use Committee. This work was supported by JSPS KAKENHI Gant Number 15K00818 and 18K11099. (COI: No)

1P-111

Age-dependent attenuation of hypothalamic sensitivity to thermogenic melanocortin signals

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Obesity is often developed with age due to attenuation of energy expenditure, such as from metabolic thermogenesis. However, the mechanism of age-dependent attenuation of metabolism is unknown. In this study, we focused on age-dependent alteration of the central neural circuit controlling metabolic thermogenesis in brown adipose tissue (BAT). Our *in vivo* physiological experiments revealed that skin cooling-induced BAT thermogenesis was attenuated in older (6 months old) male rats compared with younger (9 weeks old) ones. Neurons in the dorsomedial hypothalamus (DMH) are known to mediate thermogenic sympathetic outflow to BAT and express melanocortin-4 receptors (MC4Rs), which play essential roles in the regulation of appetite and energy homeostasis. Then, we hypothesized that melanocortin signaling mediated by MC4Rs in the DMH is altered in older animals. Supporting this hypothesis, nanoinjection of melanotan-2 (MT-2), an MC4R agonist, into the DMH induced blunted BAT thermogenesis in older rats, compared to younger rats. Indicating unaltered ability of BAT thermogenesis, disinhibition of DMH neurons with a $GABA_A$receptor antagonist elicited comparable, intense BAT thermogenesis in these two groups. These results suggest that attenuation of MC4R-mediated potentiation of BAT sympathetic outflow from the DMH causes age-dependent attenuation of energy expenditure leading to obesity. (COI: No)

1P-112

Intake of caffeine in the morning exhibits anti-obesity effect on mice fed with high-fat diet

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Caffeine is one of the nutritional foods which have anti-obesity effect. Previous study in mice showed that high-fat diet (HFD) contained caffeine (CHFD) inhibited obesity. However, there was no study on intake timing which effectively exhibits anti-obesity effect by caffeine. Therefore, we aimed to clear the effective caffeine intake timing for anti-obesity effect. Mice were fed 2 times per day (ZT8-16 and ZT20-4 were regarded as breakfast and dinner, respectively; ZT0 and 12 indicate light-on and off time, respectively). Control group was fed on HFD, breakfast group was fed on CHFD at breakfast, dinner group was fed on CHFD at dinner, and breakfast and dinner group was fed on CHFD. Only the breakfast group showed tendency to inhibit the increase of body weight and fat. Moreover, we found that the breakfast group consumed more lipid as an energy source during latter half of active period than other groups. These results suggest that breakfast may be effective timing for anti-obesity effect by caffeine and that caffeine intake at dinner may suppress anti-obesity effect by caffeine. Based on the prediction that caffeine intake at dinner delays the biological rhythm, which may be cause of suppression of anti-obesity effect, we are trying to examine serum lipid and lipid metabolic genes circadian rhythm. This work was supported by Grant-in-Aid for JSPS Research Fellow (17J10069) and by Early Bird Program for Waseda Research Institute for Science and Engineering. (COI: No)

1P-113

Effect of suppression of oral sweet-sensing with gymnema sylvestre on food motivation in humans

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[Purpose] We previously reported that oral stimulation with *Gymnema sylvestre* (GS), which is a plant known to suppress the sweet-sensing in humans, delayed gastric emptying rate during/after oral glucose ingestion. However, the influence of oral sweet-sensing on postprandial appetite and sensory-specific satiety (SSS) is not well understood. We, therefore, tested the hypothesis that suppression of oral sweet-sensing with GS would modulate SSS both during and after the prandial phase. [Methods] 15 healthy subjects rinsed their mouth with 25 ml of water (control) or 2.5% GS solution (GS group) and then consumed a muffin (97 g), yogurt with sucrose (100 g), and a banana (100 g). Using visual analog scales, the subjective appetite scores (i.e., hunger, fullness, satisfaction, and prospective consumption), SSS scores for sweetness, and subjective sweet taste intensity scores were evaluated during/after the prandial phase. [Results] Decreased subjective sweet taste intensity was observed in the GS group after consuming all foods. The scores of satisfaction (but not hunger, fullness, and prospective consumption) and SSS for sweetness were lower in the GS group than in the controls during and after the prandial phase. [Conclusions] These results suggest that the suppression of oral sweet taste sensation modulates food motivation and SSS for sweetness during/after the prandial phase. (COI: Properly Declared)

1P-114

Impact of Aerobic Exercises on Hunger, Satiety and Food Intake in Type 2 Diabetes Mellitus (T2DM)

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Purpose: Exercises increase insulin sensitivity and energy expenditure in T2DM patients.The effects of exercises on hunger and satiety are less known in T2DM. Understanding the impact of regular exercises on hunger, satiety and food consumption is important in diabetic management.

Method: Seventy five diabetics were randomly assigned into an exercise and a control group. Brisk walking, 30 min/day, 4-5 days/week for 3 months was introduced to the exercise group. Both groups maintained a diet diary. Hunger and satiety were assessed subjectively by a Visual Analogue Scale, at -30 min, +30 min,+60 min in relation to a standard breakfast. Food consumption was assessed by Nutrisurvey2007 (EBISpro) software. Data was analyzed by paired sample t-test.

Results: Level of hunger decreased at -30 (44.78/±23.76 vs 21.83/±19.48, p=0.000) and +30min (33.94/±14.83 vs 21.31/±13.9, p=0.003),and satiety increased at -30(29.06/±19.81 vs 47.75/±28.28, p=0.005) and +30min (44.22/±24.09 vs 56.19/±15.21, p=0.01) in the exercise group, compared to the baseline. Both groups showed reduced intake of total calorie, carbohydrates, fat and proteins with significant results for total calorie(1899.13/±665.1 vs 1719.36/±520.4 kcal, p=0.015)and carbohydrates (243.11/±103.98g vs 209.35/±68.54g, p=0.05) in the exercise group after 3 months. No significant differences were seen in controls. **Conclusion:** Regular aerobic exercises reduce hunger and increase satiety leading to less energy intake in T2DM. (COI: No)

1P-115

Possible improvement of cognitive function by long-term dark chocolate ingestion in young subjects

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Our prior research suggested that in mice, theobromine ingestion for ~1 month improves cognitive function. This study investigated whether long-term intake of dark chocolate, which contains a high level of theobromine, improves cognitive function in humans. Eighteen healthy subjects (20~31 years old) were divided into two groups; dark chocolate (DC; n=10) and theobromine-free white chocolate (WC; n=8) intake groups. Subjects took chocolate daily for ~30 days. Before and after chocolate intake and 3 weeks after the end of chocolate intake (Post), cognitive function of all subjects was assessed using a modified Stroop color word test (modified SCWT) and Digital Cancellation Test (D-CAT). Blood samples were taken to measure neurotrophin. For modified SCWT, the subjects look at a paper with color words written in a variety of random colors, and read words (Words test) or name the ink colors as quickly and accurately as possible for 1 minute. The D-CAT was carried out with a standard method. DC intake significantly increased the number of correct answers in the Words test and the number of total performance in D-CAT (both, P<0.017). The effects were persistent in the Post. DC intake significantly elevated plasma nerve growth factor levels (P=0.006). However, these responses were not seen after WC ingestion. The results suggest that long-term DC intake improves cognitive function in young subjects. This effect seemed to last for at least 3 weeks after the end DC intake. (COI: No)

1P-116

Maternal low-protein-diet alters the glucose metabolism and its intestinal mechanism of offspring

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Purpose Maternal low-protein-diet maybe related to the impaired glucose tolerance and type 2 diabetes of offspring. As the important interface of glucose entering the body, the intestine maybe an important target to explore. The effect of fetal programming on glucose homeostasis and intestinal mechanisms was investigated.

Methods Sprague-Dawley rats were feed with chow diet and protein restricted (PR) diet during pregnancy and lactation. Food intake and body weight of offspring were measured weekly. The oral glucose tolerance tests were tested and the duodenum, jejunum and ileum were collected for gene and protein expression analysis in 3- and 12-week-old of offspring.

Results PR rats presented decreased glucose concentrations after glucose administration and smaller areas under the glucose curves (AUG) in 3-week-old. In 12-week-old, they showed the enhancement in glucose level and higher AUC. There was a worse trend of the glucose tolerance in PR offspring from 3- to 12-week-old. Additionally, The mRNA and protein expression of T1R3, SGLT1 and GLUT2 in the intestine increased greatly which are related to augmented intestine sweet perception and glucose absorption of PR rats.

Conclusions These results suggest that maternal consumption of PR diet during critical developmental windows influences offspring glucose metabolism, which may be associated with dysregulation of taste receptor T1R3, and glucose transporter SGLT-1, GLUT2 mRNA and protein expression in the gut. (COI: No)

1P-117

Importance of RANTES/CCR5 signaling in lipid oxidation and adaptive thermogenesis in mice

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Our study conducted with RANTES / C-C chemokine receptor 5 (CCR5) double knockout mice (DKO) and wild type mice (WT) showed that adipose RANTES and CCR5 expression are markedly reduced by cold exposure in mice and by a β-adrenergic agonist in brown and beige adipocyte cell lines. Deletion of RANTES and CCR5 facilitates the thermogenic function of BAT, a morphological "browning" of inguinal white adipose tissue (iWAT) and enhanced oxygen and cold adaptability following cold exposure (CE, 4℃). The number of genes in response to cold exposure was significantly increased in both BAT or iWAT of DKO compared to WT by using RNA sequencing. Accordingly, the increased UCP-1 mRNA and protein levels in BAT and iWAT were noted in DKO compared to WT under room temperature (RT). The expression of genes involved in the mitochondrial oxidative function such as cox7a1, atp5b, cys were dramatically increased, especially in iWAT of DKO compared to WT under both CE and RT. The expressions of genes related to fatty acid metabolism such as elovl3, ATGL, cpt1b, scd1 and also lipolysis proteins such as ATGL, perilipin and phosphorylation of HSL were significantly increased in DKO compared to WT under RT and these genes were also significantly increased in BAT and iWAT of WT and DKO mice under CE. The present study suggested a negative regulatory role for adipose RANTES/CCR5 signaling in regulating lipid metabolism and mitochondrial thermogenesis under physiological condition.

(COI: No)

1P-118

Estradiol protects decrease in energy intake under psychosocial stress in ovariectomized rats

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We examined whether estradiol regulates energy intake under chronic psychosocial stress (CPS; Resident-intruder paradigm) by mediating ghrelin receptor (GHSR) levels in ovariectomized (OVX) rats. Female rats aged 11 wk were ovariectomized and assigned either to a placebo (Pla) group or a group treated with 17β-estradiol (E2) implanted with E2 pellet. At 14 wk of age, rats were given high-fat diet (HFD) in addition to standard diet. Food intake and body weight of the rats were measured every day for 21 days. Half of the rats in each group aged 15 wk were daily exposed to 120-min CPS for 14 days. On day 15, each rat received a bolus injection of ghrelin (40 μg/kg) intraperitoneally to compare orexigenic effect of ghrelin among the groups. After the rats were euthanized intracardiac bloods and gastric mucosa were sampled for measuring plasma corticosterone levels and for evaluating ghrelin and GHSR protein levels by western blotting, respectively. CPS decreased total energy intake and inhibited body weight gain in Pla group, but not in E2 group. Orexigenic effect of ghrelin was attenuated in CPS-Pla group compared to stress-free (control) Pla group. Moreover, GHSR protein level in gastric mucosa was lower in CPS-Pla group than that in control-Pla group. After CPS exposure, plasma corticosterone level was increased only in Pla group. These results suggest that estradiol protects CPS-induced depression in energy intake by restoring reduction of GHSR in OVX rats. (COI: No)

1P-119

Involvement of phosphoinositide 3-kinase in leptin signaling in sweet sensitive taste cells

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Anorectic hormone leptin is known to suppress behavioral, neural and taste cell responses to sweet substances in mice. Subpopulation of taste cells expressing sweet receptor component T1R3 also expresses leptin receptor Ob-Rb and K_{ATP} channel subunit SUR1. In sweet sensitive taste cells, sweet suppressive effect of leptin was inhibited by leptin receptor antagonist and K_{ATP} channel inhibitor glibenclamide. These results suggest that leptin may act via Ob-Rb and K_{ATP} channels present in T1R3 expressing taste cells to selectively suppress their responses to sweet compounds. However, intracellular signaling pathway linking from Ob-Rb to K_{ATP} channel in taste cells is still unclear. Here, we tried to elucidate leptin signaling pathway in taste cells. In taste cell responses, sweet suppressive effect of leptin was significantly inhibited by phosphoinositide 3-kinase (PI3K) inhibitors such as wortmannin and LY294002 but not affected by STAT3 inhibitor Stattic. Using antibody for phosphatidylinositol-trisphosphate (PIP3), we observed PIP3 production after leptin stimulation in some T1R3-positive taste cells but not in GAD67-positive taste cells, which represent sour sensitive taste cells. Leptin-induced PIP3 production in T1R3-positive taste cells was not observed after treatment of PI3K inhibitor. These results suggest that PI3K is a key mediator linking Ob-Rb to K_{ATP} channel in sweet sensitive taste cells. (COI: No)

1P-120

CRF circuit involved in the regulation of food intake

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Corticotropin-releasing factor (CRF) neurons in the paraventricular hypothalamic nucleus (PVH) send axons to the median eminence as neuroendocrine cells, but they also send axons to various other brain regions. We previously showed that the treatment that selectively ablate histamine H1 receptor-expressing neurons in the PVH also ablated CRF neurons. This treatment increased food intake of the mice, indicating that CRF neurons were involved in feeding regulation.

To study the role of CRF neurons, we classified PVH CRF neurons according to their differential projections to brain regions. First, we injected AAV vector to the PVH of CRF-Cre mice to express GFP and WGA (wheat germ agglutinin); GFP was used for tracking neural pathways and WGA for the target neurons. Potential target regions were found to be the solitary nucleus (NTS), locus ceruleus (LC), parabrachial nucleus (LPBN), dorsal raphe (DRN), and lateral hypothalamus (LH). Secondly, we injected retrograde viral vector to each target region to selectively express GFP in the CRF neurons projecting to each site. The CRF neurons were distributed differently in the PVH according to their projection sites. These projecting neurons were found to be mostly non-endocrine. Thirdly, we expressed functional protein (tetanus toxin) selectively in each CRF neuron to find physiological functions. This strategy is effective in classifying CRF neurons, tracking each neural pathway, and finding their role in feeding behavior. (COI: No)

1P-121

Withdrawn

1P-122

EID1 inhibits adipogenesis through reduction of GPDH expression

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The obesity is related with the increased triglycerides in the adipocyte and is a chief factor for developing lifestyle diseases such as type 2 diabetes and cardiovascular diseases. Recently, we have shown that EP300-interacting inhibitor of differentiation 1 (EID1) reduces the accumulation of triglycerides in mouse pre-adipocyte 3T3-L1 cells. Here, we show that EID1 suppresses fat accumulation in preadipocytes 3T3-L1 cells through down-regulation of glycerol 3-phosphate dehydrogenase (GPDH), which is a key enzyme in the synthesis of triglycerides. Preadipocytes were induced into mature adipocytes with 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, and rosiglitazone. During adipocyte differentiation, EID1 was overexpressed in the cells. After 9 days of induction, almost all 3T3-L1 cells without EID1 transfection were observed the accumulation of lipid droplets. In contrast, 70% of cells transfected with EID1 were inhibited lipid accumulation in response to its expression levels. To clarify the inhibitory mechanism of EID1 on lipid accumulation, intracellular glucose concentration and GPDH activity were measured. We found that EID1 inhibits a lipid accumulation through the down regulation of GPDH, whereas, EID1 is not involved in the regulation of intracellular glucose. These findings provide a molecular explanation for the inhibitory effect of EID1 to the lipid accumulation in the adipocytes. (COI: No)

1P-123

Macrophage Raptor deficiency-induced lysosome dysfunction exacerbates non-alcoholic steatohepatitis

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Non-alcoholic steatohepatitis (NASH) is an increasingly prevalent non-alcoholic fatty liver disease, characterized by inflammatory cell infiltration and hepatocellular damage. Mammalian target of rapamycin complex 1 (mTORC1) has been extensively investigated in the context of cancer, including hepatocellular carcinoma. However, the role of mTORC1 in NASH remains largely unknown. We report that in humans bearing NASH, macrophage mTORC1 activity was lower in livers experiencing severe versus mild NASH liver. Moreover, macrophage mTORC1 disruption exacerbated the inflammatory response in two diet-induced NASH mouse models. Mechanistically, in response to apoptotic hepatocytes (AHs), macrophage polarization toward a M2 anti-inflammatory phenotype was inhibited in Raptor-deficient macrophages. Lysosomal lipolysis is vital for the M2-like response of macrophages. During the digestion of AHs, macrophage mTORC1 was activated, which is essential for lysosome acidification and subsequent lipolysis. In conclusion, persistent mTORC1 deficiency in macrophages contributes to the progression of NASH by causing lysosome dysfunction and subsequently attenuating anti-inflammatory M2-like response in macrophages during clearance of AHs. (COI: No)

1P-124

Capsaicinoid Nonivamide ameliorates hepatic injury on non-alcoholic fatty liver disease in rat model

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Non-alcoholic fatty liver disease (NAFLD) is increasing rapidly in worldwide population. An ectopic lipid deposition of NAFLD leads to hepatic injury. Capsaicinoid nonivamide (PAVA) is present in chili peppers. PAVA have been recommended as an effectively reduced body fat accumulation and also maintained metabolic rate. Thus, PAVA could be an appropriate agent for treatment of NAFLD and related disorders. Male SD rats (Permitted number of IACUCs: 50/2559) were divided into 5 groups; normal-chow-diet (NCD), NCD plus PAVA (N+PAVA), high-fat-diet (HFD), HFD plus PAVA (H+PAVA) and HFD plus rosuvastatin (H+RSV). The 65% fat of HFD fed was used to induction of NAFLD for 16 weeks and another 4 weeks for PAVA treatment (1 mg/kg) and RSV treatment (10 mg/kg). Blood and livers were collected and metabolic parameters were measured includes hepatic injury markers, morphological change and lipid contents. Furthermore, hepatic injury leads to cell death through oxidative stress was determined. The NAFLD increased degrees of injury and were decreased in HFD treated with PAVA and RSV. Moreover, disorganized morphological changes were obviously decreased in HFD treated with PAVA and RSV. In addition, PAVA and RSV could ameliorate NAFLD by reduced ROS production, indicating that the oxidative stress and apoptosis were suppressed. Treatments of PAVA could be suggested that the alternative medicine for NAFLD and as these treatments can attenuate the severe progression of liver disease. (COI: No)

1P-125

Regulation of mitochondrial respiration, energy metabolism, and obesity by neuronal Ca^{2+}-sensor-1

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Obesity is a risk factor of life threatening diseases such as stroke and myocardial infarction, thus it is important to identify novel mechanisms of its regulation. Recent evidence suggests an involvement of Ca^{2+}-signaling. Neuronal Ca^{2+}-sensor-1 (NCS-1) is an EF-hand Ca^{2+} binding protein, playing crucial roles in excitable tissues via activation of Ca^{2+} signals. During characterization of NCS-1 deficient (KO) mice, we found that these mice become obese with age. This study was aimed to clarify the mechanism of NCS-1-dependent obesity. Analyses using the metabolic cages indicated that both food intake and locomotor activity were similar between WT and KO groups, but energy metabolism, assessed by O_2 consumption and CO_2 emission, was significantly decreased in KO group. At the cellular level, mitochondrial respiration rate and thermogenesis were significantly decreased in KO cells. Indeed, the levels of proteins involved in mitochondrial thermogenesis and biosynthesis, and its Ca^{2+}-dependent upstream regulator were all decreased in the brown adipocytes in KO mice, suggesting that decrease in mitochondrial respiration followed by decrease in energy metabolism are major mechanisms of obesity in KO mice. Metabolome analysis in both brown and white adipocytes from WT and KO mice further specified the related pathways as the cause of obesity in KO mice. Taken together, these results demonstrate a novel mechanism of energy metabolism mediated by a Ca^{2+} regulatory protein NCS-1.
(COI: No)

1P-126

Pioglitazone ameliorates senescence related markers in visceral adipose tissue of obese mice

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Background and Purpose
Senescence related markers were reported to be increased in white adipose tissue of obese animals. The purpose of this study was to investigate the effects of pioglitazone ingestion on senescence related markers in visceral adipose tissue of high fat diet induced obese mice.
Animals and Methods
5-week-old male ICR mice were assigned to normal control diet (NCD), high fat diet (HFD) or HFD + Pioglitazone ingestion (PGZ) groups. The mice were fed the HFD or the normal control diet from 5 to 17 weeks of age. The PGZ group was given 12-week dietary ingestion of HFD with 0.02 % (w/w) pioglitazone. At the end of experimental period, abdominal fat tissues were collected for the analysis of adipocyte cellularity and senescence related markers.
Results and Discussions
Body weights and abdominal fat weights were significantly greater in the HFD and PGZ groups than in the NCD group, but no significant difference was found between the HFD and PGZ groups. The averaged adipocyte sizes and senescence related markers (i.e. p53, p21, p16 mRNA expressions and SA-β-Gal staining) in epididymal adipose tissue were greater in the HFD group than in the NCD and PGZ groups, and no difference was observed between the NCD and PGZ groups.
These results suggested that the pioglitazone ingestion might ameliorate senescence related markers in obese adipose tissue, and that is not related to the body weight or adipose tissue volume, but is related to the fat cell diameter. (COI: No)

1P-127

Remote ischemic preconditioning affects gluconeogenesis via the brain-liver route

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Purpose: Our previous study revealed that murine remote ischemic preconditioning (RIP) activates cardiomyocte acetylcholine (ACh) synthesis, enhanced glucose uptake and ACh-induced cardioprotection from ischemic insults, which modified CNS phenotypes. However, the mechanisms underlying the glucose metabolism by RIP remain unclear *in vivo*. Therefore, the mechanisms of RIP were studied under pathophysiological conditions. Methods: The effects of RIP on blood sugar (BS), glucose uptake, central parasympathetic nervous system (PNS) activity, hepatic gluconeogenesis and those of ACh on hepatocellular glucose uptake were assessed. Results: RIP reduced BS levels with increased c-fos signals around the solitary tract and dorsal motor nucleus, a center of PNS. RIP specifically decreased hepatic gluconeogenesis by glucose-6-phosphatase and phosphoenolpyruvate carboxykinase and enhanced hepatic glucose uptake. Hepatic branch vagotomy reversed RIP-induced BS decrease and suppressed the glucose uptake. Suppression of gluconeogenesis was reversed by intra-cerebroventricular injection of a choline acetyltransferase inhibitor, suggesting that this effect is through the central nervous system. RIP significantly suppressed hyperglycemia of murine model of type 1 and 2 diabetes mellitus (DM). Conclusion: RIP provides a therapeutic option for treatment of DM along with its resilience from cardiac ischemia and advocates an adjunctive mode rectifying disturbed glucose metabolism.
(COI: No)

1P-128

Systemic glucose oxidation is enhanced in acquired liver and muscle insulin receptor knockout mice

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The ^{13}C-glucose breath test (^{13}C-GBT) is an oral glucose test using ^{13}C-glucose, a glucose labeled with stable isotopes of carbon. The $^{13}CO_2$ quantity and the time course of its appearance in expired air correlate with systemic glucose metabolism because respiratory $^{13}CO_2$ is derived almost entirely from orally ingested ^{13}C-glucose. We first performed ^{13}C-GBT in young ZDF fatty rats, a substrain of Zucker rats characterized by homozygous mutation of the leptin receptor gene, before they became hyperglycemic. We found that fasted ZDF fatty rats produced more $^{13}CO_2$ during ^{13}C-GBT which was accompanied by decreased hepatic ^{13}C-glucose clearance, compared to control rats. To study whether decreased actions of insulin in the liver actually enhances systemic glucose oxidation, we created mice with an acquired insulin receptor knockout in the liver alone (iLIRKO mice). In fasted iLIRKO mice, blood ^{13}C-glucose concentration and respiratory $^{13}CO_2$ excretion were markedly increased. We next created mice with an acquired insulin receptor knockout in both the liver and skeletal muscles (iLMIRKO mice). Surprisingly, respiratory $^{13}CO_2$ excretion during ^{13}C-GBT was increased even more in iLMIRKO mice. Thus, in both the liver and skeletal muscles, impaired action of insulin enhances systemic glucose oxidation. Our novel findings suggest that tissue(s) other than the liver or skeletal muscles play major role in oxidizing glucose in fasted states. (COI: Properly Declared)

1P-129

CCL5 Deficiency Protect against High-fat Diet-induced Insulin Resistance

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Purpose: CCL5 (CC-chemokine ligand 5) mediated leukocyte recruitment has been demonstrated in high-fat diet induced whole body inflammation and insulin resistance. In this study, we aimed to investigate the mechanisms of CCL5 deficiency in the protection of high-fat diet induced insulin resistance.
Methods: WT and CCL5 knockout mice were randomly assigned to fed with chow diet (WTC and L5KOC) or high fat diet (WTH and L5KOH) for 20 weeks.
Results: The body weight gain of L5KOC was slighter compared with WTC after 20 weeks feeing. WTH mice exhibited obesity, hyperglycemia, and hyperinsulinemia; whereas, L5KOH mice have relative less body weight gain with lower plasma insulin level. However, fat pad weight (subcutaneous fat, epididymal fat and brown fat) and liver weight in L5KOH were comparable with WTH animals. Intraperitoneal insulin tolerance test revealed that CCL5 knockout can dramatically improve HFD-induced insulin resistance. Simultaneously, the HFD-induced pancreatic islet enlargement was mitigated by CCL5 deficiency. However, the glucose intolerance in L5KOH mice was augmented compared with WTH, and this can be explained by the decreased insulin secretion from the smaller pancreatic islet in L5KOH mice.
Conclusions: CCL5 deficiency can protect against high fat diet-induced hyperinsulinemia, insulin resistance and islet enlargement. Therefore, inhibition of CCL5 provide a novel therapeutic strategy to develop new treatment for insulin resistance and type 2 diabetes. (COI: No)

1P-130

The effects of insulin signaling on mouse taste bud organoid

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Insulin is an essential hormone for our whole body energy metabolism, but its expression and function in peripheral taste system has not been studied well. In this study, we report that insulin receptor (IR) mRNA and protein are expressed in mouse taste bud cells. In immunohistochemical study, IR was expressed in taste bud broadly, including type II and III taste cells. The newly developed *ex vivo* 3-Dimensional stem cell culture system 'taste bud organoid' revealed that the number of taste cells and mRNA expression levels of taste cell markers, such as nucleoside triphosphate diphosphohydrolase-2 (NTPDase2), Tas1R3 (T1R3), gustducin, and carbonic anhydrase 4 (CA4), were significantly decreased in an insulin concentration-dependent manner. The expression levels of several glucose transporters (GLUT8 and SGLT1) were also decreased by insulin application. In addition, taste bud expressed mammalian target of rapamycin (mTOR) protein which is known to be activated by insulin or other mitogen and act as a key molecule for the regulation of cell growth, protein synthesis, and autophagy. A mTOR inhibitor rapamycin application drastically diminished the insulin effects on taste cell generation in organoids. Altogether, insulin may be an important regulator of taste cell growth and/or proliferation via the activation of the mTOR pathway. (COI: No)

1P-131

Anti-hyperglycemic Effect *Gynura Procumbens* (Lour.) Merr. in *In vivo* and *In vitro* Studies

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Gynura procumbens (GP) has grown attention for using as alternative drugs in treating diabetes mellitus. The present study aimed to find out the antihyperglycemic effect of *GP* and to elaborate the possible mechanism of action in insulin signaling. The antihyperglycemic action of GP was investigated in normal mice which were divided into 4 groups (n=6) (i.e treated with normal diet-ND, normal diet+ 1%GP powder-ND+GP, high fat diet-HFD and high fat diet+1%GP powder-HFD+GP) and OGTT test was done at 1, 3 and 5 month.GLUT4 translocation in myc-GLUT4-ECFP stably expressing cells was detected by immunocytochemistry.The effect of GP extracts on the GLUT-4 and phosphorylation of AMPK1α and Akt1 in C2C12(muscle cell) and 3T3-L1 (adipocyte) was determined by Western Blot.The results showed significant raised serum glucose level in HFD group was reduced by giving GP power. Moreover, the GP extracts promoted GLUT4 membrane translocation predominantly in C2C12 as compared with 3T3-L1 cells. GLUT4 expression and AMPK phosphorylation in 3T3-L1 cells were significantly increased by aqueous extract. Although there was no significant increased GLUT4 expression in C2C12 cells, both extracts of GP significantly increased AMPK phosphorylation. In conclusion, GP may improve glucose intolerance and both ethanolic and aqueous extracts of GP have potent antihyperglycaemic effect and its possible mechanism of action may be through the AMPK mediated signalling pathway in muscle and adipose tissues. (COI: No)

1P-132

Whole organism chemical screening identifies modulators of pancreatic β cell function

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(Background and aims)
Pancreatic β cell loss and dysfunction play a critical role in the progression of type 1 and type 2 diabetes. Identifying new molecules and/or molecular pathways that improve β cell function should significantly contribute to the development of new therapies for diabetes. Our research aim is to identify new molecules and/or new molecular pathways which promote β cell function by zebrafish chemical screening.
(Methods)
We screened 4640 small molecules using a zebrafish line expressing firefly luciferase under the *insulin* promoter (*ins:Luc2*). Following hits selection, we measured free glucose levels to identify enhancers or repressors of insulin expression which have a functional effect on glucose levels. Furthermore, we characterized our insulin enhancers and repressors by conducting time course studies.
(Results and Conclusion)
After small molecules screening, we identified 84 stimulators of *insulin* expression, which simultaneously reduced glucose levels. The *insulin* promoter activation kinetics for 32 of these stimulators were consistent with a direct mode of action. Notably, Kv1.3 inhibitors increased β cell mass in larval zebrafish and stimulated β cell function in adult zebrafish and in the STZ induced hyperglycemic mouse model. In addition, our data indicate that cytoplasmic Kv1.3 regulates β cell function. Thus, using whole organism screening, we have identified new small molecule modulators of β cell function and glucose metabolism. (COI: No)

1P-133

Colonic smooth muscle injury ameliorates via SIRT1 activator in STZ-Induced Diabetic Micee

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AIMS: Diabetes mellitus (DM) is one of the major diseases which is seriously threatening human health. The present study investigated the effects of SIRT1 activator on colonic smooth muscle injury by hyperglycemia.
METHODS: The effects of SIRT1 activator on diabetes rats and NIH cells were assessed immunohistochemistry, and western blot analyses. Additionally, oxidative indicators (such as catalase, superoxide dismutase, reactive oxygen species, and malondialdehyde), the deacetylase activity of SIRT1 and protein expressions of SIRT1, FOXO3a, and acetylated-FOXO3a were measured.
RESULTS: After 12 weeks of RSV treatment, RSV treatment increased SIRT1 deacetylase activity, subsequently decreasing the expression of acetylated-FOXO3a and inhibiting the oxidative stress caused by hyperglycemia both in vivo and in vitro. The silencing of SIRT1 in NIH cells aggravated the high glucose-induced oxidative stress and overexpression of acetylated-FOXO3a.
CONCLUTIONS: SIRT1 activator ameliorates the colonic smooth muscle injury by hyperglycemia via modulating the FOXO3a pathway
Key word: SIRT1 activator; Colon smooth muscle; Diabetes; FOXO3a

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1P-134

Evaluation of anti-hyperglycemic efficacy of *Lactobacillus paracasei* HII01 in type 2 diabetic rat

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Hyperglycemia and insulin resistance are major features in type 2 diabetes. Recently, several studies have highlighted an anti-diabetic effect of probiotics in diabetic patients and animals. This study was conducted to evaluate the anti-hyperglycemic effect of *L. paracasei* HII01 supplementation and to further examine whether this advantage may lie in its ability on skeletal glucose uptake. The total 36 rats were randomly into 6 groups: normal diet control (NDC), normal rats supplemented with *L.paracasei* 10^{10} CFU (ND-L), diabetic rats control (DMC), diabetic rats supplemented with *L.paracasei* 10^{10} CFU (DM-L), diabetic rats treated with metformin 30 mg/kg BW (DMM) as a positive control and diabetic rats supplemented with combination of *L.paracasei* and metformin (DMM-L) for 16 weeks. Supplementation with either *L.paracasei* HII01 alone or combined with metformin significantly decreased plasma glucose levels and also ameliorated the insulin resistance compared to DMC. Likewise, the insulin-stimulated glucose uptake assessed *in vitro* study by the isolated hemi-diaphragm significantly enhanced in DM-L, DMM and DMM-L compared with DMC while the basal glucose uptake did not affect via *L. paracasei* HII01 supplement in diabetic rats. The supplement of *L. paracasei* HII01 possessed an anti-hyperglycemic effect on the type 2 diabetic rats. Its beneficial effect may partly mediated via an enhancing of insulin-stimulated muscle glucose uptake and thereby improving insulin sensitivity. (COI: No)

1P-135

White-skinned sweet potato stimulates insulin secretion from pancreatic β cells

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[Aims] White-skinned sweet potato (WSSP) has been reported to exhibit hypoglycemic effect. However, it is not yet shown how WSSP cause hypoglycemic effect. In this study, we investigated the effects of WSSP on the regulation of blood glucose levels in normal and diabetic rats and on insulin secretion from pancreatic β cells.
[Methods] To evaluate glucose tolerance, oral glucose tolerance test (OGTT) was performed using normal or streptozotosin (STZ)-induced diabetic rats. WSSP DW extracts were orally administered 15 min before glucose injection. To measure insulin secretory capacity, batch incubation experiment was performed using rat pancreatic β cell line, INS-1 cells, in the presence or absence of WSSP DW extracts. In addition, WSSP DW extracts were divided into three fractions of molecular weight (more than 100 kDa, 10~100 kDa, and less than 10 kDa) by ultrafiltration.
[Results] *In vivo*, WSSP DW extracts significantly lowered elevation of blood glucose levels in not only normal but also STZ diabetic rats. *In vitro*, WSSP DW extracts increased insulin secretion from pancreatic β cells dose-dependently. After ultrafiltration WSSP DW extracts, the fraction of less than 10 kDa only increased insulin secretion.
[Conclusions] It is revealed that WSSP increases insulin secretion from pancreatic β cells and lowers elevation of blood glucose levels in rats. In addition, the molecules stimulating insulin secretion are shown to have the weight of less than 10 kDa. (COI: No)

1P-136

Correlation between hie-sho score and progesterone, fat intake in the pre- and post-menopausal women

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INTRODUCTION Japanese menopausal women experience "hie-sho"; however, the association between the magnitude of coldness and female hormones, fat intake, and menopausal symptoms is unknown. The aim of the present study was to elucidate the relationship between the hie-sho interview scores and female hormones, fat intake, Kupperman index in pre- (pre group) and post- (post group) menopausal women. **METHODS** The hie-sho interview scores, the questionnaire of Kupperman index, dietary survey for 2 days to analyze fat intake, and body weight were analyzed, and plasma estradiol, progesterone, and lipid levels were measured in the subjects in the pre and post groups. **RESULTS** The hie-sho score was not different between the pre and post groups. No correlation was observed between plasma estradiol and hie-sho score in the pre and post groups. Plasma progesterone was positively correlated with the hie-sho score only in the post group. Plasma triglyceride was positively correlated with the hie-sho score only in the pre group. Intake of cholesterol, arachidonic acid, and docosapentaenoic acid was negatively correlated with the hie-sho score only in the pre group. The positive correlation between total Kupperman index and hie-sho score was observed only in the pre group. **CONCLUSION** Progesterone level was related the magnitude of coldness in post-menopausal women. Fat intake, triglyceride, and menopausal symptoms may be involved the magnitude of coldness in the pre-menopausal women. (COI: No)

1P-137

Action mechanisms of sex steroids during puberty on sexual differentiation of the brain in mice

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Sex steroids play an important role in brain sexual differentiation. We reported that prepubertal castration disrupts masculinization of the sexually dimorphic nuclei composed of calbindin neurons in the preoptic area and the principal nucleus of the bed nucleus of the stria terminalis (BNSTp), which are termed the CALB-SDN and CALB-BNSTp, and that prepubertal ovariectomy disrupts feminization of the ventral part of the BNSTp (BNSTpv). However, the action mechanisms of sex steroids during puberty on the formation of these nuclei are not well understood. In this study, we examined the effects of compensatory treatment with sex steroids [testosterone (T), estradiol (E) and dihydrotestosterone (DHT)] on postnatal day (PD) 20 to 70 on the CALB-SDN, CALB-BNSTp, and BNSTpv in male and female mice that were gonadectomized on PD20. After brains were sampled on PD70, the brain sections were immunostained for calbindin and NeuN, a neuron marker, to measure neuron number in the target nuclei. As the results, the number of calbindin neurons in the CALB-SDN and CALB-BNSTp in prepubertally castrated males was significantly increased by treatment with T or DHT, but not E. Treatment with E significantly increased the number of non-calbindin neurons in the BNSTpv of prepubertally ovariectomized females. These results suggest that testicular androgens during puberty act on the CLAB-SDN and CALB-BNSTp directly for masculinization, and ovarian estrogens during puberty act to feminize the BNSTpv. (COI: No)

1P-138

Role of Sphingosine-1-phosphate on the proliferative effect of Estrogen in Human Osteoblast cells

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Estrogen (E_2) and Sphingosine-1-phosphate (S1P) play important role in bone metabolism involving with both bone resorption and formation. Estrogen modulates S1P production in many cells including astroglia, breast, and endothelium. The relation of estrogen and S1P signaling in bone is poorly studied. We studied role of S1P on the proliferative effect of E_2 in human osteoblasts (hOB). We showed the expression of Sphingosine-1-phosphate receptors (S1PR) 1, 2, and 3 mRNAs in hOB but S1PR4 or S1PR5 were not found. At 24 hours, hOB proliferation was increased by 10 nM E_2, 1 μM S1P, and 1 μM of the S1PR1 agonist (SEW2871). Expression of S1PR1 was increased by E_2 or S1P, whereas S1PR2 mRNA was unaffected in proliferating cells. S1PR3 was not affected by E_2 or S1P. As S1P is synthesized from sphingosine by sphingosine kinases (SPHK), we examined the role of SPHK on the effect of E_2 in hOB. Interesting, inhibiting SPHK activity with sphingosine kinase inhibitor (Ski) greatly reduced the proliferative effect of E_2. Moreover, both E_2 and S1P increased SPHK mRNA at 24 hours in hOB and also increased S1P synthesis in a fluorescent S1P assay. Interaction of E_2 and S1P signaling was indicated by up-regulation of E_2 receptor mRNA after S1P treatment. Therefore, we demonstrate that the SPHK system is a co-mediator for osteoblast proliferation complementary to E_2, whose effect is mediated at least by S1PR1 and S1PR2. (COI: No)

1P-139

Neonatal motor coordination is impaired by moderate perinatal hypothyroidism in mice

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Thyroid hormones play important roles in regulating metabolism of various organs and development of brain. Cretinism (severe form of congenital hypothyroidism) causes growth disorder and intellectual disability. However, neuronal phenotypes caused by mild/moderate perinatal hypothyroidism have not yet been fully studied. To evaluate the effect of mild/moderate perinatal hypothyroidism on early postnatal mice development, we induced hypothyroidism by administering propylthiouracil to dams from embryonic day 14 to postnatal day (PND) 21. Then motor coordination and vestibular function of newborn pups were examined by righting reflex test (PND3 to 12), negative geotaxis test (PND8), and rotarod test (PND21). Moderate hypothyroid animals showed impaired motor coordination, whereas mild hypothyroid animals showed normal motor coordination. Then, we measured mRNA levels of glutamate and GABA receptors in the cerebellum on PND0, 5, 8, 15, and 21. The expression levels of AMPA receptor and *Gabrb2* subunits were altered initially, followed by the alteration of NMDA and GABA receptor subunits in the later period. These results indicate that moderate perinatal hypothyroidism impairs motor coordination during early postnatal developmental stage. The altered mRNA expression levels of glutamate and GABA receptor subunits in the cerebellum may be involved in such impairments. (COI: No)

1P-140

Mifepristone upregulates vimentin expression in human hepatic stellate cells

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Vimentin functions as the intermediate filament to maintain a lipid droplet in lipid-storage cells such as hepatic stellate cells. The protein expression of vimentin in liver is shown to be up-regulated in non-alcoholic steatohepatitis; however, its cellular source remains unclear. Our previous study showed that the 12-week administration of mifepristone, which is known as a steroid hormone receptor antagonist and is clinically used as an anti-cancer agent, as a mixture with regular diet, markedly promoted a non-alcoholic steatohepatitis. This study aimed to determine whether or not the vimentin upregulation is associated with mifepristone-induced steatohepatitis and which cell type of liver is responsible for the vimentin upregulation. Here, we examined the effect of mifepristone on mRNA and protein expression of vimentin in hepatic parenchymal cell (HepG2) and human hepatic stellate cell (HHSteC). The mRNA and protein levels of vimentin were evaluated with real time RT-PCR and western blot analysis, respectively. The treatment with 10 μM mifepristone had no effect on the mRNA and protein levels of vimentin in HepG2. On the other hand, mifepristone significantly increased the protein expression of vimentin (21.1 fold) in HHSteC. These results suggest that the mifepristone-induced steatohepatitis is associated with upregulation of vimentin and a hepatic stellate cell plays more important role in the upregulation of vimentin. (COI: No)

1P-141

CDK5 regulates estrogen receptor and breast cancer cell growth

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Breast cancer is the most commonly occurring cancer and the leading cause of cancer deaths in women worldwide. Cyclin-dependent kinase 5 (CDK5) plays an important roles in cancer progression. Purpose: Previous results demonstrate Cdk5 regulates growth of various kinds of cancer cells through specific regulators, such as STAT3 or androgen receptor (AR). We specifically investigated the roles and mechanisms of Cdk5 in regulating breast cancer growth and the expression of CDK5 proteins and kinase activity in ER-positive breast cancer cell lines. Results: Due to estrogen receptor α (ERα) plays key roles in the development of breast cancer, we first identified that Cdk5 might phosphorylate ERα and increase its transactivation to promote breast cancer growth. Second, Promotion of breast cancer cells (MCF-7 and BT-474) proliferation and degradation of ERα proteins were observed by overexpressing CDK5 and P35. In contrast, blocking the activity or silencing the expression of CDK5, prevented the degradation of ERα proteins. And the estrogen response element (ERE) report genes analysis reveal overexpression of p35 could induce CDK5 activity and induce downstream expression of ERE. Therefore, CDK5 was activated by p35 might accelerate breast cancer cells growth by promoting the ERα transcription and inducing ERE downstream expression. Conclusion: CDK5 might play critical roles in breast cancer cell growth and also regulate ER in cancer progress. (COI: No)

1P-142

Effect of Blood Donation on Insulin Resistance and Lipid Peroxidation Product

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Background: Several studies related to blood donation are targeted on iron deficient conditions and its adverse outcomes. On the contrary, reduction in iron stores following repeated blood donation can impact positive effects also.
Objectives: This study was conducted to determine the effect of blood donation on insulin resistance and lipid peroxidation product in regular male blood donors.
Methods: It was a comparative cross-sectional analytical study. A total of 60 male subjects, 30 non-donors and 30 regular blood donors (donation frequency between 5 to 10 times) participated in the study. Serum ferritin, fasting glucose, serum insulin, serum malondialdehyde (MDA) and homeostatic model assessment for insulin resistance (HOMA-IR) were determined.
Results: The mean serum ferritin, serum insulin, HOMA-IR and serum MDA of non-donors were 112.37±70.88 ng/mL, 31.47±18.46 μIU/mL, 4.78±3.67 and 1.76±0.35 μmol/L respectively. In blood donors, mean serum ferritin, serum insulin, HOMA-IR and serum MDA were 19.7±19.33 ng/mL, 18.3±9.18 μIU/mL, 2.26±1.49 and 1.66±0.50 μmol/L respectively. There was a significant difference in serum ferritin, MDA, insulin and HOMA-IR ($p<0.05$) between two study groups. Serum ferritin level was significantly correlated with HOMA-IR but not with serum MDA.
Conclusion: These findings suggested that reduction in iron stores seems to improve insulin sensitivity and have some remarkable effect on serum MDA level in repeated blood donors. (COI: No)

1P-143

Ghrelin modulates duration or number of wakefulness, NREM and REM sleep event

Ryosuke Okumura; Toshiki Tajima; Takuya Mukai; Taiga Yamashita; Taichi Kakizawa; Juhyon Kim; Kazuki Nakajima (*Division of Bio-Information Engineering, Faculty of Engineering, University of Toyama, Japan*)

Ghrelin, an endogenous peptide which is produced in the stomach and some regions of brain, facilitates growth hormone secretion and food intake. Recent study demonstrated that centrally administrated ghrelin also acts as wake-promoting substance. However, only total time spent in wakefulness, non-rapid eye movement (NREM) sleep and REM sleep has been compared, and the detail of changes in each state has not been clarified. Therefore, we examined effect of ghrelin not only on total time, but also on each duration and number of entry into wakefulness, NREM and REM sleep events using ICV administration in rats. Total time spent in the wakefulness was increased, and in the both NREM and REM sleep were decreased by ghrelin administration. Ghrelin induced no significant change in number of entry into the wake state, but increased in the duration. On the other hand, numbers of entry into the NREM and REM sleep state were decreased, but no change was observed in the duration. The present result indicates that ghrelin-induced wake-promotion consists of duration extension of the wake event, and decrease of number of the NREM and REM sleep event. (COI: No)

1P-144

Estrogen deficiency leads to decreased water channel aquaporin 4 expression in skeletal muscle

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Older women exhibit more loss of skeletal muscle mass and strength than older men do. Current theories suggest that the lack of estrogen after menopause might significantly contribute to the reduction in muscle mass and strength in older women. Interestingly, the loss of intracellular water in the muscle tissue of older women is greater than that in older men. However, an increase in extracellular water in skeletal muscle is typically associated with a reduction of estrogen. It is believed that estrogen regulates muscular water; however, the mechanism by which this occurs is unknown. Aquaporin 4 (AQP4) is a water channel protein that regulates water homeostasis of skeletal muscle and contributes to the physiological functions of skeletal muscle. Female adult rats were divided into three groups: a sham group, an ovariectomy group, and an ovariectomy plus estrogen treatment group. Estrogen deficiency was induced by ovariectomy. At 10 days postoperatively, quadriceps muscles were collected and screened for AQP4 expression by western blotting and immunohistochemical (IHC) staining. The data showed that AQP4 protein expression in the quadriceps was decreased by ovariectomy, and estrogen treatment after ovariectomy reversed the decrease in AQP4. Notably, IHC staining demonstrated that AQP4 protein was not detectable in large muscle fibers in the ovariectomy group. This result demonstrates that estrogen deficiency leads to decrease in AQP4 expression in skeletal muscle. (COI: No)

1P-146

The expression of the arginine vasopressin gene in the rat hypothalamus of EAE model

Kentaro Tanaka; Haruki Nishimura; Kazuaki Nishimura; Satomi Sonoda; Hiromichi Ueno; Takanori Matsuura; Reiko Saito; Mitsuhiro Yoshimura; Takashi Maruyama; Koichi Kusuhara; Yoichi Ueta (*Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Japan*)

Purpose: The hypothalamo-pituitary adrenal (HPA) axis is activated by various stressful conditions. Experimental allergic encephalomyelitis (EAE) is thought to be one of chronic stress models. Previous study demonstrated that HPA axis was activated, but the expression of the CRH gene in the hypothalamus was down-regulated in EAE model rat. In the present study, we investigated whether the gene expressions of the AVP as well as CRH in the hypothalamus are modulated in EAE model rat.
Methods: Adult male Lewis Rats were used. EAE was induced by subcutaneous injection of myelin basic protein and complete Freund's adjuvant and *mycobacterium tuberculosis*. They were decapitated at day 6, 12 and 18, and the brains were removed. The expressions of the AVP and CRH gene in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) were quantified by using *in situ* hybridization histochemistry.
Results: The mRNA levels of AVP in the parvocellular (p) PVN but not in the magnocellular (m) PVN at day 12 in EAE rats were increased significantly. The mRNA levels of CRH in the pPVN at day 12 and 18 in EAE rats were decreased significantly in comparison with controls.
Conclusions: We confirmed down-regulation of the CRH gene expression in the pPVN at the peak of paralysis in EAE rats. Meanwhile, the AVP gene expression in the pPVN and the SON was up-regulated in EAE rats. These results suggest that EAE-induced activation of HPA axis may contribute to AVP expression in the rat hypothalamus. (COI: No)

1P-147

Effect of persistent nicotine exposure on cell differentiation in rat pituitary gland

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Nicotine, a toxic component of smoking, affects negatively growth and reproductive through a decreased secretion of anterior pituitary hormones. However, it has not been clarified whether nicotine inhibits the differentiation from stem/progenitor cells into hormone-producing cells in the pituitary gland. The present study investigated the effect of nicotine on cell differentiation in rat pituitary gland. Three-week-old male Wistar rats were injected intraperitoneally with nicotine (1 mg/kg body weight/day) for 7 days. Gene expression, DNA methylation, number of stem/progenitor and hormone-producing cells in the pituitary gland were analyzed using qPCR, bisulfite sequence and immunohistochemistry on the next day (4-week-old) and 4 weeks after treatments (8-week-old). Persistent nicotine exposure inhibited expression of *Prrx1*, a progenitor cell marker, by first intron DNA hypermethylation. On the other hand, the expression level of a stem cell marker *Sox2* was not changed by nicotine. Immunohistochemical analysis showed a nicotine-mediated decrease in the proportion of PRRX1/SOX2-double positive cells. In addition, the expression level of growth hormone (*Gh*) and the proportion of GH-positive cells were decreased by nicotine, and the difference was remarkable at 8 weeks of age. These results suggest that the persistent nicotine exposure causes the delay of cell differentiation through a down-regulated expression of *Prrx1*, followed by a decreased number of GH-producing cells. (COI: No)

1P-148

Identification and functional analysis of inhibin βE (*INHBE*) as a hepatokine

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[Background] Hepatokines secreted from the liver regulate systemic metabolism. Previously, we have identified selenoprotein P and LECT2 as hepatokines. To identify novel hepatokine associated with insulin resistance, we performed a comprehensive analysis of gene expression profiles using a DNA chip method in liver biopsy samples from humans with varying degrees of insulin resistance. In addition, gene expressions in independent liver samples were analyzed using quantitative real time-PCR method.
[Results] Inhibin βE (*INHBE*) was emerged as a novel hepatokine from the analysis of two independent cohort. The hepatic gene expression was positively correlated with HOMA-IR and body mass index in humans. *Inhbe* gene expression in liver and protein levels in serum of db/db mice were higher than those of C57BL/6J. To screen the function of *Inhbe* in whole-body energy metabolic status, hepatic mRNA was knocked down with siRNA for *Inhbe* (siINHBE) in db/db mice. Treatment with siINHBE results in decrease of body fat percentage and respiratory quotient as well as increase of plasma total ketone bodies compared with treatment with non-targeting siRNA. These results suggest that inhibin βE accelerates fat oxidation.
[Conclusion] Obesity cause overproduction of inhibin βE, which would suppresses fat oxidation. This could be one of factors for hard to lose weight in obese people. (COI: Properly Declared)

1P-149

Serum leptin adiponectin and their effects on obesity among adolescents in Colombo district Sri Lanka

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Background
Leptin and adiponectin are adipokines with opposing effects in the body including insulin sensitivity. This study aimed to assess serum leptin and adiponectin levels in obese(OB)and normalweight(NW)children and to describe its relationship with body fat percentage(BF%) and insulin resistance
Methodology
Obese and overweight children(n=121)and healthy normal weight children(n= 263)aged 10 to 16 years were recruited after informed written consent. Definitions of overweight and obesity were based on WHOZ scores ofBMIfor age. Serum leptin and adiponectin were measured by enzyme linked immunosorbent assay
BF%was obtained from Bioelectrical Impedance Analysis(BIA) Homeostasis model(HOMAIR)was applied to estimate IR.
Results
The mean(SD)age of the sample was 13.1(1.9) years and mean BMIZ score in OB and NW children were 1.5(0.8) and -0.2(0.5) respectively. Mean BF%in OB and NW children were 26.4(4.6) and 20.1(4.5). In OB, serum leptin(25.0 ± 12.3ng/mL), and HOMA–IR(3.4 ± 0.7) were significantly higher whereas adiponectin (9.0 ±6.1) was significantly lower . Serum leptin positively correlated with BF%(r=0.725),BMIZ(r=0.667) and HOMA IR (r= 0.582) while serum adiponectin correlated negatively(r= -0.578, -0.720 and -0.752) respectively
Conclusion
Serum leptin was higher and adiponectin was lower in OB children. The association of Leptin and adiponectin with BMI, BF% and IF was opposite
There is no actual or potential conflict of interest in relation to this presentation (COI: Properly Declared)

1P-151

Targeting FGF/FGFR axis ameliorates endometriosis progression

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Fibroblast growth factor (FGF)/FGF receptor (FGFR) system regulate a broad spectrum of signaling in embryonic development, implantation, and malignant transformation. However, surprise little information of aim-at-targeting FGF/FGFR system exploration to alleviate endometriosis, thus, we aimed to delineate their roles and contribution to endometriosis progression. Our results showed that, via bioinformatics screening of public available clinical database of endometriosis, we identified an unique feature of amplified FGF1/2/9/18 concordantly with their high affinity receptors FGFR1/2/3 in endometriosis. Employing selective small molecule of FGFR TKIs BGJ398 and AZD4547, effectively against FGFR1/2/3, reduced FGF9-augmentated endometriotic stromal cells proliferation by MTT assay. FGF9 enhanced endothelial cell migration and proliferation. FGF9-induced tube formation of in vitro cultured HUVEC. We then also determined that FGF9 significantly induced invasion of newly blood vessel by directed in vivo angiogenesis assay. Against FGFR2/3 signaling by FGFR TKIs significantly block FGF9-augmentated in vitro and in vivo vascular remodeling. Furthermore, we also established in vivo surgery-induced endometriosis of C57BL/6NCrj mice model. Mice received BGJ398 and AZD4547 magnificently abolishing FGF9-enhanced endometriotic lesion multiplicities of recipient mice. Our data provided novel evidence to support that therapeutic potential of against amplified FGF/FGFR axis in endometriosis. (COI: No)

1P-152

Subepithelial synchronous interstitial cells drive spontaneous contractions in the seminal vesicle

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In most visceral hollow organs, known pacemaker cells that drive spontaneous contractions are distributed in the muscular layer. However, we recently demonstrated that the mucosa of guinea pig seminal vesicles (SVs), a pair of male accessory glands, is required for generating periodical electrical and Ca^{2+} activity in the SV smooth muscle to develop spontaneous contractions. Here we further explored spontaneously active mucosal cells that drive the autorhythmicity in SV smooth muscle using intracellular recording techniques and fluorescent Ca^{2+} imaging. Their morphological properties were examined by focused ion beam/ scanning electron microscopy (FIB/SEM) tomography and fluorescent immunohistochemistry. In the basal surface of the SV mucosal preparations dissected free from the smooth muscle layer, two populations of cells developed spontaneous activity: 1) epithelial basal cells that irregularly generated spontaneous transient depolarizations and asynchronous Ca^{2+} transients and 2) subepithelial synchronous interstitial (SSI) cells that periodically generated electrical slow waves and synchronous Ca^{2+} transients. In SV smooth muscle preparations whose epithelium were partially removed, the synchronous Ca^{2+} transients occurred in the SSI cells preceded the synchronous Ca^{2+} flashes and associated contractions in the attached smooth muscle cells. Thus, it is likely that SSI cells directly drive the spontaneous activity in SV smooth muscle by sending electrical signal. (COI: No)

1P-153

Chronological change in concepts and symptoms of premenstrual syndrome of female university students

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Introduction. Premenstrual syndrome (PMS) is defined as physical and emotional symptoms that occur before women's menstruation period and often vary between women and resolve around the start of menstruation. Its etiology is still under investigation, and therapy and measures have not been established. We performed an identical questionnaire survey on PMS for Japanese female university students in both 2008 and 2016, and compared the results. Methods. We conducted an anonymous questionnaire survey involving 131 respondents. We asked about their profile, daily life, and PMS symptoms. We also asked about PMS recognition and knowledge on PMS. Furthermore we asked about 16 physical, mental, and behavioral symptoms. Results. After having added a definition of PMS at the beginning of the questionnaire, the recognition of PMS was 58% of the respondents (recognition rate). Forty-eight percent of the respondents answered that they suffered from PMS (disease rate). Comparison of the results in 2008 and 2016 revealed that the recognition rate significantly increased in 2016 compared with that in 2008 ($p<0.05$). The rate of PMS symptoms also increased in 2016 compared with 2008 without significant changes in rates of each PMS symptom. Respondents that obtained information on PMS from smart phones and social networking service (SNS) increased in 2016. Conclusions. Recognition and rates of PMS increased more in 2016 compared with 2008. The spread of SNS may increase PMS.
(COI: No)

1P-154

Expression and function of GLUT1-4 in mouse endometrium during the preimplantation period

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In this study, we have investigated the expression and localization of GLUT1-4 in mouse endometrium, and explored the role of GLUT1-4 in maintaining a proper glucose content in intrauterine milieu, which is beneficial for early embryonic development and subsequent implantation. The results showed that GLUT1-4 was spatiotemporally expressed in mouse endometrium during preimplantation period detected by PCR, immunoblotting, immunohistochemistry and confocal microscopy. Functional blockage or knockdown of some of GLUTs (such as GLUT4) significantly decreased glucose content in uterine cavity fluid and down-regulate the expression of leukocyte inhibitory factor(Lif), and then impaired embryo development and implantation. Moreover, functional blockage or knockdown of GLUT4 expression can decrease the uptake of 2-NBDG and cell proliferation in cultured mouse endometrial epithelial cells(EECs) detected by Flow cytometry and Edu incorporation. Results of this study indicate spatiotemporal expression of GLUT1-4 in mouse endometrium involved in maintaining appropriate glucose concentration in uterine cavity fluid, which is essential for embryo development and implantation. (COI: No)

1P-155

The dynamic expression of PTEN in the development of mouse spiral limbus

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The Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a critical negative regulator of extracellular signal-induced PI3K activities, which can regulate various cell processes including proliferation, growth, neural and glioma stem/progenitor cell renewal. PTEN also plays a critical role in the differentiation of the cellular pathways in inner ear development, including cochlear neurons and hair cells, as well as various cells in the cochlear lateral wall, which is crucial for regulating K^+ homeostasis. In this study we use immunofluorescence, western blot and RT-PCR to reveal the expression of PTEN in the stellate fibrocytes (SFs) and the interdental cells (IDCs) of the spiral limbus (SL) in the early postnatal cochlea. PTEN is expressed in differentiating SFs and IDCs at postnatal days P4 and P7. At P14, PTEN levels decrease in the SFs but remain high in IDCs. In the mature cochlea, the expression of PTEN is low or undetectable in SFs but it remains high in IDCs. This expression pattern for PTEN in the developing cochlea suggests that PTEN plays a dynamic role in the development of the SL. (COI: No)

1P-156

The effect of post-natal PFOS exposure on cerebellar development and motor coordination

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Background: Perfluorooctane sulfonate (PFOS) is an organic pollutant, and was widely used in industries and consumer products. PFOS has been detected in umbilical cord blood, breast milk and serum. Although previous studies reported the significant neurotoxicity of PFOS, the underlying mechanism has not yet been clear. The aim of this study was to examine the effect of early postnatal PFOS exposure on motor coordination and memory, and further reveal the molecular mechanisms involved.
Methodology: PFOS (1mg/kg) was orally administered to dams from post partum day (P) 1 to P14 so that pups would be exposed through breast milk. After postnatal 7-10 weeks, we performed Rotarod test using male offspring for 5 consecutive days. We also measured several mRNA levels in the cerebellar cortex at P2, P7, P14, P21 and P28 by quantitative real time PCR.
Results: PFOS treated male mice showed significant decrease in time-to-fall latency comparing with control group. However, their motor learning skill was conserved in consecutive trials. PCR analysis showed a significant decrease in mRNA levels of several genes responsible for cerebellar function and neurodevelopment only on P21.
Conclusion: Our study showed that postnatal PFOS exposure has profound permanent effect on cerebellar development, and consequently leads to motor coordination deficiency without affecting motor learning. Furthermore, we found that PFOS peak effect in cerebellum may be on P21. (COI: No)

1P-157

The effects of thyroid hormone on development of hippocampal neurons in vitro

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Thyroid hormone (TH) plays essential roles in the growth and development of many organs including the brain. TH deficiency in early life stage causes morphological and functional disorder in the hippocampus. However, the mechanisms of TH action in the developing hippocampus are not fully understood. In this study, to investigate the effects of TH in developing hippocampal neurons using primary culture, we compounded 3,5,3'-tri-iodo-l-thyronine (T3)-free neural supplement based on the original (Chen et al., 2008), and compared the development of hippocampal neurons with or without T3. Morphological analysis and quantitative real-time PCR were performed on 7, 10, and 14 days *in vitro* (DIV). On 7DIV, no evident morphological differences between +T3 and -T3 group were observed, whereas a significant decrease in dendrites arborization was observed in -T3 group on 10 DIV. Such difference was disappeared on 14 DIV. Among mRNAs we measured, *Bdnf* mRNA levels decreased significantly in -T3 group on 10 DIV. Therefore, to examine the involvement of BDNF on dendrites arborization, we added 10 ng/mL BDNF to -T3 group on 8 and 9 DIV. The exogenous BDNF normalized the status of dendrites on 10 DIV same as +T3 group. These results indicate that TH deficiency induced abnormal maturation of primary hippocampal neurons on 10 DIV, and this retardation might be caused by decreased *Bdnf* mRNA expression. (COI: No)

1P-158

Perceptions towards health and care giving among elderly with loneliness, living in aged-care homes

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Purpose
This study was conducted among three different elderly homes in Colombo district, Sri Lanka, aiming to identify health status of the elderly and their perceptions towards health and care giving.
Methods
The research design was a combination of quantitative and qualitative approaches. Quantitative part was conducted among 75 randomly selected elderly, from three elderly homes. Structured interviews were conducted to collect socio-demographic data and loneliness was measured using Revised University of California at Los Angeles (R-UCLA) scale. 15 in-depth interviews were conducted to collect qualitative data. Statistical Package for Social Sciences version 20 and content analysis were used in quantitative and qualitative analysis.
Results
The results indicate that the elderly felt lonely to a certain degree, as medians on the R-UCLA loneliness scale showed 45 (range 29 min-77 max). 61% of them perceived their health as good or very good, 25% as general and only 13% perceived as poor health. Loneliness is not significantly associate with the health status (p= 0.526). Elderly with higher degree of loneliness, perceived their health as poor and uncertain about the later life disabilities. Also, they were satisfied with the care given by the staff at the aged care homes.
Conclusions
Involvement of the caregivers, nurses, other health care professionals in concerning health and providing satisfied care is important to improve the well-being of the elderly living in aged care homes. (COI: No)

1P-159

Krüppel-like factor 5 regulates proliferation of neural precursor cells in the developing brain

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Krüppel-like factor (*Klf*) family proteins are DNA-binding transcriptional factors, which share highly conserved sequences and redundant functions including cell cycle regulation, cell differentiation and tissue organization. Among Klf family, *Klf5* is indispensable for early embryonic development. *Klf5* also maintains the pluripotency of ES cells and enhances reprogramming somatic cells to generate mouse iPS cells in place of *Klf4* with other three factors. Although these previous studies suggest the broad roles of *Klf5* in organogenesis, its roles in the central nervous system has poorly understood despite of its expression in the developing brain. By using *in utero* electroporation and *Klf5* conditional knockout mouse, we clarified *Klf*s-dependent regulation of neural stem/progenitor proliferation and differentiation. Overexpression of *Klf5* by *in utero* electroporation promoted the proliferation of the apical progenitors, whereas shRNA-mediated knockdown of *Klf5* showed little effects, possibly due to the redundant functions by other *Klf*s family proteins. Indeed, the triple knockdown of *Klf2*, *Klf4* and *Klf5* resulted in the significant reduction of the apical progenitor proliferation and impaired migration of NPCs. Furthermore, self-renewal of NPCs was also suppressed in *Klf5* conditional knockout mouse brain.
Our data suggest that *Klf5*, together with *Klf2* and *Klf4*, plays important roles in the neural development. (COI: No)

1P-160

Rescue of craniofacial defects with therapeutic hedgehog target chemical in ECO syndrome mouse model

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Endocrine-cerebro-osteodysplasia (ECO) syndrome is a recessive genetic disorder in human associated with congenital defects in endocrine, cerebral, and skeletal systems that are caused by a missense mutation of the intestinal cell kinase (ICK) gene. ICK is important for ciliogenesis. Previous findings have shown that craniofacial defects with cleft palate/lip and tooth malformation in Ick mutant mice closely resemble ECO syndrome, similar to ciliary disorders. Cleft palate/lip is the most common congenital defect. Ick mutant results in cleft palate and reduced sonic hedgehog signaling, but not palatal adhesion and fusion. Ick deficiency affects palatal cell proliferation. However, the regulatory effects of cilia on craniofacial development and therapeutic attempt have not yet been reported. Therefore, we intraperitoneally treated with smoothened agonist (SAG) into pregnant Ick mutant mice to examine its therapeutic effect. Exogenous stimulation of Hh signaling restored palate development. These data implicate that Hh agonist is a therapeutic drug for the development of novel therapies for cleft palate/lip and possibly other symptoms of the ciliopathies. (COI: No)

1P-161

Nicotine layer-specifically modulates synaptic plasticity in the mouse insular cortex

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Nicotinic acetylcholine receptors (nAChRs) in the insular cortex play an important role in nicotine addiction, but its synaptic mechanisms still remain unresolved. In layer 5 pyramidal neurons of the mouse insular cortex, activation of nAChRs suppresses synaptic potentiation through enhancing GABAergic synaptic transmission. However, it has not been addressed whether and how activation of nAChRs modulates synaptic plasticity in layers 3 and 6 pyramidal neurons of the insular cortex. In this study, we demonstrate that activation of nAChRs oppositely modulates synaptic potentiation in layers 3 and 6 pyramidal neurons of the insular cortex. In layer 3 pyramidal neurons, activation of nAChRs depressed synaptic potentiation induced by combination of presynaptic stimulation with postsynaptic depolarization through enhancing GABAergic synaptic transmission via activation of β2-containing nAChRs in non-FS interneurons. By contrast, in layer 6 pyramidal neurons, activation of nAChRs enhanced synaptic potentiation through activating postsynaptic β2-containing nAChRs. These results indicate, in different layers of the mouse insular cortex, paired training-induced synaptic potentiation is oppositely regulated by activation of nAChRs which are located on GABAergic interneurons (layer 3) and on pyramidal neurons (layer 6). Thus, layer-specific modulation of synaptic potentiation may be involved in synaptic mechanisms of insular cortical changes in nicotine addiction. (COI: No)

1P-162

Large volume electron microscopy and neural microcircuitanalysis

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One recent technical innovation in neuroscience is microcircuit analysis using three-dimensional reconstructions of neural elements with a large volume electron microscopy (EM) data set. Large-scale data sets are acquired with newly-developed EMs such as automated tape-collecting ultramicrotomy (ATUM) with scanning electron microscopy (SEM), and the others. Currently, projects are also underway to develop computer applications for the registration and segmentation of the serially-captured electron micrographs that are suitable for analyzing large volume EM data sets thoroughly and efficiently. Here, we focus on the ATUM method which allows the collection of many serial ultrathin sections of consistent quality quickly and automatically by producing ultrathin sections on a tape that can be repeatedly imaged using SEM. We found that plasma-hydrophilized-carbon nanotube tape is optimal for the ATUM-SEM method due to its extremely high surface conductivity and low endogenous signal, and it can provide high quality images of tissue sections with SEM. We labeled the thalamo-corticalaxon terminals with a viral vector injection into individual motor related thalamic nuclei: the ventral medial nucleus, the ventral anterior nucleus, or the ventral lateral nucleusand analyzed their target in primary motor cortexusing the ATUM-SEM method. We found that they innervated the cortical microcircuit with a different manner.
(COI: No)

1P-163

Stimulated single fiber electromyography in orbicularis oculi muscle in profenofos poisoned patients

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Self poisoning with organophosphates (OP) like profenofos (PF) is common in Sri Lanka with high respiratory failure (RF) rates due to intermediate syndrome (IMS). OP inhibits acetylcholine esterase at the neuromuscular junction (NMJ). After cholinergic features subside, the characteristic weakness in bulbar and respiratory muscles due to NMJ receptor blockade is described as IMS. Our study assessed this impairment in PF ingestion using concentric needle single fiber electromyography (SfEMG) to predict IMS, and to describe SfEMG changes over time.
Patients with > 2 cholinergic symptoms after PF ingestion were subjected to SfEMG < 24 hrs and alternate days. Patients with 3/4 clinical criteria: neck weakness, proximal muscle weakness, opthalmoplegia and RF within 24-96 hrs were diagnosed as IMS. A branch of the facial nerve was stimulated to record SfEMG jitter from orbicularis oculi muscle (OOM).
Total number of patients=54(males: 46, mean age= 41± 16.4 yrs). IMS patients=53.7%. Patients tested with SfEMG < 24 hrs= 31(IMS=15). Odds of a patient with increased jitter <24 hrs developing IMS was not significant (OR=2.7, p = 0.27). But IMS patients had significantly increased median jitters compared to those without IMS at time windows <24 hrs (p=0.011), 24-48 hrs (p=0.009), 48-72 hrs (0.012), and 72-96 hrs (p=0.039).
Normal jitter of OOM =33.4µs. A higher median jitter with SfEMG was recorded up to 96 hrs in the IMS group following PF ingestion. (COI: No)

1P-164

Conduction filtering of synaptic currents via dendrites by SK channels in cerebellar Purkinje cells

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Whether synaptic inputs onto dendrites transmit to the soma depending on excitability of branches is elusive. Here, we performed simultaneous voltage-clamp recordings from the soma and dendrite of single Purkinje neurons, and we analyzed the spontaneous excitatory postsynaptic currents (EPSCs) on both sites. First, while EPSCs recorded on the dendrite close to the soma were correlated to EPSCs on the soma, EPSCs recorded on a distal dendrite had a significant discrepancy in the amplitude. And, EPSC recordings from different somata were not correlated, either. Next, we analyzed the ratios between the EPSC amplitudes concurrently recorded on the soma and dendrite, and we found that the ratios (*i.e.*, $EPSC_{dend}$ / $EPSC_{soma}$) were not unique, suggesting the various attenuation in different dendritic paths to the soma. Given the location of synaptic inputs were restricted in dendritic branches, the ratios would be discrete. Our data and simulation suggest that the synaptic inputs on Purkinje cells were localized in three or four areas, at least. Further, we recorded co-events on soma and dendrite after the intrinsic plasticity or under small conductance Ca^{2+}-activated K^+ channel (SK channel) blocker, and the ratios were uniformly-distributed. Our results suggest the difference of the excitability among the dendritic branches were inheritited from the locally-expressed plasticity or SK channel downregulation, which may give computational power branch-specifically to the neurons.
(COI: No)

1P-165

Bidirectional dopamine-dependent synaptic plasticity at IPSC of SNr GABA neurons in young rat slice

Takefumi Miyazaki (*Department of Physiology, Tokyo Medical University, Japan*)

Deep brain stimulation (DBS) is a general therapeutics for patients of progressed Parkinson's disease, while several side effects, such as depression, are reported. I analyzed the effects on the striat-nigral synaptic transmission of the high frequency stimulation onto a subthalamic nucleus (STN-HFS) in slices from young rats instead of DBS. In the slices from normal rats, STN-HFS for 20 min induced a IPSC-LTP at substantia nigra pars reticulata GABA neurons evoked by an electrical stimulation onto the internal capsule in the half of neurons tested (9 out of 17), under whole-cell voltage clamp condition. In the residual 8 neurons, an IPSC-LTD was induced by STN-HFS. These LTP and LTD were observed even at 120 min after STN-HFS. LTD (n=8) or LTP (n=9) was induced by STN-HFS in the solutions with D_1- or D_2-antagonist, respectively. In the slices from dopamine deficient rats, an acute model of Parkinson's disease, STN-HFS did not induce such synaptic plasticity (n=10). Even in those slices, STN-HFS with D_1- or D_2-agonist induced LTP (n=7) or LTD (n=7), respectively. The LTP or LTD was accompanied with the increase (n=5) or the decrease (n=5) in frequency of spontaneous IPSC suggesting the presynaptic mechanism of synaptic plasticity. These results indicate that STN-HFS leads to the LTP if D_1-receptor is activated predominantly in the slice, while it also leads to the LTD if D_2-receptor is activated predominantly. Thus, the synaptic plasticity may be a reason for side effects. (COI: No)

1P-166

Miniature inhibitory postsynaptic current in cerebellar Purkinje cells of old dystrophic *mdx* mice

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Duchenne muscular dystrophy (DMD) is caused by the mutations in the X-linked dystrophin gene resulting in a deficiency in the dystrophin protein. About 1/3 of boys with DMD display some degree of cognitive impairments. In the cerebellum, dystrophin is normally localised at the postsynaptic membrane of GABAergic synapses of Purkinje cells. Previously, we showed a significant reduction in both the frequency and amplitude of miniature inhibitory postsynaptic current (mIPSCs) in Purkinje cells of young (3-4 months old) *mdx* compared with littermate control (LC). Here, we investigated the mIPSCs of young and old *mdx* mice (23-26 months old). These aging mice were chosen because earlier reports showed brain degenerative progression in old *mdx* mice resembles that found in DMD patients. All experiments were conducted in accordance with the international guidelines on the care and use of experimental animals and approved by the Animal Care and Ethics Committee of Western Sydney University. Using whole-cell patch clamp recording, we showed reduced frequency and amplitude of mIPSCs in *mdx* mice when compared to LCs. These results imply that lack of dystrophin disrupts the synaptic transmissions at GABAergic synapses and it may be similar to human disease progression and contribute to the cognitive dysfunction in boys with DMD. Cognitive impairment in DMD boys is non-progressive, and it is of interest that the frequency and amplitude of mIPSCs were similar between young and old *mdx* mice. (COI: No)

1P-167

Src kinase regulates the presynaptic transmitter release in avian cochler nucleus

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Src kinase is expressed in many neurons and involved in the regulation of synaptic transmission. In hippocampal neurons, long-term potentiation is induced by increasing the expression of NMDA receptors at postsynapse via Src kinase activity. However, it is not well understood whether and how Src kinase regulates presynaptic transmission. In this study, we examined the contribution of Src kinase to the synaptic transmission at avian cochlear nucleus, where an auditory nerve fiber (ANF) forms a large end-bulb synapse. We made brainstem slice from posthatch chicks and recorded EPSC in cochlear nuclear neurons, while stimulating the ANF. We found that application of Src kinase inhibitor (PP2) increased EPSC amplitude. On the other hand, mEPSC amplitude did not change with PP2, suggesting that the increase of EPSC amplitude would be mediated by presynaptic mechanisms. The cumulative analysis of EPSC amplitude revealed that the readily releasable pool size might be the target of Src kinase regulation. During the continuous train stimulation, gradual decrease of EPSC amplitude was observed, but not in PP2 pre-treated slice, suggesting that Src kinase was regulated through the ANF activity. Thus, we concluded that Src kinase might suppress the neurotransmitter release through activity dependent mechanisms in avian cochlear nucleus. We will further examine the localization of Src kinase and discuss the physiological roles of Src kinase activity in cochlear nucleus. (COI: No)

1P-168

The mGluR1 contributes strengthening and maintenance of developing lemniscal synapses

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The metabotropic glutamate receptor subtype 1 (mGluR1) is a major component of the group I mGluRs, which contributes synaptic refinement and plasticity. In the sensory thalamus, a thalamocortical (TC) neuron receives excitatory inputs from sensory afferents and corticothalamic (CT) fibers, and mGluR1 is more concentrated in CT synapses. We have reported that mGluR1 is required for the maintenance of the afferent synapse after maturation in the visual thalamus (Narushima et al., 2016). We hypothesized that mGluR1 played a general role for strengthening and/or stabilization of excitatory synapses irrespective of the developmental phases. Therefore, we investigated the effects of mGluR1 knockout (KO) on development of lemniscal synapses, the somatosensory afferent synapse, in the somatosensory thalamus (VPm) where mGluR1 was expressed from birth. We found that mGluR1-KO mice exhibited delayed developmental strengthening, incomplete elimination and remodeling after maturation in lemniscal synapses. While blockade of the somatosensory cortical activity from P12 or P21 for one week in wild-type mice perturbed elimination and maintenance of lemniscal synapses respectively, the same manipulation to mGluR1-KO mice failed to induce additional effects on lemniscal synaptic connectivity. These results suggest that mGluR1 is necessary for strengthening and refinement of immature synapses as well as maintenance of matured synapses in the somatosensory thalamus. (COI: No)

1P-169

Inhibition expands dynamic range of inputs in low-tuning frequency neurons in avian cochlear nucleus

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Avian cochlear nucleus, nucleus magnocellularis (NM), receives excitatory inputs from auditory nerve fiber (ANF) and conveys temporal information of sound bilaterally to the coincident detector neurons of nucleus laminaris (NL) for sound localization. Fine adjustment of EPSP size to an appropriate level is crucial for accurate coincident detection. NM receives feedback inhibition from superior olivary nucleus which is also controlled by ANFs and large amount of inhibitory terminals are observed particularly around neurons with low-tuning frequency (low-CF neurons). However, how the inhibition regulates the output of low-CF neurons is still elusive. To address this, we made thick slice preparation (2.5 mm), where the whole circuit to NM was preserved, and synaptic responses were recorded from low-CF neurons with ANF stimulation. Whole-cell recording revealed that IPSC appeared at a similar level with EPSC threshold and increased gradually with the increase of EPSC for a wide range of intensity. We evaluated contribution of inhibition to the firing of NM neurons in cell-attached recording with $GABA_A$ receptor blocker and found that the blocker increased the firing probabilities from the firing threshold and narrowed the dynamic range of inputs. Thus, we concluded that the inhibition finely adjusts the output of low-CF NM neurons, which may ensure the binaural computation of NL neurons for a wide range of sound intensity. (COI: No)

1P-170

5-HT-induced inhibition of excitatory transmission onto basal forebrain cholinergic neurons

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Cholinergic neurons in the basal forebrain (BF) project to various brain regions including cortex and hippocampus. BF received various inputs such as serotonergic fibers in the dorsal raphe nuclei. However, serotonin (5-HT)-induced modulatory effects on the excitatory synaptic transmission in the BF are unknown. This study was aimed to elucidate 5-HT-induced modulation of glutamatergic synaptic transmission onto BF cholinergic neurons. BF cholinergic neurons were identified with Cy3-192IgG and investigated in P12-20 rat brain slices. Excitatory postsynaptic currents (EPSCs) were evoked by focal electrical stimulation. 5-HT, a 5-HT_{1A} receptor agonist or a 5-HT_{1B} receptor agonist inhibited the amplitude of EPSCs. A 5-HT_{1A} receptor antagonist or a 5-HT_{1B} receptor antagonist partially antagonized 5-HT-induced inhibition of EPSCs. In the presence of both antagonists, most of 5-HT-induced effect disappeared. 5-HT-induced inhibition was significantly smaller in the presence of ω-agatoxin TK than that without ω-agatoxin TK, whereas 5-HT could still inhibit the EPSCs in the presence of ω-conotoxin GVIA. 5-HT reduced synaptic strength by changing AMPA/ NMDA ratio. These results suggest that 5-HT inhibits glutamatergic transmission onto BF cholinergic neurons and that both 5-HT_{1A} and 5-HT_{1B} receptors contribute to 5-HT-induced inhibition. They also suggest that 5-HT inhibits glutamatergic transmission by selectively blocking CaV2.1 (P/Q-type). (COI: No)

1P-171

Electrophysiological comparison between zebrin-positive and -negative Punkinje cells

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Subsets cerebellar Purkinje cells (PCs) that have a particular molecular expression profile are arranged into separate longitudinal stripes, which have different topographic afferent and efferent axonal connections to be involved in different functions. Expression levels of many molecules such as glycolysis enzyme aldolase C and glutamate transporter EAAT4 are linked together among PC subsets, suggesting different physiological properties among them. In this study, we recorded from PCs of different "zebrin types" (zebrin-positive = aldolase C-positive = Z+; and Z-) in identified neighboring stripes in vermal lobule VIII in cerebellar slice preparations from Aldoc-Venus mice. No significant differences were observed in input resistance or in occurrence probability of types of firing patterns between Z+ and Z- PCs. Also, no significant differences were observed either between Z+ and Z- PCs in interval dependency of paired-pulse facilitation or in time course of synaptic current in parallel fiber (PF)-PC synapse. These results indicate that molecular expression differences associated with the zebrin type do not directly affect basic electrophysiological properties of PCs or PF-PC synapse. The results suggest that differences in climbing fiber (CF) activity and in CF-PC synaptic property, as well as some region (lobule)-dependent differences in PC properties, may be involved in heterogeneous electrophysiological properties in PC subsets reported in *in vivo* preparation. (COI: No)

1P-172

Actin-associated tropomyosins in the dendritic spine play a role in synaptic function

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Tropomyosins regulate the interactions of other proteins with actin. As reorganization of the actin cytoskeleton is essential for developing and remodeling neurons, it follows that tropomyosins must play a role in neuronal function. Two isoforms, Tpm3.1 and Tpm4.2, are enriched in dendritic spines. We investigated if modulation of tropomyosin expression alters synaptic function or morphology.

We compared synaptic function and structure in neurons from transgenic mice overexpressing Tpm3.1 with WT controls. No differences were seen in amplitude or frequency of miniature excitatory postsynaptic currents (mEPSCs), or dendritic spine morphology, suggesting that increasing Tpm3.1 has no impact on basal function. However, the degree of long-term potentiation (LTP) evoked by high frequency stimulation (100 Hz 1s) was bigger in brain slices prepared from Tpm3.1 overexpressing mice (n = 16) than in WT (n = 15, p = 0.04; RM-ANOVA), suggesting Tpm3.1 overexpression augments LTP.

Overexpression of Tpm4.2 had no impact on basal synaptic function (mEPSC amplitude and frequency) or spine morphology. Deletion of Tpm4.2 decreased mEPSC amplitude (p = 0.001) and frequency (p = 0.002; unpaired t-test, Tpm4.2KO n = 16, WT n = 17). However, there were no changes in spine morphology.

Our data supports our hypothesis that Tpm3.1 and Tpm4.2 affects synaptic function. The finding that overexpression enhances plasticity also suggests they may be a future target for treatments of neurodegeneration.

(COI: No)

1P-173

New method to prevent the visually-evoked somatic depolarization for spine imaging

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Understanding how neurons integrate thousands of synaptic inputs is critical to discern cortical information processing. Substantial evidences suggest the importance of spatial arrangement of synaptic inputs onto dendrites for neuronal computation. However, the principle of spatial arrangement and integration mechanisms of inputs remain largely unsolved. Recent advances of functional imaging technique enable recordings of activities of individual spines using various calcium sensors. Due to the low time resolution of calcium signal transient, back propagating action potential (bAP) invades spines and makes the correct spine analysis difficult. To solve this problem two methods are currently used. (1) Subtract the contaminated bAP signal from the spine signal and estimate the 'true' spine signal. (2) Prevent the somatic action potential generation by voltage-clamping the imaged neuron. However, both methods have some technical problems. In this study we conducted a new method to overcome this problem by using inhibitory optogenetics. We sparsely co-expressed GCaMP6s and inhibitory optogenetic protein in mouse primary visual cortex by AAV and recorded visually evoked spine signals from neurons. Without inhibition, spine signals were contaminated with bAP. By activating inhibitory optogenetic protein, we successfully suppressed the somatic depolarization and individual spine signal was detected. This new method should be valuable for the accurate analysis of spine imagings. (COI: No)

1P-174

Fndc3b promotes climbing fiber synapse elimination partly by inhibiting STAT3 in the cerebellum

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Purkinje cells (PCs) in neonatal mouse cerebellum receive synaptic inputs from multiple climbing fibers (CFs). A single CF is strengthened in each PC out of multiple CFs from postnatal day 3 (P3) to P7. Then, redundant CFs are eliminated in each PC from P8 to around P11 (early-phase CF elimination) and from around P12 to P17 (late-phase CF elimination), and most PCs become innervated by single strong CFs. While a number of molecules responsible for the late phase of CF elimination are known, molecular mechanisms of the early phase of CF elimination remain largely unknown. Among candidate molecules that are expressed in developing PCs and that might possibly be involved in CF synapse elimination, we focused on Fndc3b (Fibronectin type-III domain containing protein 3b). We knocked down (KD) Fndc3b in neonatal PCs by injecting lentivirus containing a microRNA against Fndc3b under the control of a PC-specific promoter into the cerebellum at P0 to P3. We found that Fndc3b KD impaired CF synapse elimination, which became obvious during P8 to P11 and persisted into adulthood. Since Fndc3b is reported to inhibit signaling of STAT3(Signal Transducers and Activator of Transcription 3) in melanoma cells. We investigated STAT3 is involved in CF synapse elimination by miRNA-mediated KD of STAT3 in neonatal PCs. We found that STAT3 KD accelerated CF synapse elimination from P10 to P14. This result suggests that Fndc3b promotes CF synapse elimination partly through inhibiting STAT3. (COI: No)

1P-175

Distinct kinetics of synaptic vesicle replenishment mediated by synaptotagmin 1, 2 and 7

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In peripheral neurons, fine-tuning mechanisms of synaptic vesicle (SV) recycling dependent on dynamin (Dyn) isoforms allow synapses to maintain stable neurotransmission in response to various action potential (AP) firing patterns. However, whether there is a spatial synaptic organization to Dyn isoform-dependent recycling pathways remain unclear. One of the candidate molecules is synaptotagmin (Syt), playing a role as a Ca^{2+} sensor in SV replenishment. Thus, we examined the role of Syt 1, 2 and 7 in activity sensing in the presynaptic superior cervical ganglion (SCG) neurons. Syt 1, 2 or 7 expressed in SCG neurons was acutely knocked down by microinjection of the specific siRNA. Two days later, after depletion of SVs in the readily releasable pool by a train of 4-min APs at 5 Hz, the refilling rate was monitored by measuring the EPSP amplitude every 1 s. We found that the recovery rate of fast and slow phase was delayed with each Syt-knockdown (Syt-KD), while Syt7-KD or Syt7/Dyn1-double KD indicated the slow EPSP recovery with delaying rates. These results suggest that, in sympathetic neurons, Syt1 and Syt2 mediates recovery with both fast and slow kinetics following Dyn1- and Dyn3-mediated endocytosis, whereas Syt7 mainly mediates recovery with fast kinetics following Dyn1-mediated endocytosis, respectively. (COI: No)

1P-176

Synaptic clustering regulates the auditory coincidence detection in low tuning frequency neurons

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The synaptic location along dendrite has strong impact on integrative properties. Neurons in nucleus laminaris (NL) of birds are the coincidence detector of binaural inputs and involved in processing of interaural time difference (ITD) for sound localization. NL neurons with low tuning frequency (low-CF neurons) have prominently long dendrites, which were proposed to be preferable for binaural coincidence detection. However, synaptic location was not well examined in low-CF neurons and the contributions of dendrites are still controversial. In this study, we examined the dendritic location of synapses in low-CF neurons. We analyzed the distribution of glutamate receptors with focal uncaging and found that large currents were generated at distal dendrites. As the amplitude of mEPSC was uniform along dendrites, synaptic terminals might be clustered at distal dendrites. We recorded voltage responses at soma and found that responses generated at distal dendrites were strongly attenuated particularly at the strong stimulation. Model study revealed that the clustered inputs at distal dendrite generated large depolarization at the site, which decreased driving force of synaptic currents and increased shunting conductance, then increased the extent of attenuation in an intensity-dependent manner. We concluded that the synaptic clustering at distal dendrite would regulate the size of synaptic potential reaching to soma and maintain the ITD processing according to the input intensity. (COI: No)

1P-177

Function of type 1 metabotropic glutamate receptors in the neonatal rat hippocampal marginal zone

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Among the metabotropic glutamate receptors (mGluRs), group I mGluRs couple to G_q protein and increase intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through the release of Ca^{2+} from intracellular stores. mGluR1 and mGluR5 are subtypes of group I mGluRs and regulate neuronal excitability and play an important role in synaptic plasticity and memory formation. Cajal-Retzius cells (C-R cells) in hippocampal marginal zone are early-generated neurons which regulate neuronal migration by secretion of glycoprotein, reelin. These cells also project their dendrites to hippocampal neurons and modulate network activity. In the present study, characteristics of the mGluR1-mediated Ca^{2+} mobilization in hippocampal marginal zone were determined by fluorescence imaging using acute slices of neonatal rat hippocampus. $[Ca^{2+}]_i$ elevation was induced by group 1 mGluR-specific agonist in the presence of mGluR5 specific antagonist in C-R cells. Contrarily, mGluR1-specific antagonist prevented the $[Ca^{2+}]_i$ increment caused by Group I agonist. These results demonstrate that neonatal hippocampal C-R cells express functional mGluR1. The mGluR1-mediated component of glutamate-induced $[Ca^{2+}]_i$ elevation was characterized and source of Ca^{2+} mobilization by mGluR1 activation was determined. In addition, effects of GABA and NMDA application on $[Ca^{2+}]_i$ were studied in mGluR1-expressing neurons of hippocampal marginal zone. (COI: No)

1P-178

Sodium channel-independent components of axonal afterdepolarization in hippocampal mossy fibers

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Axonal afterdepolarization (ADP) is small and prolonged depolarization following action potentials. We recently have reported that sodium channel contributes to shaping ADP in hippocampal mossy fiber terminals. Unexpected from this notion, however, substantial component of ADP was left after application of sodium channel blocker tetrodotoxin (TTX), suggesting that additional mechanisms are involved. In this study, we adopted whole-cell recordings from mossy fiber terminals in mouse hippocampal slices and examined the mechanisms underlying TTX-resistant components of ADP. Brief current injection into the axon terminal during focal application of 0.5 μM TTX elicited transient depolarization like action potential followed by prolonged depolarization with similar time course of ADP. Further application of 2 mM 4-aminopyridine (4-AP), a blocker of potassium channel, increased the amplitude and duration of the prolonged depolarization. This finding suggested that 4-AP sensitive potassium channel is curtailing the time course of ADP. Prolonged depolarization recorded in the presence of TTX and 4-AP is likely to represent capacitive discharge of axonal membrane, since the time course was similar with that of membrane potential change in response to negative current pulse injection. Taken together, capacitive discharge of axonal membrane, as well as sodium and potassium channel-dependent mechanisms, is suggested to contribute to shaping ADP in hippocampal mossy fiber terminals. (COI: No)

1P-179

Different taste sensitivity to salt and amiloride relates localization in the rat rNST neurons

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A previous study has reported that in taste receptor cells (TRCs) that respond to low concentration of NaCl, their responses are reduced by amiloride, epithelial sodium channel (ENaC) antagonist. But TRCs that only respond to high concentration are unaffected by amiloride. In the present study, we investigated whether (1) two neuronal properties as well the TRCs were maintained for the first-order taste relay, the rostral nucleus of the solitary tract (rNST), (2) two neuronal groups had distinct histological distributions. Here, we recorded extracellular single unit activities in the rNST neurons using multi-barrel glass micropipettes while under urethane anesthesia. Taste solutions were applied to the tongue and the oral cavity, and rinsed by distilled water or 10 μM amiloride. The rNST neurons that were reduced by amiloride exhibited the sensitivity to low concentrations (0.1 or 0.2 M). In contrast, the neurons unaffected by amiloride responded from the higher concentrations (0.4 or ≥ 0.8 M). The property originated from the ENaC would be principally maintained in the rNST. The amiloride-sensitive neurons with the low threshold for NaCl was located in a center of the rNST neurons on horizontal plane constructed from histological preparations, and surrounded by the amiloride-insensitive neurons with the high threshold. The localization for the rNST neurons may be advantageous to convey information for hypotonic or hypertonic NaCl to the higher-order brain. (COI: Properly Declared)

1P-180

Olfactory marker protein controls cAMP-throughput capacity via cAMP-gated channels in normosmia

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Hyposmia has been recently highlighted as an overlooked prodrome of neurodegenerative diseases. The genetic ablation of olfactory marker protein (OMP), which reportedly decreases in early-stage Parkinson's disease, has been shown to cause impairments in odour discrimination. However, biological functions of OMP remain elusive. Here, we show that OMP directly captures an olfactory second messenger cAMP. OMP rapidly sequesters cAMP actions via cyclic nucleotide-gated channels, which secures the high-fidelity response of olfactory neurons under repetitive chemo-mechanical stimulation, otherwise OMP-knockout neurons fell silent by overburden. OMP-heteroknockout mice, but not OMP-knockout mice, were capable of locating hidden food by continual sniffing, but rendered incapable of recognizing visible food when intranasal cAMP was overloaded, indicating that OMP-deficiency causes latent cAMP-vulnerable hyponosmia. Our results reveal that OMP controls the cAMP-throughput capacity in sustaining normal olfaction during active sniffing. These findings will advance pathophysiological understanding, diagnosing and treating hyposmia due to the temporal discoordination of OMP/cAMP-dynamics. (COI: No)

1P-181

Melatonin does not protect the brain against cardiac ischemia/reperfusion injury

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Purpose: To test the hypothesis that melatonin administration at various time points including pretreatment, during cardiac ischemia, and at the onset of reperfusion attenuated brain inflammation and brain mitochondrial dysfunction in rats with cardiac ischemia/reperfusion (I/R) injury.

Methods: Twenty Male rats were subjected to either sham operation (n=5) or cardiac I/R injury (30 min-ischemia and 120 min-reperfusion: n=20). Melatonin (10 mg/kg, i.v.) was given to the rats at various time points of cardiac I/R (n=5/time-point) including: 1) Pretreatment, 2) During ischemia, and 3) Onset of reperfusion. Normal saline solution was used as a control (n=5). After reperfusion, all rats were sacrificed, and the brain was rapidly removed. The number of infiltrated microglia and resident microglia were determined using flow cytometry. Brain mitochondrial oxidative stress, membrane potential changes, and swelling were determined as brain mitochondrial function.

Results: Cardiac I/R lead to brain inflammation as indicated by increasing number of infiltrated microglia, and reducing number of resident microglia. Moreover, brain mitochondrial dysfunction was observed after cardiac I/R. Treatment with melatonin at all time points did not affect the number of infiltrated and resident microglia, and it did not alter brain mitochondrial function in rats with cardiac I/R.

Conclusion: A single dose of melatonin administration failed to reduce brain damage following cardiac I/R injury. (COI: No)

1P-182

Developmental regulation of Ca channel expression in avian cochlear nucleus

Kensuke Muto; Rei Yamada; Hiroshi Kuba (*Department of Cell Physiology, Graduate School of Medicine, Nagoya University, Japan*)

In developing neurons, calcium ion plays significant roles in maturation of cellular functions including ion channel expression. In avian cochlear nucleus, the expression of Kv channels increases around hatch and regulates the excitability of neurons for precise temporal coding. Activity dependent influx of Ca might contribute to this Kv channel refinement. However, the regulation of Ca influx during development is not well understood. In this study, we examined developmental changes of Ca channel in chicken cochlear nucleus by recording Ca currents with patch-clamp technique in brainstem slices from different ages. Ca channels are categorized into low-voltage activated (LVA) and high-voltage activated (HVA) channels according to their activation voltages, and we evaluated them separately. During the embryonic days, both currents increased gradually and reached maximum just before hatch. Interestingly, LVA current showed drastic decrease after hatch, but HVA current did not. Moreover, deprivation of auditory input by removing the cochlea precluded the reduction of LVA current. We concluded that in avian cochlear nucleus both LVA and HVA channels are present during embryonic period and they might contribute to the maturation of neuronal functions. Our results further suggested that the expression of LVA channel might be regulated through auditory activity. (COI: No)

1P-183

Mechanisms underlying WNK3 kinase mediated regulation of neuronal excitability in prefrontal cortex

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The WNK kinases are a family of serine/threonine kinases. WNK3 is expressed in both fetal and postnatal brain and its expression level increases during development.To elucidate its role in neurons, here we used WNK3 knockout mice and compared the chloride homeostasis, passive membrane characteristics and the properties of single and repetitive action potential (AP) firing of layer V pyramidal neurons in slice preparations of the medial prefrontal cortex(mPFC) at postnatal day 21-27. We found that loss of WNK3 significantly reduced neuronal excitability, as evidenced by hyperpolarized resting membrane potential(RMP), decreases in input resistance, thereby increasing the rheobase for AP generation. Both the rising and falling phases of an AP were significantly slowed, resulting in a spike width broader than that in wildtype littermates. Voltage-clamp recordings revealed the enhanced inwardly rectifying potassium (IRK) conductance caused the hyperpolarized RMP. Indeed, introduction of the active form of WNK3 proteins into the knockout neurons suppressed the enhancement. The enhanced conductance was not due to the GIRK channels coupled to $GABA_B$ receptor activation.Contrastingly E_{GABA} levels were depolarized. Phosphorylation of alternative WNK1 and its downstream cascade SPAK/OSR1 were studied. Regulation of IRK subunits was also evaluated by western blotting. To conclude WNK3 kinase regulates $[Cl^-]_i$ and IRK mediated potassium conductance in layer V pyramidal neurons of the mPFC.

(COI: No)

1P-184

Ca^{2+} signaling and ion channel activation in embryonic neurons in the medial ganglionic eminence

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Most of the GABAergic interneurons in the cerebral cortex are born primarily in a region in the ventral telencephalon, called the medial ganglionic eminence (MGE), and they migrate toward the neocortex during prenatal and perinatal periods. Several chemoattractive and chemorepulsive guidance cues for the migration, like neuregulin-1 and semaphorin 3A, respectively, have been identified. On the other hand, since the intracellular Cl^- concentration in the embryonic neurons is raised, compared with mature neurons, by the activity of Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1), activation of $GABA_A$ receptor Cl^- channels induces membrane depolarization that can elicit voltage-gated Ca^{2+} entry, and the resulting rises in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) are known to facilitate the migration. However, whether the actions of guidance cues may regulate $[Ca^{2+}]_i$ and/or may interact with the action of GABA is as yet unknown. In addition, the migration must be accompanied by the changes in cell volume, and the changes are driven by ion movements across the cell membrane. The types of ion channels activated by the guidance cues to induce ion movements for the migration also remain to be elucidated. We will report our recent attempts to address these issues by $[Ca^{2+}]_i$ imaging and patch-clamp recording in acutely-isolated mouse MGE neurons. (COI: No)

1P-185

GABA in the suprachiasmatic nucleus refines circadian behavioral rhythms

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In mammals, the circadian rhythms are regulated by the central clock located in the hypothalamic suprachiasmatic nucleus (SCN). In the SCN, an inhibitory neurotransmitter, γ-Aminobutyric acid (GABA) is expressed in almost all neurons. Nevertheless, the role of GABA in the circadian physiology of the central clock is still elusive, because all previous studies were performed by pharmacological approaches using different recording methods. In the present study, we genetically disrupted the GABA functions by knocking out the vesicular GABA transporter (*Vgat*), and simultaneously measured molecular circadian oscillation (PER2::LUC), spontaneous firing (Multi-electrodes array dish), and cytosolic calcium (GCaMP6s) in the cultured SCN. Circadian PER2 rhythms in *Vgat*$^{-/-}$ SCN were not different from those of the wild type. On the other hand, spontaneous firing from *Vgat*$^{-/-}$ SCN showed burst firings with a firing rate higher than 35 Hz appearing at 2-3 min intervals together with calcium spikes overlain the circadian fluctuation. Finally, to understand roles of GABA in the SCN for circadian behavior, we expressed Cre recombinase in the SCN of *Vgat*$^{flox/flox}$ (SCN-*Vgat*$^{-/-}$) mice using adeno-associated virus injection, and measured locomotor activity rhythms. SCN-*Vgat*$^{-/-}$ mice showed unstable circadian behavioral rhythm under constant darkness, and their rhythm amplitude was decreased. These results indicate a novel function of GABA in the SCN, the refinement of circadian output rhythms. (COI: Properly Declared)

1P-186

Withdrawn

1P-187

P2X7 receptor-pannexin-1 channel interaction in rat trigeminal ganglion neuron

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Extracellular ATP has been suggested to be associated with neuropathic pain. Ionotropic P2X7 receptors needs high concentration of extracellular ATP to be activated and plays important roles in generation and/or regulation of nociceptive and neuropathic pain. When Bz-ATP binds to the P2X7 receptor on cell membrane, cation flows into the cell via the P2X7 receptor. This should be observed as Bz-ATP-induced fast inward current (first component). However, various cells, a slow inward current has been observed following the fast inward current. We investigated how the second component of this biphasic inward current is occurred in rat trigeminal ganglion (TG) neurons.

In TG neurons, 100 μM Bz-ATP induced biphasic inward current. These currents were significantly suppressed by P2X7 receptor antagonists (10 μM A-740003 and 10 μM A-438079). The Bz-ATP-induced current densities in both components were increased by concentration-dependent manner (EC_{50} = 27.6 μM for first, and 27.5 μM for second component. The appearance frequency of second component in Bz-ATP-induced inward current increased by concentration- and time-dependent manner. Pannexin channel blocker (mefloquine) dose-dependently reduced the current density and duration in the second component of Bz-ATP-induced current.

The functional expression of P2X7 receptor in rat TG neurons suggests that extracellular ATP released by pannexin-1 channel is capable to re-activate P2X7 receptor, resulting the biphasic P2X7 current. (COI: No)

1P-188

Oxygen affects simple circuit for cold acclimation via KQT potassium channel and HADH in *C. elegans*

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We are studying temperature acclimation by using nematode *C. elegans* (Ohta et al., ***Nature commun***, 2014; Sonoda et al., ***Cell reports***, 2016; Ujisawa et al., ***PNAS***, 2018). We show here that oxygen affects temperature acclimation through KCNQ potassium channel KQT-2. *kqt-2*mutant showed abnormal temperature acclimation depending on cultivation-space, which is caused by abnormality of ADL sensoryneuron known as aversive chemo-sensoryneuron. Calcium imaging revealed that ADL is responsive to temperature changes via TRP channels OSM-9 and OCR-1, 2, in which OCR-1 functions as inhibitory subunit. KQT-2 assembles with KQT-3 to form a heteromeric channel, where KQT-2 acts as negative regulator for opening its channel. Beta-oxidation of fatty acid metabolism in mitochondrial matrix maybe regulate cold acclimation in ADL. Abnormal temperature acclimation and response of ADL in *kqt-2*mutant were suppressed by mutation in GCY-35 oxygen receptor in URX sensoryneuron. Low oxygen also enhanced abnormal temperature acclimation of *kqt-2*mutant. Altogether, integrating two different information, temperature and oxygen, affects temperature acclimation via KQT-2 channel. We also recently found that cold experience of parental generation influences cold tolerance of animal in next generation. Then, we are decoding an epigenome for transgenerational transmission of cold information by deep sequencing. (Correction: M.F. is first author and A.K. is last author of this presentation) (COI: No)

1P-189

Corticocortical mechanisms underlying perceptual memory consolidation during NREM sleep

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Non–rapid eye movement (NREM) sleep plays a vital role in memory consolidation. Recently, we found that top-down projection from the secondary motor cortex (M2) neurons to the primary somatosensory cortex (S1) initiated dendritic activity and persistent firing of S1 layer 5 (L5) neurons (Manita *et al*., 2015, ***Neuron***), and that the top-down cortical information flow during NREM sleep is required for perceptual memory consolidation (Miyamoto *et al*., 2016, ***Science***). The dendritic activity may cause an increase in intracellular concentrations of Ca^{2+}, which acts as a secondary messenger in neurons and induces an activity-dependent increase or decrease in synaptic strength. Therefore, we hypothesized that the activation of such top-down circuits during NREM sleep induces dendritic activities and subsequent growth of dendritic spines in individual pyramidal neurons. To test this hypothesis, transgenic mice with L5-specific expression of GCaMP6s were used to perform a perceptual memory task. We measured Ca^{2+} activity during NREM sleep in single dendrites of L5 neurons. Furthermore, to examine the effects of top-down control on dendritic activation, subsequent spine growth, and perceptual memory-based behavior, we chemogenetically inactivated the top-down inputs and observed the resulting perception inaccuracies, dendritic activity, and spine dynamics. These data demonstrated that top-down inputs are necessary for the dendritic dynamics underlying perceptual memory consolidation. (COI: Properly Declared)

1P-190

Physiological and anatomical organization of cortico-striatal inputs in the basal ganglia

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The striatum and subthalamic nucleus (STN) receive cortical efferents as input stations of the basal ganglia. To understand the functional difference between trans-striatal and trans-STN circuits, it is a key to examine how different information the cortex sends to the striatum and STN. In this study, we applied optogenetics to reveal physiological and anatomical organization of cortico-striatal neurons. Lentiviral vector termed neuron-specific retrograde gene transfer (NeuRet) was injected into the mouse striatum to express Cre recombinase in cortico-striatal neurons. Then, adeno-associated viral vector containing a double-floxed channelrhodopsin-2 (ChR2) was injected into the mouse motor cortex. ChR2 was expected to express specifically in cortico-striatal neurons. We gave photo stimulation to the motor cortex and recorded neuronal activity in the external segment of the globus pallidus (GPe) and substantia nigra pars reticulata (SNr). We observed triphasic response composed of early excitation, inhibition and late excitation. The early excitation and inhibition are mediated by cortico-STN-GPe/SNr and cortico-striato-GPe/SNr pathways, respectively. These results suggest that cortico-STN as well as cortico-striatal projections were photo-stimulated. Nerve terminals of cortico-striatal neurons were visualized by ChR2, and found also in the STN. Our results suggest that cortico-striatal inputs are mediated by cortico-striatal neurons and collaterals of cortico-STN neurons. (COI: No)

1P-191

Effects of acute kidney dysfunction on arginine vasopressin in transgenic rats

Hiromichi Ueno; Kenya Sanada; Kentaro Tanaka; Haruki Nishimura; Kazuaki Nishimura; Satomi Sonoda; Yoshihiro Yoshimura; Takashi Maruyama; Yutaka Otsuji; Yoichi Ueta (*Department of Physiology, University of Occupational and Environmental Health, Japan*)

Acute loss of kidney function is a critical internal stressor. The paraventricular nucleus (PVN) of the hypothalamus is an integrative site of the neuroendocrine and autonomic nervous systems that deals with a variety of aversive stressors. Hypothalamic arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH) in the parvocellular division of the PVN (pPVN) plays an important role in the regulation of stress responses. However, hypothalamic AVP dynamics under acute kidney dysfunction remain unclear. We generated transgenic rats that express the AVP-enhanced green fluorescent protein (eGFP) fusion gene. The eGFP fluorescent intensity is a quantitative indicator of AVP synthesis in the transgenic rats. Here, first we evaluated AVP-eGFP fluorescence in the hypothalamus after bilateral nephrectomy. Second, we examined the gene expression by *in situ* hybridization histochemistry. Finally, we quantified Fos-like immunoreactive (IR) cells in the several brain regions that are involved in the biological responses to severe stressors.

After bilateral nephrectomy, eGFP fluorescent intensities and the mRNA levels of *eGFP*, *AVP*, and *CRH* were significantly increased in the pPVN. Bilateral nephrectomy also caused a marked increase in Fos-IR in the brainstem neurons that are responsible for modulating sympathetic nerve activity. Further studies are needed to identify the neural and/or humoral factors that activate AVP synthesis and neural circuits under dysfunction of kidney. (COI: Properly Declared)

1P-192

How does the cerebellum control thalamocortical activity?

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The cerebellum plays a crucial role in controlling voluntary movements. The cerebellum receives information from the cerebral cortices, and projects back to the original cortices through the thalamus. To understand roles of the cerebellum in control of thalamocortical activity, channelrhodopsin-2 or halorhodopsin was expressed in neurons of the deep cerebellar nuclei, the output station of the cerebellum, in macaque monkeys by viral vector injections. Activities of thalamocortical neurons were examined in response to selective activation or inhibition of cerebellar inputs by light stimulation to the thalamus. Short-duration activation of cerebellar inputs induced a biphasic response composed of short-latency brief excitation immediately followed by strong inhibition. During repetitive activation, a train of biphasic responses was observed. Inhibition of cerebellar inputs largely decreased firing rates of thalamocortical neurons. We also recorded activities of thalamocortical neurons during hand reaching task. Thalamocortical neurons increased their firings during the task. Activation of cerebellar inputs enhanced increase in firing rates during the task in the half of thalamocortical neurons and diminished that in the other half. These results suggest that cerebellar inputs play a major role in controlling thalamocortical activities and inducing cortical activities at accurate timing, contributing to fine and accurate voluntary movement control. (COI: No)

1P-193

The perioral sensory signaling pathway for complex spike generation in cerebellar Purkinje cells

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Perioral tactile signals are first transmitted through the infraorbital nerve (ION) to the ipsilateral trigeminal nuclei. Purkinje cells (PCs) in the cerebellum receive these signals from the contralateral inferior olive (IO), which generates complex spikes (CSs). However, little is known about the anatomical pathway from the trigeminal nuclei to the IO. In the present study, we examined anatomical pathways from the trigeminal nuclei to the IO in mice. CSs evoked by electrical stimulation of ION were recorded from PCs in the right cerebellar Crus II by the single unit recording. CS generation by stimulation of ION ipsilateral to the recorded PCs was strongly suppressed by a $GABA_A$ receptor agonist, muscimol, injection into the contralateral 'area parafascicularis prerubralis (PfPr)', in the mesodiencephalic junction. CS generation by contralateral ION stimulation was also partially suppressed by muscimol injections into the caudal part of the PfPr. CSs evoked by air-puff stimulation to the ipsilateral whisker region were also suppressed by contralateral PfPr inhibition. We also found that inhibition of the primary motor cortex enhanced the CS generation by ION stimulation. These results suggest that PfPr relays perioral tactile signals transmitted to the IO, and the primary motor cortex provides inhibitory influence on this signaling pathway. (COI: No)

1P-194

Examination into effects of stimulation of the lateral habenula on cardiovascular responses in rats

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It has been known that neurons in the lateral habenula (LHb) show stress-related responses. However, in stress events, functional roles of the LHb neurons to the cardiovascular autonomic nervous system are still unclear. Therefore, in this study, we tried to examine the effects of LHb stimulation on the cardiovascular responses in anesthetized rats. In this study, we used male Wistar rats (250-300g, n = 5) with anesthetized by urethane (1-1.25 g/kg, *i.p.*). Mean arterial pressure (MAP) was monitored from a right femoral artery. Heart rate (HR) was calculated from electrocardiogram. The left LHb was electrically stimulated (300 μA intensity, 0.5 ms duration, 100 Hz for 10 s). As a result, stimulation of the LHb (n = 13) increased MAP by 10.4±1.9 mmHg (15.1±9.5% *vs* before stimulation, p = 0.001) and decreased HR by 9.4±2.1 bpm (2.8±2.4% *vs* before stimulation, p = 0.007). Onset latencies of the bradycardia preceded those of pressor responses by 0.66±0.47 s ($p < 0.001$). On the other hand, when we stimulated the brain at a distance of 0.25 mm lateral or ventral from the LHb, MAP and HR did not change. These data suggested that the LHb neurons regulate cardiovascular autonomic nervous system to modulate cardiovascular functions. This system may have a crucial role to induce cardiovascular responses in stress events. (COI: No)

1P-195

NMDA receptor-mediated activation of excitatory networks in rat interstitial nucleus of Cajal

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Gaze holding is primarily controlled by neural structures including the prepositus hypoglossi nucleus (PHN) for horizontal gaze and the interstitial nucleus of Cajal (INC) for vertical gaze. Our previous studies showed that the application of a transient, high-frequency electrical stimulation (100 Hz, 20 pulses) to a nearby site of a recorded neuron in the PHN and INC induced an increase in the frequency of spontaneous excitatory postsynaptic currents (EPSCs) that lasted for several seconds. These findings indicate excitatory networks that activate sustained EPSC responses are essential for generation of the signals for gaze holding. Although the activation of excitatory networks in the PHN were primarily mediated via Ca^{2+}-permeable AMPA (CP-AMPA) receptors, the contribution of CP-AMPA receptors was small in the activation of INC excitatory networks. In this study, we explored other mechanisms that participate in the activation of excitatory networks in the INC using whole-cell recordings in rat brainstem slices. The duration of the increased EPSC frequency of INC neurons decreased by the application of an antagonist of NMDA receptors, AP5. The percentage of duration reduction by AP5 in the INC (57.6 ± 14.5%, n = 10) was significantly larger than that in the PHN (14.5 ± 5.3%, n = 10, $p < 0.0001$). This result suggests that the activation of INC excitatory networks is primarily mediated via NMDA receptors. (COI: Properly Declared)

1P-196

Topographic representation of saccade vector in frontal eye field of common marmoset

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Frontal eye field (FEF) is an important brain area controlling saccades. However, because it is deep in the rostral bank of arcuate sulcus and difficult to access in macaques, whether it contains a topographic representation of saccade vectors remains undetermined. Here, we used the flat cortical surface in common marmoset and systematically mapped the FEF with electrical stimulation.

We applied stimulations with biphasic current at 300 Hz for 30 trains in potential cortical areas identified by the atlas during the gap period when marmosets performing gap saccade. We varied the stimulation amplitude in some cases but most of our stimulations were kept at 0.07 mA. We also varied the initial fixation locations to identify whether the evoked saccades were vector-based or endpoint-based coding.

We successfully evoked saccades ranging from 2-20 degrees in areas 8 and 45. We observed a systematic decrease in amplitude and changing of direction from upper to lower visual field of the evoked saccades when moving the stimulation sites from medial to lateral. The evoked saccades in these areas were vector-based. However, if the stimulation site was posterior to this region, the saccades became more endpoint-based coding.

Together, we found a continuous topographic representation of saccade vectors in the marmoset FEF. By using the marmosets' flat cortex, we can advance our knowledge of the areas deep in the sulci of macaques and understand more about the oculomotor systems. (COI: No)

1P-197

Measurement of multiple cerebellar mossy fiber activities by calcium imaging in mouse

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The cerebellar mossy fiber is one of the two major inputs to the cerebellum, providing excitatory inputs to Purkinje cells via granule cell-parallel fiber pathway. Our purpose in this study is to investigate spatial and temporal properties of the mossy fiber activities during voluntary movements. For this purpose, we perform calcium imaging from individual mossy fiber terminals in Thy1-G-CaMP7 transgenic mouse in vivo. We first confirmed G-CaMP7 localizes in the cerebellar mossy fiber terminals by using immunocytochemistry. Next, we measured the G-CaMP7 signals in the granular layer from acute cerebellar slices, and the signal depended on sodium spike, supporting that the G-CaMP7 signal reflects spikes of the mossy fiber. We next observed sensory responses of the mossy fibers by electrical stimulation of forelimb in anesthetized mouse with wide-field calcium imaging, and found that the mossy fiber activities are evoked in the cerebellar vermis and hemispheres. To monitor the mossy fiber activity during movements, we used forelimb movement tasks and observed mossy fiber activities correlating with forelimb movements. Activities of single mossy fiber terminals during forelimb movements can also be clearly observed by two-photon imaging. Further analyses of the spatiotemporal pattern of the mossy fiber activity during forelimb movements will reveal how the motor and sensory information are represented by ongoing mossy fiber activities. (COI: No)

1P-198

Activity-dependent formation and restoration of callosal axon projections in developing neocortex

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The developing cerebral cortex exhibits robust spontaneous network activity, ranging from highly synchronous to less correlated patterns. Such activity has been thought to be critical for the formation of cortical circuits, but its role remains unclear. We previously reported that the development of callosal axon projection, one of the major long-range axonal projections in the brain, is activity-dependent (Mizuno et al., Journal of Neuroscience, 2007; Tagawa and Hirano, Neural Plasticity, 2012). Here, reducing and restoring spontaneous network activity in mouse cerebral cortex by genetic methods, we show that spontaneous cortical network activity contributes to the region and lamina specific projections of visual callosal axons. We also demonstrate that there is "a critical period" for the formation of callosal axon projection: restoring neuronal activity during the second postnatal week was able to resume the projection whereas that after the period failed. Restored activity pattern was mainly less-correlated network activity, and we are speculating that this activity pattern reflects the correlated sub-network activity of callosal projection neurons. Our findings suggest that spontaneous cortical network activity during a limited period in development contributes to the formation of long-range axonal projections in the developing cerebral cortex. (COI: No)

1P-199

Neural ensemble dynamics during P-waves in mice

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Rapid eye movement (REM) sleep is characterized by hippocampal theta waves and phasic sub-second waves in the brainstem, so-called ponto-geniculo-occipital (PGO) waves in cats or pontine (P) waves in rodents. Although the sub-second pontine wave during REM sleep was originally described in the 1960s, we still do not know occurrence of P-waves in mice. Using in vivo electrophysiological approaches in mice, we investigated the temporal evolution of P-waves and underlying neural ensemble dynamics in the brainstem. First, to examine how P-waves appear during the sleep-wake cycle, bipolar electrodes were implanted into the sublaterodorsal nucleus (SLD) of head-fixed mice. The density of P-waves was typically at ~ 0.8 Hz during REM sleep, consistent with that in cats and rats. Second, to monitor neural ensemble dynamics during P-wave, we inserted a 4-shank silicon probe to cover multiple brainstem nuclei simultaneously. While a rich structure of neural activity was apparent across brainstem nuclei, we found that P-waves during REM sleep were associated with highly synchronous burst firing across nuclei. Next, we also investigated functional interactions between the brainstem and hippocampus during P-wave genesis. While P-waves were seen during both REM and non-REM sleep, the functional connectivity between two structures was changed depending on sleep states. In conclusion, our findings suggest that P-waves were part of state-dependent coordinated activity across brain regions. (COI: No)

1P-200

The neural connections between the oculomotor neural integrators and the vestibulo-cerebellum

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Gaze holding is primarily controlled by the oculomotor neural integrators, which are separated into the prepositus hypoglossi nucleus (PHN) for horizontal gaze and the interstitial nucleus of Cajal (INC) for vertical. Although it has been argued that the neural connections between the integrators and the cerebellar cortex are significant in gaze holding, the distributions of the integrator neurons that project to the cortex have not been well defined. In this study, we examined the distributions of PHN and INC neurons that were retrogradely labeled by injecting the dextran-conjugated Alexa 488 tracer into the cerebellar flocculus (FL) or uvula/nodulus (UN) using choline acetyltransferase (ChAT)-tdTomato transgenic rats. When the tracer was injected into the FL unilaterally or center of the UN, the retrogradely labeled neurons were observed in the PHN but not in the INC. The proportion of cholinergic PHN neurons that projected to the UN (22.2 ± 1.1 % in 4 rats) was significantly larger than the proportion of the neurons that projected to the FL (9.8 ± 1.8 % in 6 rats, P = 0.0008). The proportion of cholinergic PHN neurons that projected to the UN differed depending on the rostral-caudal position of PHN. These results indicate that the projection to the vestibulo-cerebellum from the neural integrator is restricted to the PHN, in which the proportion of cholinergic projection neurons differs dependent on the projection sites. (COI: No)

1P-201

Serotonin regulated the fetal movement-like activity in the spinal cord

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Fetal movement-like activity (FMA) is suppressed by strychnine-sensitive glycine receptor after birth (Robinson SR. 2000; Shimomura et al., 2015). We understood that the FMA generator was in the spinal cord; however, the FMA generator had not fully understood. In this study, we examined the location and the properties of the FMA generator. We compared the responses of isolated whole spinal cord preparation to that of regional spinal cord (cervical: C or thoracic: Th or lumbar: L or sacra: S) preparation under strychnine application. The FMA in whole spinal cord showed more continuously and more frequently than the regional spinal cord. In the whole spinal cord, the timing of the FMA in each segment showed synchronously, however, interestingly, the FMA in each C-S regional spinal cord showed independently and produced its rhythm. It is well known that 5-HT has strongly affected the spinal rhythmic activity such as locomotion. We examined the effect of 5-HT on the spinal activity. When we added 5-HT and strychnine applications to these preparations, the FMA in each regional spinal cord showed some regularity. These results suggested that the FMA generator existed in C6-8, L4-5, and S of the spinal cord and each spinal region produced their rhythm even if the spontaneous spinal wave. The FMA generator can produce own rhythm in segmental preparation and can be accelerated by 5-HT. 5-HT modulated their origin of FMA. (COI: No)

1P-202

Function of inhibitory neurons in the solitary nucleus in the control of respiration

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Visceral sensory information affects respiratory rhythm, which is generated by interaction among interneuron populations within the ventral respiratory column (VRC). Sensory information from the lung stretch receptor is transmitted to GABAergic/glycinergic inhibitory neurons in the lateral part of the nucleus of the solitary tract (lateral NTS). These inhibitory neurons send their axons to the VRC and may cause the transition from inspiration to expiration. However, it remains unknown how these inhibitory neurons affect the respiratory rhythm. In this study, we examined the effect of selective stimulation of the inhibitory lateral NTS neurons on respiratory rhythm using the optogenetic technique. We made mice expressing Channelrhodopsin-2 (ChR2) in the inhibitory lateral NTS neurons and optically stimulated these neurons. Single pulse photo-stimulation during the inspiratory phase shortened the inter-inspiratory interval. On the other hand, single pulse photo-stimulation during the early or late expiratory phase did not alter ongoing respiratory rhythm. Within the VRC, we found overlapping distribution of axon terminals of ChR2 expressing neuron and somatostatin containing neurons, a part of neuronal population thought to be the primary source of rhythmic inspiratory excitatory drive. These results suggest that the inhibitory lateral NTS neurons affect the rhythmogenic inspiratory interneuron population within the VRC.

(COI: No)

1P-203

Information processing in brainstem bitter taste-relaying neurons

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Bitter is the primary taste modality that elicits aversive and displeasing responses. Bitter taste detection is mediated by taste receptor cells that coexpress multiple T2Rs. We combined genetic tracing with electrophysiological recordings and immunohistochemistry to functionally characterize brainstem bitter taste-relaying neurons, which were fluorescently labeled by the transneuronal tracer tWGA-DsRed originating from T2R-expressing cells. In the solitary tract nuclei, the neurons labeled by tWGA-DsRed were located posteriorly. In the parabrachial nuclei that receive input from the solitary tract nuclei, the tWGA-DsRed-labeled neurons were located rostrally in the external lateral parabrachial nuclei, and caudally in the medial parabrachial nuclei. The tracer-labeled neurons in the solitary tract nuclei are heterogeneous, and can be classified into catecholamine and POMC neurons. The tracer-labeled neurons in the medial and the external lateral parabrachial nuclei exhibit the differential responsivities to noradrenaline and alpha-MSH. Mapping the induction of immediate early genes to detect the neurons activated by different stimuli suggests that the tracer-labeled neurons in the solitary tract nuclei and in the medial parabrachial nuclei may be selectively activated by bitter taste, whereas the tracer-labeled neurons in the external lateral parabrachial nuclei may receive the convergent inputs of bitter taste and aversion-eliciting information from the gut. (COI: No)

1P-204

Inhibitory local connection of parvalbumin-expressing neurons in the rat globus pallidus

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The globus pallidus (GP) is known as a relay nucleus of the indirect pathway of the basal ganglia network. GP plays an important role for the control of action. The GABAergic GP neurons can be classified into two subtypes: a prototypic neuron and an arkypallidal neuron. In vivo recording showed that about half of prototypic neurons showed antiphasic firing pattern with arkypallidal neurons during movement. One possible mechanism to generate antiphase rhythm in two neuronal groups is mutual inhibition. However, it is unclarified whether the probability and strength of the intra-GP connection depend on the subtypes. To address this question, we firstly investigated local synaptic inputs from parvalbumin (PV) expressing GP neurons (PV-GP neurons), because PV-GP neurons were the most numerous population of prototypic neurons account for 70% of GP neurons. AAV expressing ChR2 was injected into GP of PV-Cre rats to selectively stimulate PV-GP neurons. As a result, the optogenetically evoked inhibitory postsynaptic currents (oIPSCs) were elicited in both subtypes of post-synaptic GP neurons. The oIPSCs were mediated by $GABA_A$ receptors. The connection probability and oIPSC amplitude were not significantly different between the subtypes of post-synaptic GP neurons. Our data suggest that the local inhibition by PV-GP neurons might not directly induce cell type characteristic activity phases of prototypic and arkypallidal neurons. (COI: No)

1P-205

Effects of hypovolemia and osmotic challenge on arginine vasopressin synthesis in transgenic rats

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Both acute hypovolemia and osmotic challenge is known to increase arginine vasopressin (AVP) synthesis in the paraventricular (PVN) and the supraoptic nuclei (SON) of the hypothalamus and activates neuronal circuits related to body fluid homeostasis. In the present study, we examined the effects of intraperitoneal (ip) administration of polyethylene glycol (PEG) for hypovolemia and 3% hypertonic saline (HTN) for osmotic challenge on AVP synthesis in the PVN and the SON, using a transgenic rat expressing the AVP enhanced green fluorescent protein (eGFP) fusion gene. After ip administration of PEG as well as HTN, AVP-eGFP intensities, which is a quantitative indicator of AVP, were significantly increased in the magnocellular division of the PVN and the SON. Surprisingly, AVP-eGFP intensities appeared markedly in the parvocellular division of the PVN (pPVN). AVP and Corticotropin-releasing hormone (CRH), which are both synthesized in the pPVN , have an important role in the regulation of stress responses via adenohypophyseal systems. Furthermore, PEG induced a remarkable increase in Fos-IR in the not only circumventricular organs regulating water balance but also the brainstem neurons responsible for modulating sympathetic nervous system activity.

These results suggest that acute hypovolemia upregulates AVP synthesis in both neurohypophyseal and adenohypophyseal systems and activates broad neuronal circuits. (COI: No)

1P-206

Sex difference of oxytocin and vasopressin dynamics in the hypothalamus of rats

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OBJECTIVE: Oxytocin (OXT) and arginine vasopressin (AVP) are well known for posterior pituitary hormones. The objective was to elucidate the dynamics of hypothalamic OXT and AVP by gender and estrus cycle using OXT-mRFP1 and AVP-eGFP transgenic rats. METHODS:①Male and female group were divided into 5 groups of estrus cycles (proestrus, estrus, postestrus and diestrus). ②Ovariectomy (OVX) group was added to females. ③After OVX was administered, hormone supplement (low dose estradiol (E2), high dose E2) was added. In each case, the brain was analyzed in the hypothalamus supraoptic nucleus (SON), paraventricular nucleus (PVN) and median eminence (ME). RESULTS: The mRFP1 red fluorescence of the OXT neuronal cell bodies localized in the SON and PVN of OXT transgenic rats was significantly higher in the estrus stage and lower in the OVX group. In hormone supplementation, the fluorescent intensities increased in order of low dose E2 and high dose E2 administration. The number of AVP-eGFP granules in external layer (e) of the ME was significantly larger in all estrus cycles of females than in males, and the AVP-eGFP granules in the OVX group disappeared. In the high-dose E2-administered group, the AVP-eGFP granules in the eME were significantly increased compared to other groups. CONCLUSION: OXT synthesis in the SON and the PVN was estrogen-dependent compared with AVP synthesis. It is worth noting that AVP-eGFP granules in the eME were estrogen-dependent. (COI: No)

1P-207

Projection-specific cortico-cortical transformations in the mouse visual system

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Visual information is first received in the retina and then sent to the visual cortex via the lateral geniculate nucleus of the thalamus. The mouse visual cortex consists of a primary visual area (V1) and at least 9 higher-order visual areas (HVAs). Both V1 and HVAs are retinotopically organized. Neurons in V1 have more diverse receptive fields than those in HVAs whereas HVAs are more specialized to specific features of the visual scene. For example, neurons in AL, one of the areas in HVAs, specialize in processing fast moving, low spatial frequency visual stimuli, while neurons in PM, another area in HVAs, specialize in processing slow, high temporal frequency stimuli. Little is known, however, about how the functional specializations of HVAs occur. Two-photon imaging of V1 axon terminals projecting to different HVAs has revealed that the function of V1 neurons projecting to each HVA matched to the function of its target area. From these results, we infer that receptive fields of V1 neurons projecting to each HVA should be generated from target-specific integration of presynaptic inputs such as subcortical inputs, local computations, and feedback inputs from other cortical areas. Here we mapped the direct synaptic inputs to V1 neurons projecting to a different target HVA with trans-synaptic tracing of G-deleted rabies viral vectors in mice. (COI: No)

1P-208

Presynaptic H3 heteroreceptor in nucleus accumbens mediates anxiolytic effect of histamine

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Anxiety is one of the most prevalent psychiatric disorders and usually entangled with abnormal synaptic transmission, such as increased glutamatergic neurotransmission. Intriguingly, dysfunction of the central histaminergic system may result in psychiatric conditions. Yet little is known about the underlying neural mechanisms. Here, we report that hypothalamic histaminergic neurons in rats directly project to the core of nucleus accumbens (NAc), a pivotal structure in the mesolimbic system. Activation of presynaptic histamine H3 receptor in the NAc core produces an anxiolytic-like effect, which is due to selective inhibition of glutamatergic rather than GABAergic synaptic transmission. Moreover, H3 receptor is expressed on the glutamatergic terminals apposing to the principle GABAergic neurons in the NAc core. Blockage or downregulation of H3 receptor in the NAc core induces anxiogenic-like behaviors. Furthermore, histamine reverses anxiogenic-like behaviors induced by selective optogenetic activation of prelimbic cortex-NAc core glutamatergic pathway. These results suggest that histaminergic afferent inputs in the NAc core may selectively suppress the glutamatergic neurotransmission from prelimbic cortex by presynaptic H3 heteroreceptor and consequently produce an anxiolytic effect. The findings define a novel mechanism for the central histaminergic system in anxiety regulation, and shed light on the strategies of targeting H3 receptor for treatment of emotional disorders. (COI: No)

1P-209

VTA neurons targeting cortical motor areas exhibit highly diffuse collateral projections

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Ventral tegmental area (VTA) neurons, including dopaminergic neurons, play a crucial role in mediating a variety of reward-based learning through projections to cortical and subcortical areas. However, it is unclear how the axon collaterals of VTA neurons that are related to motor skill learning are distributed at the whole-brain scale. Here, we attempted the double infection of retrograde and anterograde viral vectors combined with Cre-loxP system in rats in order to label the whole axonal trajectories of a subpopulation of VTA neurons that targeted the cortical motor areas. To find the best viral combination, four kinds of retrograde viral vectors carrying Cre recombinase were injected into primary and secondary motor areas of the cerebral cortex, and three kinds of anterograde viral vectors carrying EF1a-DIO-ChR2-EYFP sequence were injected into VTA of rats. The highest efficiency of labeling the VTA neurons including dopaminergic neurons, as evidenced by immunostaining with antibody against tyrosine hydroxylase, was achieved by the double infection of retrograde AAV2retro-CAGGS-Cre and anterograde AAVDJ-EF1a-DIO-ChR2-EYFP. Furthermore, the best viral combination showed that VTA neurons that targeted the cortical motor areas sent axon collaterals to various brain regions. Therefore, this result suggests that VTA neurons targeting the cortical motor areas may coordinate the individual functions of various brain structures through the dopamine-dependent plasticity. (COI: No)

1P-210

Phasic increase of interleukin 1 in the dorsal raphe nucleus affects inter-male aggressive behavior

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It has shown that the level of interleukin 1 beta (IL-1β) in the periphery or cerebrospinal fluid correlates with aggression trait in human. We therefore aimed to study biological mechanisms in the relationship between IL-1β and individual difference of aggression using mouse model. Like humans, there are large individual difference of aggression in mice, and some animals consistently show aggressive behavior during the agonistic confrontations (AGG) but some rarely show aggressive behavior (NON). We measured the peripheral and central IL-1β using ELISA, and found that aggressive encounter caused a phasic increase of IL-1β in both the blood and brain, especially in the dorsal raphe nucleus (DRN). We found that NONs showed higher level of IL-1β than AGGs in the DRN. Local injection of IL-1 receptor antagonist in the DRN caused an increase of aggressive behaviors in the males. Knockdown of IL-1 receptor IL-1R1 specifically in the DRN using shRNA expressing AAV also increased inter-male aggressive behavior, suggesting that endogenous IL-1β in the DRN has suppressive effect on aggressive behavior. By using c-Fos immunohistochemistry, we found a positive correlation between activation of 5-HT neurons in the DRN and aggressive behaviors, and this difference of 5-HT neural activation was mediated by IL-1R1. These results suggest that phasic increase of endogenous IL-1β modulates activity of DRN 5-HT neurons and affect aggressive behavior of male mouse. (COI: No)

1P-211

Cerebellar integration of neocortical somatosensory signals

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Somatosensory signals from the facial area are conveyed to the cerebellar granule cells as mossy fibers both via direct trigeminocerebellar pathway and indirect cortico-ponto-cerebellar (CPC) pathway. Climbing fibers from the inferior olive also transmit sensory signals to the Purkinje cells. To reveal how these multiple types of signals are integrated in the cerebellar cortical circuit, we made whole-cell recordings from granule cells and unit recording from Purkinje cells in anesthetized transgenic mice, whose CPC pathway could be blocked by light illumination to the somatosensory cortex. When tactile stimulation was given to the upper lip, excitatory synaptic currents in the granule cells appeared in two distinct timings. The early response (~10 ms latency) was not affected but the late response (~30 ms latency) was suppressed by the light illumination, suggesting that they were the trigeminal and CPC responses, respectively. Essentially the same result was obtained in the field potential recordings in awake mice. Similarly, in Purkinje cells, the early simple spikes were not affected, but the late simple spikes and the complex spikes are suppressed by cortical block. Furthermore, by using knock-in mice in which aldolase C bands were visualized by fluorescent protein, we found that each band in crus II had different proportion of trigeminal and CPC responses. These results demonstrate the manner in which the cerebellar cortex integrates signals from distinct origins. (COI: No)

1P-212

Phox2b-expressing neurons in the rat reticular formation dorsal to the trigeminal motor nucleus

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Transcription factor Phox2b is essential for the development of the autonomic nervous systems. Histological studies demonstrated that the Phox2b-expressing neurons are also distributed in the reticular formation dorsal the trigeminal motor nucleus (RdV), which is considered as jaw-movement related region. However, the nature of Phox2b neurons is unclear. In this study, we examined physiological and morphological properties of Phox2b neurons in Phox2b-EYFP knock-in rats. Almost all of Phox2b-positive RdV neurons were glutamatergic, whereas Phox2b-negative RdV neurons consisted of a few glutamatergic, many GABAergic, and glycinergic neurons. The majority (86%) of Phox2b-positive neurons exhibited low-frequency firing, whereas most of the Phox2b-negative neurons (76%) were characterized as high-frequency firing responding to intracellularly injected currents. The majority of Phox2b-positive (76%) and half of the Phox2b-negative neurons (48%) did not respond to stimulations of the mesencephalic trigeminal nucleus, the trigeminal tract, and the principal sensory trigeminal nucleus. About half of the Phox2b-positive (42%) and Phox2b-negative RdV neurons (50%) send their axons to the trigeminal motor nucleus (MoV). These results suggest that the neurotransmitter-phenotypes and firing properties are quite different between Phox2b-positive and Phox2b-negative RdV neurons, and they might play important roles in feeding-related functions including suckling and possibly mastication. (COI: No)

1P-213

Neural activity underlying mismatch negativity generation in macaque temporal and frontal cortices

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The mismatch negativity (MMN) is a negative deflection of the auditory event-related potential (ERP) elicited by an abrupt change of sound stimulus after repetition of the same sound. Although the MMN is estimated to be generated in the temporal and frontal cortices involving mainly a "deviance detection" component, how it is distributed within the areas remains unclear. To address this issue, we investigated the localization of MMN components using Electrocorticogram (ECoG) electrodes which was implanted in the temporal, lateral prefrontal and orbitofrontal cortices of macaque monkeys. We recorded ECoG from three monkeys during the presentation of auditory stimuli consisting of two different frequency tones, a standard tone and a deviant tone, with two stimulus sequences, the standard oddball condition and the many standard condition. To distinguish among MMN components, we extracted three components, tone difference, adaptation and deviance detection. We confirmed that the deviance detection component in the anterior part of the auditory cortex occurred at the same timing as the MMN in the human. Furthermore, in the orbitofrontal and the anterior part of the lateral prefrontal cortices, there was a "deviance detection" component around 100ms. These results suggest that the "deviance detection" component is processed in the orbitofrontal and the prefrontal cortex around 100ms, which was earlier than that of the front end of the auditory cortex. (COI: No)

1P-214

CRH release regulation by GABAergic projection from arcuate nucleus using chemogenetic model

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Corticotropin-releasing hormone (CRH) plays a key role in the endocrine stress response. We have previously shown that the steady-state release of CRH at the axon terminals of CRH neurons in median eminence (ME) is mediated by excitatory GABAergic input from arcuate nucleus (ARC). In ARC, the agouti-related peptide (AgRP) neuron is GABAergic and related with energy homeostasis and feeding behavior. In the current study, we aimed to investigate physiological roles of AgRP neuronal projection to the axon terminals of CRH neurons in ME. We generated AgRP cre:: DREADD transgenic mice in which AgRP neurons were selectively activated by i.p. administration of Clozapine-N-oxide (CNO). We measured corticosterone level by radioimmunoassay and c-fos expression was immunohistochemically confirmed .Upon CNO i.p. injection, c-fos expression was detected in AgRP neurons in ARC, endorsing selective activation of ARC AgRP neurons by CNO i.p. administration. Serum corticosterone level was also increased by CNO. Increases in corticosterone and c-fos expression in AgRP neurons in ARC by CNO indicate that selective activation of ARC AgRP neurons directly projecting to CRH nerve terminals in ME is successful. (COI: No)

1P-215

Exploring the roles of calbindin-D28K in the medial preoptic nucleus in sexual behavior of male rats

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The medial preoptic nucleus (MPN) is known as a regulatory center of male sexual behavior in rats. The central part of the MPN (MPNc) contains a male-biased sexually dimorphic nucleus that is composed of neurons expressing calbindin-D28K (Calb). Calb is a calcium-binding protein serving as an intracellular buffer and sensor of calcium, although the physiological roles of Calb neurons in the MPNc remain to be uncovered. To explore the roles of Calb neurons in the rat MPNc in male sexual behavior, first we examined the expression of c-Fos, a neuronal activity maker, in the MPNc of male rats after they displayed sexual behavior. The number of c-Fos-immunoreactive cells in the MPNc significantly increased after copulation compared with the control males that had no contact with females. Approximately 25% of total Calb neurons in the MPNc co-expressed c-Fos. Next we examined the effects of Calb knockdown in the MPNc by an adeno-associated virus vector to express shRNA targeting Calb mRNA in sexual behavior of male rats. Male rats bearing Calb knockdown exhibited a performance of male sexual behavior that was comparable to that in the control males. These results suggest that the activity of a subpopulation of Calb neurons in the MPNc increases during copulation. However, it is likely that Calb protein in Calb neurons of the MPNc contributes less to the regulation of sexual behavior in male rats. (COI: No)

1P-216

Withdrawn

1P-218

Withdrawn

1P-219

Ventral hippocampus inactivation facilitates the attenuation of olfactory neophobia in rats

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Animals tend to hesitate to consume a new food due to prevent the ingestion of large amounts of potentially toxic stimuli (food neophobia). In the absence of negative post-ingestive consequences, the consumption increase over exposures (attenuation of neophobia). The neural mechanisms of the neophobia to food-related odor stimuli are not yet clear. Here, we examined whether ventral hippocampus (VH) inactivation influences olfactory neophobia. Wistar rats were exposed to the almond odor solution (0.15% benzaldehyde) for 20 min for four consecutive days (odor session). Before the first exposure, they received a microinjection of a gamma aminobutyric acid type A ($GABA_A$) agonist muscimol (50 ng/0.25 μl) or saline into the bilateral VH. After the end of the odor session, the same procedure was repeated with exposures to the sweet taste solution (0.5% saccharin) instead of the benzaldehyde for the taste session. At the first odor test, the benzaldehyde consumption in both groups were lower than compared with their water baseline and a difference between groups was not significant. At the second odor test the muscimol-injected rats showed higher consumption than controls did, suggesting that the VH inactivation facilitates the attenuation of olfactory neophobia. On the other hand, muscimol injection did not influence the saccharin consumption. These results indicate that the function of VH has an inhibitory role in the learning of the odor-safe association. (COI: Properly Declared)

1P-220

Effect of Castration on Electrophysiological Properties of LMAN Neurons in Adult Male Zebra Finches

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To investigate the effects of androgen on electrophysiological properties of LMAN neurons in adult male zebra finches .Neurons were recorded by whole-cell patch-clamp technique.The mian results were as follows:

The data of electrophysiology showed that, the spiking of ADI neuron (adapting typeI cell) was regular while ADII (adapting typeII cell) had a single spike which was unregular then follwed a long and low depolarization ,when injected 100pA,500ms current. The curve of V-I showed that ADI neuron emerged rectification .ADII neuron were not used for further anaysis as only 7 ADII neuron was detected.

When applied to 300pA,5ms current,LMAN neuron had a single spike whose AHP peak amplitude and AP threshold decreased after castration.It indicated that castration increased the excitability of LMAN neuron.When applied to 100pA.500ms current,the evoked AP latency decreased of LMAN neuron .It indicated that LMAN neuron tended to be evoked easily,meanwhile the normalized spike rate increased after castration. The membrane time constant and input resistance of LMAN neuron decreased after castration,which indicated that the ability of intergration increased and the ion channel opened after castration .

In conclusion,LMAN neuron was more excited after castration .Castration increased the excitability of AFP and LMAN-RA .Low level of androgen increased the plasticity of birdsong. The elecctrophysiological properties of LMAN was regulated by androgen. (COI: Properly Declared)

1P-221

MMP-9 activity is required for the NMDA induced endocytosis of AMPA receptor

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Synaptic plasticity is the cellular basis for memory and learning. The Long-term depression (LTD) is one form of synaptic plasticity, in which the efficiency of synaptic transmission can be reduced for a long term. It has been clarified that the LTD is induced by the endocytosis of AMPA type glutamate receptor (AMPA receptor) on dendrites. However, the precise molecular mechanism which regulates the endocytosis of AMPA receptor remains still unclear. Here we showed that Matrix metalloproteinase-9 (MMP9) is required for the induction of LTD. We knocked down MMP-9 and examined whether the endocytosis of AMPA receptor was affected or not by using immunocytochemical analysis. In the control neurons transfected with scramble RNA, the infusing of NMDA induced the endocytosis of AMPA receptor, whereas, in the siRNA transfected neurons, NMDA induced endocytosis of AMPA receptor was not observed. Moreover, we showed that Tissue inhibitor of metalloproteinase-1 (TIMP1), which inhibit the activity of MMP-9, was internalized after NMDA treatment. On the other hand, the amount of extracellular MMP-9 was not affected by the activation of NMDA receptors. These results suggest that the activation of MMP-9 induced by the internalization of TIMP-1 was required for the AMPA receptor endocytosis. (COI: No)

1P-222

Impairment of Long-term Plasticity in Purkinje Cell with Dominant-negative Thyroid Hormone Receptor

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Thyroid hormone (TH) action is mainly mediated by nuclear TH receptors (TRs), which are the ligand-dependent transcription factor. Mutation of TRB gene, predominantly expressed in cerebellar Purkinje cells (PCs), causes resistance to TH (RTH), which is an inherited syndrome of disrupted TH action. Some patients with RTH display motor impairment throughout the life. However, its specific mechanism and effective treatment have not been well established. To investigate the cellular basis for motor impairment due to the disrupted TR action in the cerebellum, we utilized adult male transgenic mice expressing human dominant-negative TRB1 (Mf-1) specifically in PCs, the sole output neurons in the cerebellum to control motor performance. Motor performance tests revealed the impairment in motor coordination and motor learning in Mf-1 mice. The electrophysiological study at synapses between parallel fiber (PF) and PCs showed that long-term synaptic plasticity was postsynaptically impaired in PCs of Mf-1 mice, whereas presynaptic (PF-origin) short-term plasticity was intact in Mf-1 mice. Thus, the motor impairment in Mf-1 mice could be attributed to PCs with *Trb* mutation through the disrupted cellular basis. The present study may suggest that the impairment of long-term plasticity in PCs would be one of the contributing factors to motor impairment in RTH.

Keywords: Thyroid hormone receptor, Cerebellum, Motor coordination, Motor learning, Long term depression, Long term potentiation (COI: No)

1P-223

Remote memory traces in the mouse hippocampus revealed by *Arc*-based functional labeling

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The hippocampus, including the dentate gyrus (DG), is crucial for spatial and contextual memories in rodents. While the role of the hippocampus on acquisition of memory is well established, its contribution on memory retrieval has not yet reached a consensus especially when the age of memory becomes old. Here, we attempted to evaluate the possible roles of the DG in new memory (i.e., recent memory) and old memory (i.e., remote memory) by using a novel functional cell labeling system in mice, which utilizes the neuronal activity-dependent enhancer of the immediate early gene *Arc*. Mice were trained in a contextual fear-conditioning paradigm. We first investigated the neuronal ensembles that were activated during the recall of contextual fear memory, and found that a small but significant population of DG neurons were reactivated during the recalls of recent and remote contextual fear memories. We then found that both the DG neurons activated during recent and remote memory recalls were sufficient to induce fear responses in an unconditioned context when they were artificially activated by an optogenetic means. These findings suggest that a subpopulation of hippocampal DG neurons may play a sufficient role in memory recall processes even a long time after the acquisition. (COI: No)

1P-224

Plasmalogens enhance spatial memory in mice by increasing the gene expression in hippocampus

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Plasmalogens (Pls) are special lipids present in the brain tissue and known to be reduced among Alzheimer's disease (AD) brains and by ageing. The role of these lipids in the hippocampus in regulating the memory was mostly unknown. The clinical study showed that oral intake of sPls (Pls extracted from the scallop) improved the cognition among mild AD patients. *In vivo* study showed that sPls enhance the spatial memory in mice and a reduction of Pls in the murine hippocampus triggered a loss of spatial memory, suggesting that Pls have memory enhancing effects. In the microarray study, we noticed an upregulation of various synaptic function-related genes in neuronal cells treated by the sPls. In addition, sPls drinking improved memory by enhancing the BDNF-TrkB signaling in the mice hippocampus. The Pls treatments increased the dendritic spines in the cultured neuronal cells, suggesting a direct effect of Pls to the neuronal morphology. Pls-mediated memory was reduced when the hippocampal TrkB was knockdown by shRNA. We also found that Pls-drinking in triple transgenic AD model mice reduced the accumulation of amyloid beta proteins in the brain and improved ageing-induced reduction of spatial memory. Our research outcome may help us to understand the sPls-mediated cognition improvement among the AD patients. (COI: Properly Declared)

1P-225

Reaction time property of visual working memory to adjacent two-lever task in standing rats

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In order to compare visual working memory (VWM) between rodents and primates, we have developed an operant task in rats. Because there has been controversial; the longest delay period of VWM seems to be 30 min or more for rodents in maze tasks, and 1 min or less for primates in operant tasks. As in the title, 2 LED light-lever sets were separated by 13 mm gap at 170 mm above worktop in an operant chamber. The LEDs were synchronically turned-on/off. The delayed alternation interval of lever-release was 1 or 2 sec including reward drink period. Exception of free choice at the first trial, rats needed to release one lever to the other in alternate sequence over circulating task intervals of 1, 1 to 2, 2, and 2 to 1 sec. The method allowed testing VWM in rats for 2 kinds of delay period lengths within a day. The trained rats ($n = 10$) finally exhibited stable performance (for 10 days) as 92.1 ± 0.6 and 80.0 ± 1.5 % correct response for 1 and 2 sec, respectively. Lever release before LED-off was defined as premature response but not incorrect, i.e., any errors resultantly prolonged delay periods. We observed negative correlation between correction % and premature No. for 1 or 2 sec ($p < 0.01$ in each). Then we planned to further analyze 16 reaction times; (1 or 2 sec) x (correct or incorrect) x (sufficient or premature response) x (left or right), in order to investigate effect of innate dominant arm on the performance and/or the reaction time. (COI: No)

1P-226

Gut Dysbiosis Induced Brain Pathological Changes and Cognitive Decline in HFD-Fed Rats

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Purpose: High-fat diet (HFD) consumption caused not only gut dysbiosis but also hippocampal oxidative stress and cognitive decline at different time-points. However, no single study demonstrated a potential causation of brain pathologies or a specific time-point in the development of these pathologies following HFD consumption. The present study hypothesized that gut dysbiosis developed at the early stage of HFD consumption, followed by brain pathology and cognitive impairment.

Methods: Twenty-four male Wistar rats were divided into 2 groups to receive either a normal diet (ND) or HFD for 2, 8, and 12 weeks (n=4/dietary group of each time-point). At the end of each time-point, cognitive function was assessed by the Morris Water Maze test. Then, rats were sacrificed and brains were removed. Gut dysbiosis, hippocampal reactive oxygen species (ROS) production, and hippocampal dendritic spine density were investigated.

Results: HFD-fed rats developed gut dysbiosis as indicated by increased gut Enterobacteriaceae at week 2 whereas the elevation in hippocampal ROS levels, the reduction in hippocampal dendritic spine density, and cognitive decline were observed at week 12 of HFD feeding.

Conclusion: Gut dysbiosis might be a causative factor in the development of brain pathologies and cognitive decline following HFD consumption. (COI: No)

1P-227

PKD1 promotes functional synapse formation coordinated with N-cadherin in hippocampus

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Functional synapse formation is critical for the wiring of neural circuits in the developing brain. The cell adhesion molecule N-cadherin plays important roles in target recognition and synaptogenesis. However, the molecular mechanisms that regulate the localization of N-cadherin and the subsequent effects remain poorly understood. Here, we show that protein kinase D1 (PKD1) directly binds to N-cadherin at amino acid residues 836-871 and phosphorylates it at Ser 869, 871, 872, thereby increasing the surface localization of N-cadherin and promoting functional synapse formation. Accordingly, either disruption the binding between N-cadherin and PKD1 or preventing the phosphorylation of N-cadherin by PKD1 leads to the reduction in synapse number and impairment of long-term potentiation (LTP). Strikingly, *in vivo* disrupting PKD1-N-cadherin interaction leads to a reduction in dendritic spine density but an improvement in spatial learning and memory of adolescent rats. Together, this study demonstrates a novel mechanism of PKD1 regulating the surface localization of N-cadherin and suggests that the PKD1-N-cadherin interaction is critical for synapse function and learning and memory. (COI: No)

1P-228

Dynamics of cell assemblies in hippocampus during memory consolidation and recall

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Cell assemblies are populations of functionally connected neurons that encode memory and can be memory engrams. Recently, experiments utilizing optogenetics revealed reactivation of engram cells in hippocampus is necessary for retrieval of "recent" memory while in neocortex is necessary for retrieval of "remote" memory. However, these experiments used behavioral tasks that can be learned in only one experience, such as contextual fear conditioning. Therefore, the activation and reactivation of engram cells which gradually encode memory over a longer time span is unclear. The objective of this study is to reveal the dynamic change of cell assemblies in gradual processes of memory consolidation and rapid processes of memory recall using a complex behavior task and multineuronal recording. We used a conditional discrimination task with tones where rats were required to discriminate between high and low pitches. Multineuronal activities were recorded from the hippocampus during the learning processes of the task. After an interval following completion of learning, we retrained the rats with the same behavioral task to make them recall the memory of the task. In the processes of learning and recalling, we detected changes in the synchronous firing of neurons which is characteristic of cell assemblies. We discuss the relation between dynamic activities of cell assemblies in the hippocampus and degrees of memory consolidation and recall. (COI: No)

1P-229

Hippocampal-prefrontal plasticity with transcranial direct current stimulation

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Transcranial direct current stimulation (tDCS) has been explored as a new treatment technique for improving cognitive and motor function. However, the neuronal mechanisms of the tDCS in modulating brain function, especially the neuronal mechanisms for modulating cognitive function, are not well understood. In the present study, we examined whether tDCS can promote neuronal plasticity between the hippocampus and the prefrontal cortex by using rats. Electrical stimulation was applied to the CA1 region of the hippocampus and evoked prefrontal activity was investigated before and after the anodal tDCS stimulation to the prefrontal cortex. As a result, evoked prefrontal activity was potentiated comparted to the pre-tDCS condition after the tDCS stimulation. The present results indicate that LTP (long term potentiation)-like plasticity was observed in the hippocampal-prefrontal pathway after tDCS, indicating that the neurotransmission efficiency improved after tDCS stimulation. (COI: No)

1P-230

D-galactose induced aging aggravates hippocampal oxidative stress in obese-insulin resistant rats

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Purpose: Either aging or obesity can lead to the development of neurodegeneration. We aimed to test the hypothesis that D-galactose induced aging aggravates brain pathology and cognitive decline in obese-insulin resistant rats.
Methods: Twenty-four male Wistar rats were divided into 2 groups to receive either normal diet (ND) or high-fat diet (HFD) for 16 weeks. At week 13, both ND and HFD rats were received either vehicle (0.9% NSS) or D-galactose (150mg/kg/d) for 4 weeks. Then, the cognition was tested by Morris Water Maze. Metabolic function was detemined by oral glucose tolerance test. Hippocampus was removed for investigating oxidative stress and synaptic function.
Results: We found that either D-galactose induced aging or obese-insulin resistant rats impaired glucose tolerance test. In addition, both D-galactose induced aging and obese-insulin resistant rats deteriorated cognitive function, increased hippocampal reactive oxygen species (ROS) production, and impaired hippocampal synaptic function. Interestingly, D-galactose induced aging aggravated only hippocampal ROS production in obese-insulin resistant rats.
Conclusion: Both D-galactose-induced aging and obese-insulin resistance induced cognitive decline via impaired hippocampal function, and 4-week D-galactose administration could aggravate only hippocampal oxidative stress in obese-insulin resistant rats. (COI: No)

1P-231

Exercise, not calorie restriction, improves cognitive function in obese rats

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Purpose: Lifestyle modification, including calorie restriction or exercise, had the beneficial effects on the improving of brain function in diabetes and Alzheimer's disease. However, the comparative effects of calorie restriction and exercise on metabolic function and cognition in obese condition have not been thoroughly investigated. We hypothesize that exercise is better than calorie restriction for attenuating brain pathologies and cognitive decline in obese condition.
Methods: Twenty-four female rats were fed with either a normal diet (ND: n=6) or a high-fat diet (HFD: n=18) for 27 weeks. At week 22, HFD-fed rats were divided into three groups (n=6/group) and each group was obtained either sedentary lifestyle, calorie restriction or exercise for 6 weeks. Then, metabolic and brain parameters were determined.
Results: HFD-fed rats developed insulin resistance and cognitive decline via increased brain oxidative stress, apoptosis, inflammation and synaptic dysfunction. Calorie restriction decreased metabolic disturbance, brain inflammation and brain oxidative stress, but failed to improve cognition in HFD-fed rats. Exercise attenuated metabolic disorders, brain oxidative stress, brain apoptosis, brain inflammation, and synaptic dysfunction, resulting to improve cognition in HFD-fed rats.
Conclusion: Exercise had better efficacy than calorie restriction on the restoring brain and cognitive functions in obese condition. (COI: No)

1P-232

Mitochondrial ATP-linked respiration in PBMCs is associated with cognition in Aged-EGAT population

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Purpose: Mitochondria respiration in isolated peripheral blood mononuclear cells (PBMCs) has been found to be associated with symptoms of neurodegeneration. The present study hypothesizes that mitochondrial respiration also has a positive correlation with cognition in the aged population.
Methods: 1199 aged Electricity Generating Authority of Thailand (EGAT) subjects (mean age: 73 ± 4 years old) were enrolled in the present study. Blood samples were obtained for mitochondrial respiration parameters and the metabolic parameters. Montreal cognitive assessment (MoCA) score was used for cognitive assessment.
Results: The participants with MoCA score less than 26 were categorized as cognitive impairments (n=616), and the participants with MoCA score greater than or equal 26 were categorized as normal cognitive function (n=583). Mitochondrial function analysis demonstrated that the ATP-linked respiration was lowered in participants with cognitive impairments (44.07 ± 25.45 vs. 48.15 ± 28.1, $p<0.05$). In addition, MoCA score was associated with age, plasma glucose level, creatinine, systolic blood pressure, and mitochondrial ATP-linked respiration. Interestingly, data from multiple regression analysis indicated that MoCA score was independently correlated with age (B=-0.28, $p<0.01$) and mitochondrial ATP-linked respiration (B=0.06, p=0.0273).
Conclusion: In addition to the age, only mitochondrial ATP-linked respiration was correlated with cognitive function in aged EGAT population. (COI: No)

1P-233

Temporal dynamics of reward cue representation in the rat paraventricular nucleus

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The paraventricular nucleus of the thalamus (PVT) is involved in the control of cue-induced motivated behaviors. However, it is unknown how PVT neurons mediate predictive and incentive properties of conditioned stimuli (CSs). It has been hypothesized that predictive and incentive information is represented by neural activity during initial onset of cues (early activity) and that just before reward delivery (late activity), respectively. **Method:** The rats were trained to lick a tube just after a CS to obtain reward, while PVT neuronal activity was recorded. Each CS predicted reward or non-reward when presented alone, but predicted opposite reward contingency when they were presented together as a configural stimulus. **Results:** The half of CS-responsive PVT neurons responded selectively to the CSs associated with reward (CS+-related). Furthermore, the early activity of these neurons reflected reward/non-reward contingency, while the late activity was related to reward value and motivation rather than reward/non-reward contingency. Population early and late activity of the CS+-related neurons also represented predictive and incentive information of the CSs, respectively. In addition, PVT neuronal activity was correlated to individual licking during reward acquisition. **Conslusion:** The PVT is involved in a series of processes from CS recognition for reward availability to motivation for reward-seeking behavior as well as hedonic reaction during ingestion of palatable solution. (COI: No)

1P-234

Modulation of Synaptic Plasticity in Hippocampal CA1 Region by Basolateral Amygdala

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Purpose: To investigate time- and activity-dependent modulation of synaptic plasticity in the hippocampal CA1 region by the basolateral amygdala (BLA) and its underlying mechanisms.
Method: Using field electrophysiological recordings in acute horizontal mouse brain slices, we studied synaptic plasticity in the CA1 area under different BLA stimulation conditions.
Results: First, we studied the effect of timing dependence of the effect of BLA co-stimulation on synaptic plasticity in hippocampal area CA1. Co-induction of late-LTP (100Hz) in BLA and in CA1 resulted in an elevated LTP while stimulation of BLA prior to or after late-LTP induction within a specific time window suppressed late-LTP. Next, we observed that the enhancement of late-LTP due to BLA co-stimulation (100Hz) ameliorated LTP impairments in the synaptic competition phenomenon in CA1. However, stronger BLA co-stimulation (200Hz) resulted in the impairment of late-LTP in hippocampal area CA1. The modulation of BLA on synaptic plasticity in CA1 required protein synthesis and NMDAR as application of the inhibitors and antagonist, respectively, impaired late-LTP maintenance.
Conclusion: Our finding confirms that amygdala has positive and negative influences on long-term synaptic plasticity in the hippocampus and it is time- and activity-dependent. Unravelling its cellular mechanisms may provide insights into the mechanisms of emotional disorders such as post-traumatic stress disorder (PTSD).

(COI: No)

1P-235

Depotentiation at the hippocampal CA1 synapse depends on the basal synaptic transmission

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In hippocampal CA1 region during the animal's exploratory behaviors, some population of pyramidal cells are synchronously firing at the frequency of theta (4-12 Hz) and each theta cycle includes a burst of 2-4 spikes. When the animals are awake and immobile, certain numbers of pyramidal cells excite synchronously at 1-2 Hz with 2-4 spikes. Other pyramidal cells of asynchronous activities exhibit spontaneous firing at the frequency of less than 0.1 Hz. We examined the role for this very low frequency spontaneous firing in the induction of depotentiation (reversal of LTP). Electrophysiological recordings were obtained from guinea pig hippocampal slices. The monitoring stimuli were applied every 20 seconds to the Schaffer collateral - CA1 pyramidal cell pathway to yield field EPSPs and population spikes. LTP was induced by tetanic stimulation (100 Hz, 100 pulses) and low frequency stimulation (2 Hz, 1000 pulses) was applied 30 minutes after the tetanus to induce depotentiation. The monitoring stimuli were halted for 20 minutes right after the tetanus or the low-frequency stimulation, then the magnitude of depontetiation was analysed.By halting monitoring stimuli, the induction of LTP was intact while the induction of depotentiation was inhibited. This result indicates that not only the inductive stimuli but also monitoring stimuli may contribute to the establishment of depotentiation, probably via the molecular mechanisms such as calcium dependent protein phosphorylation. (COI: No)

1P-236

Population Spike-Timing-Dependent Plasticity and Synaptic Tagging and Capture in hippocampal CA1

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Understanding information processing in neuronal ensembles relies on our knowledge of the rules that shape neuronal connections. In the adult rat hippocampus, it is unclear whether long-term synaptic change can be induced by spike-timing-dependent plasticity (STDP) like mechanisms in a neuronal population. Hence, we investigated whether synaptic plasticity in a population of CA1 neurons is dependent on the temporal order and interval between pre- and postsynaptic activity. Using field electrophysiology in acute rat hippocampal slices, we elicited pre- and postsynaptic activity by extracellular stimulation of Schaffer collaterals and CA1 neuron axons, respectively. Our results showed that low frequency pairing of pre- and postsynaptic activity can induce synaptic potentiation that lasts for 4 h. We also described an asymmetric population STDP (pSTDP) curve, in which the magnitude and maintenance of synaptic change vary with the order and timing of pre- and postsynaptic activity. We showed that pSTDP requires translation, NMDAR and CaMKII signaling, but not BDNF. Significantly, we showed that pSTDP engages in selective forms of long-term associativity: pSTDP strengthened tetanization-induced early-LTP into late-LTP, thus promoting synaptic tagging and capture. In sum, our data show that brief activity, varying by tens of milliseconds, can differentially shape the hippocampal CA3-CA1 circuit for hours and affect how future information is processed in the neural network. (COI: No)

1P-237

p75 neurotrophin receptor regulates hippocampal associative plasticity in aging

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Purpose: Aging is associated with decline in cognitive function, including learning and memory. Interestingly, aging of brain is characterized by higher expression levels of p75 neurotrophin receptor ($p75^{NTR}$). We aimed to elucidate role of $p75^{NTR}$ in synaptic plasticity changes including long-term potentiation (LTP) and synaptic tagging and capture (STC), and associative memory associated with aging.
Methods: $p75^{NTR}$ knockout ($p75^{NTR}$ KO) mice were used in this study. LTP was measured from CA1 regions of hippocampus and LTP was compared between old aged (24 months) $p75^{NTR}$ KO mice and age-matched control mice. The old aged mice were also compared to their respective young aged (5-7 weeks) mice. Associative memory was assessed by both the STC electrophysiology experiment and behavioural tagging experiment.
Results: Hippocampal late-LTP from old aged wild-type mice was significantly smaller compared to the young aged wild-type mice. Both STC and associative memory were impaired in old aged wild-type mice. Surprisingly, hippocampal LTP and STC were intact in old aged $p75^{NTR}$ KO mice which was comparable to the young aged wild-type and $p75^{NTR}$ KO mice. Furthermore, associative memory was not affected in old aged $p75^{NTR}$ KO mice.
Conclusions: $p75^{NTR}$ is one of the main players in maintaining homeostasis for hippocampal-dependant synaptic plasticity and function. $p75^{NTR}$ can be treated as an attractive therapeutic target for limiting the cognitive deficit as a result of aging. (COI: No)

1P-238

Role of dopamine D_3 receptor on hyper-dopamine activity-altered novel object recognition memory

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Over-activation of dopamine is thought to underlie the pathophysiology of a number of psychiatric disorders. These disorders are frequently associated with cognitive deficits or learning and memory, suggesting that persistent changes in dopamine signaling may alter neural plasticity. In order to model overactive DA transmission in novel object recognition (NOR) memory, we used DA transporter knockdown (DAT-KD) mice, which display hyper-dopaminergic phenotypes. DAT-KD mice exhibited impaired NOR memory compared to wild-type (WT) mice. This impairment was prevented by administration of FAUC365, a DA D_3 receptor (D_3R) antagonist, prior to object learning. Similarly, D_3R knockout (KO)/DAT-KD double mutant mice displayed comparable performance in the NOR test as to WT mice. GBR12909, a DAT blocker, also impaired NOR performance in WT mice. Impaired NOR performance in GBR12909-treated WT mice was also prevented by pretreatment with FAUC365. To search underlying signaling, levels of p-GSK3α and p-GSK3β in the mPFC of WT mice were significantly decreased after exposure to the novel objects. Treatment of FAUC365 or D_3 deletion restored the novelty-induced dephosphorylation in the mPFC of DAT-KD mice. Inhibition of GSK3 activity or knockdown of GSK3b disrupted NORT in WT mice. Together, these findings indicate that reduced DAT activity can impair recognition memory in NOR test and appears to link with a disruption of D_3 receptor-GSK3b signaling in the mPFC during memory encoding. (COI: No)

1P-239

Role of olfactory tubercle in the weaning of neonatal mice

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There is a close relationship between eating behavior and olfaction, and the smell of food induces eating behavior. In the brain region related to the sense of smell, "olfactory tubercle (OT)" is thought to contribute to the learning of the motivation behavior of the smell.Weaning of neonates is a typical example of dietary behavior development and learning, which proceeds between 2 and 4 weeks of age in mice. The structural and functional development of the OT in mice also occurs at the same time as weaning. Therefore, we examined whether the development of the OT plays a role in weaning. The OT receives dopaminergic signal via dopamine receptor type 1 (D1) or type 2 (D2) expressing neurons, and D1 expressing neurons (D1 neurons) in the anteromedial domain of the OT are activated during odor-induced food attractive behaviors. We therefore ablated the D1 neurons in the anteromedial domain by using mice expressing Cre recombinase in D1 neurons and viruses expressing toxin in Cre-dependent manner. Cell ablation was conducted at the weaning period (2-4 weeks after birth), and their feeding behavior was examined at 4th week of birth or later. The cell-ablated mice exhibited lower body weight and amount of food intake, impaired food seeking ability which were hidden beneath the bedding, and the tendency of enhancing palatability to nipples over food. The results indicate a crucial role of the D1 neurons in the OT domain in the physiological weaning process. (COI: No)

1P-240

The analysis of neuropsin-dependent and-independent late associativity

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Synaptic plasticity is widely accepted to provide a cellular basis for learning and memory. Synaptic associativity could be involved in activity-dependent synaptic plasticity, because it distinguishes between local mechanisms of synaptic tags and cell-wide mechanisms that are responsible for the synthesis of plasticity-related proteins. An attractive hypothesis for synapse specificity of long-term memory (LTM) is synaptic tagging: synaptic activity generates a tag, which captures the plasticity-related proteins derived outside of synapses. Previously we have been reported that neuropsin, a plasticity-related extracellular protease, was involved in synaptic tag setting. In the present study, we tested the hypothesis that neuropsinwas engaged in behavioral tagging for LTM in vivo. Behaviorally, weak training inhibitory passive avoidance task (IA) or spatial object recognition task (SOR), which induces short-term memory (STM) but not LTM, can be consolidated into LTM by exposing animals to novel but not familiar environment 1 h before training. We found that neuropsindeficient mouse impaired such transformation short-term into long-term memory by exposure to novelty in IA, but not SOR. These results suggest that the presence of neuropsin-dependent and -independent behavioral late associativity in vivo. (COI: No)

1P-241

Differentiation of spatially overlapping routes and reward zones in the monkey hippocampus

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During navigation, overlapping areas and events in space may be represented differently in the hippocampal formation (HF) according to the route. Disambiguating overlapping information is an important element of episodic memory, however it is still unknown how this process happens in the monkey HF. In the present study, we recorded monkey hippocampal neurons during performance of a virtual navigation task consisting of two different routes with partially overlapping trajectories, while acquiring rewards in spatially identical or different locations. Out of 106 recorded neurons, 57 displayed place-related activity (place-related neurons), while 18 of these showed route-dependent activity in the central path, consistent with a hippocampal role in the disambiguation of coinciding spaces. In addition, 56 neurons displayed route-dependent reward-related activity in the overlapping goal areas. Population activity of reward-related neurons could differentiate spatial location and reward delivery in overlapping areas for each route. Likewise, neuronal ensemble activity patterns more distinctly represented overlapping trajectories than non-overlapping ones. The present results provide neurophysiological evidence of spatial disambiguation in the monkey hippocampus, consistent with a hippocampal role in episodic memory and supporting the "neural differentiation" computational model, in which overlapping items are better represented by repeated retrieval with competitive learning. (COI: No)

1P-242

(-)-Festidinol: Potential Effect on Preventing Neurodegeneration in Mice

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Alzheimer'sdisease (AD) is chronic neurodegenerative disease that cause the learning and memory dysfunction. The standard treatment can only slow disease progression with expensive cost and many side effects. Alternative treatments are developed for increase efficiency and safety in treatment. (-)-Festidinol (Fes) is flavonoid from *Dracaena conferta* Ridl, traditional plant in south of Thailand, which is reported about anti-oxidant property. Therefore, this study aimed to investigate effect of Fes on aging mice with memory impairment. Male ICR mice were divided into control and D-galactose/aluminum choline ($AlCl_3$)-induced group. Each group were divided into three sub-group and received vehicle, Donepezil (5mg/kg), and Fes (30mg/kg) consequently for 90 days. Next, memory behavior were test by Morris's water maze test (MWM). Then, the mice were sacrifice and brain were collected. Amyloid-β (Aβ) production were measured by reverse transcription polymerase chain reaction and neurodegeneration was studied by Hematoxylin and Eosin staining. Fes or donepezil treatment significantly improved memory behavior when compared to vehicle. In term of Aβ production, Fes can decreased Aβ production by beta-secretase enzyme reducing. Furthermore, Fes decreased number of cell death in induced-AD mice similar to donepezil. In conclusion, Fes improve memory function in induced-AD mice similar to donepezil. Therefore, further research is necessary to prove the other neuroprotective effects. (COI: No)

1P-243

Effect of agomelatine on neurogenesis in D-galactose-induced brain aging

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Hippocampal neurogenesis is driven by neural stem cells (NSCs), which locates in specific microenvironment composes of microvascular structure. Also, vascular endothelial cells of can secrete various factor and enhance NSCs behaviors such as self-renewal and neuronal differentiation. Finally, new neurons create strong synapsis and short-term memory respectively. Unfortunately, all of these phenomenons decline dramatically with increasing age. This study, we use agomelatine (the novel antidepressant and melatonin agonist) to apply in ageing rat models. Rats were intraperitoneal injected by D-galactose for 70 days to induce ageing. Afterthat, agomelatine were intraperitoneal injected for 30 days. Then, hippocampi were dissected for immunohistochemistry and western blot analysis. The result showed that agomelatine increased microvessels density (vWF (+) cell) and CD34 expression (marker of endothelial precursor cells). Furthermore, it increased ligand Ang1 and Tie2 receptor, that induced VEGF production. Whereas, agomelatine increased expression of nestin (NSCs marker), DCX (marker of immature neurons were differentiated from NSCs) and NeuN (mature neurons marker) caused stronger neuronal connection was represented by synaptophysin and PSD95 (pre and postsynaptic protein). Learning and memory function were improved by Morris water maze test. From our data demonstrated that agomelatine enhanced hippocampal function mediated by angiogenesis and neurogenesis stimulation. (COI: No)

1P-244

Effects of 5,6,7,4'-TMF on neurodegeneration and neurogenesis in dexamethasone-induced mice

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Alzheimer's disease (AD) is neurodegenerative disease which characterized by memory loss and behavioral issue. AD pathology are related disruption of neuronal cell function and increasing of neuronal cell death. Several studies reported the effect of flavonoids from several plants showed the effective to improve memory and stimulation of neuronal cell production in several AD models. 5,6,7,4'-tetramethoxyflavanone (TMF) is a one of flavonoids isolated form *Chromolaela Odoratum Linn*. The effect of TMF in AD model was not investigated. This study aimed to investigate the effect of TMF in dexamethasone (Dex)-induces neurodegeneration in mice. Male ICR mice were divided into 4 groups: Vehicle, Dex 60 mg/kg (i.p.), TMF 40 mg/kg (oral), Dex+TMF. After 30 days, mice were investigated behavior outcomes before sacrificed and brain tissues were collected to perform the experiment. (Permitted number of IACUCs: 30/2559), Dex-treated mice showed learning and memory impairment, increased apoptotic cells by increasing the expression of caspase-3 and TUNEL positive cells when compared with vehicle. Moreover, Dex-treated mice decreased nestin, NeuN expression. TMF treatment improved learning and memory behavior, decreased neurodegeneration, increased nestin and NeuN expression when compared to Dex-treated mice. In conclusion, TMF attenuates memory impairment behavior outcome by attenuating neurodegeneration and stimulating neurogenesis in Dex-induced AD mice. (COI: No)

1P-246

Salicylate-induced changes of tuning function in AI of guinea pigs observed by optical recording

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The influence of salicylate on the tuning function and the spatial representation of sound frequencies in the primary auditory cortex (AI) of the guinea pig were investigated using optical imaging with a voltage-sensitive dye (RH795). Seven guinea pigs were anesthetized with ketamine (80 mg/kg) and xylazine (40 mg/kg). Activity patterns to pure tones (0.5, 1, 2, 4, 8, 16 kHz, 200 ms duration at 55-85 dB SPL) were recorded from the AI on both sides 0-2 and 6-8 hours after the intraperitoneal injection of 300 mg/kg salicylate. The frequency band (FB) in AI was determined from the movement and spread of the active spot in response to a pure tone. At 75-85 dB SPL, all the FBs appeared on the both sides of the AI. At 55 dB SPL, however, the 0.5 and 16-kHz FBs disappeared 2-8 hours after the salicylate injection and the 4 or 8-kHz FBs remained on the both side. These results show that the salicylate injection increased the threshold of the low and high FB and unchanged that of the medium FB in the both AI. Similar results were reported in the rat auditory cortex (Jiang et al., 2017). The over enhancement of the spontaneous activity of AI at the early stage of the salicylate effect (Stolzberg et al. 2011) may prevent the threshold increase of the medium FB and may lead to the ringing generation of this frequency (tinnitus) by salicylate. (COI: No)

1P-247

Laterality effects of the visual information processing on the sensorimotor gating system

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It is widely known that visuospatial attention has the characteristic of laterality. It is unclear whether this laterality of visuospatial attention affects the processing of auditory stimuli. The sensorimotor gating system is a neurological process, which filters out unnecessary stimuli from environmental stimuli in the brain. Prepulse inhibition (PPI) is an operational measure of the sensorimotor gating system, which a weaker prestimulus (prepulse), such as a visual stimulus, inhibits the startle reflex elicited by a subsequent robust startling stimulus (pulse) such as a tone. Therefore, we investigated whether the visual stimulus from the left or right visual space affects the sensorimotor gating system in a low attentional condition and a high attentional condition. In the high attentional condition, we found that the target prepulse presented in the left and bilateral visual fields suppressed the startle reflex more than that presented in the right visual field. By contrast, there was no laterality of PPI in the no-target prepulse condition, and there was no laterality of PPI in the low attentional condition. These results suggest that the laterality of visuospatial attention affects the sensorimotor gating system depending on the attentional condition. Moreover, the process of visual information processing may differ between the left and right brain. (COI: No)

1P-248

Neural substrates of action timing decisions

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Decision making involves the selection of goals or actions, but it also requires determination of the timing of action. Action timing is especially important when there are no immediate stimuli to trigger an action and timing must therefore rely on internal processes. How does the brain decide the timing of such self-initiated actions? We investigated this using a combination of behavior, pharmacology and electrophysiology in rats performing a waiting task where actions to abort waiting were self-initiated during a waiting period. Single unit recordings from two regions necessary for this task, medial prefrontal cortex (mPFC) and secondary motor cortex (M2), revealed an unexpected functional dissociation. mPFC neurons encoded action timing biases, which changed slowly over trials depending on trial histories. In contrast, M2 neurons tracked stochastic trial-to-trial fluctuations in action timing. The results support a two-stage model for action timing decisions: mPFC maintains history-dependent decision bias signals, while downstream circuits, including M2, translate these signals into precise action timing signals incorporating variability. We are currently investigating what form of action timing signals are transmitted from decision areas to downstream motor output circuits to eventually affect final choice. To this end, we are developing a waiting task for head-fixed mice, which allows pathway-specific recording and manipulation of neural activity during decisions. (COI: No)

1P-249

Ongoing motor information embedded in a network dynamics of primate primary somatosensory neurons

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Neurons in primary somatosensory cortex (SI) are thought to mostly represent raw afferent signals from peripheral somatosensory neurons. However, it is also known that activity of SI neurons is modulated during motor execution, suggesting the influence from motor cortices.

In the present study, we recorded the activity of primate SI neurons during tactile self-stimulation task. The monkey needed to push and pull the lever manipulandum while robotic arm moving synchronously to the lever stimulated its opposite hand. To reveal hidden factors influencing the neuronal activity, we used a dimensionality reduction technique (also known as "neuronal state space"). Principal component (PC) analysis revealed that six PCs could explain about 90% of the variance. Of those, the 4th-6th PCs were considerably different between self-stimulation and control passive-stimulation conditions. The 4th PC showed comparable transient to tactile stimulus onset which then gradually started to diverge between conditions. The 5th and 6th PCs showed between-condition difference even before the stimulation onset, suggesting that they carry information about preparation and execution of lever manipulation before the brush stimulation. These results indicate that, although the activity of SI neurons strongly reflects somatosensory input, it also conveys ongoing motor information in a high dimensional network dynamics. (COI: No)

1P-250

Chronic mild stress increases aggressive behavior in mice

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Chronic mild stress (CMS) is known to induce memory deficits, learning impairment, anxiety-like behavior, and sleep impairment. However, the mechanisms underlying the effects of CMS on mood and behavior remain unknown. In the present study, we developed a CMS mouse model by placing mice on a wire net for 3 weeks, and evaluated the behavioral changes. We carried out nine behavioral tests, including tests for anxiety-like behavior, depression-like behavior, learning and memory function, and aggressive behavior. CMS mice showed an impaired motor learning and increased aggressive behavior, although no differences were found in anxiety-like behavior and special memory/learning ability. The contents of serotonin and their metabolite were decreased in brain of CMS mice. In addition, mRNA expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) were also altered in brain of CMS mice. The expression of NGF protein in the hypothalamus and the number of aggressive attacks showed a significant negative correlation. The administration of NGF into the lateral ventricle attenuated increased aggressive behavior observed in CMS mice. These results suggested that NGF in the hypothalamus may be related to increased aggressive behavior caused by CMS in mice. (COI: No)

1P-251

Body ownership and agency altered by a robotic arm controlled by electromyography of elbow muscles

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Understanding how we consciously experience our bodies is a fundamental issue in cognitive neuroscience. We formerly reported that a sense of ownership (SO) and a sense of agency (SA) were extended to a robotic arm using myoelectric control in a rubber hand illusion task (Sato et al., 2018). In the study, we used a robotic arm that moved synchronously with participants' wrists. In the present study, we report that the same phenomena were observed in a robotic arm that moved synchronously with the participants' elbows.

Four able-bodied participants were recruited. The robotic arm, using myoelectric control with one degree of freedom (elbow flexion and extension), consisted of a prosthetic glove and an actuator. The joint positions of the robotic arm were controlled continuously by means of the participant's muscular activity on the elbow flexor and extensor. The participants took part in the in-phase and out-of-phase movement conditions for 10 min each. The participants answered a questionnaire to assess both SO and SA (Kalckert and Ehrsson, 2014), immediately after each experiment.

The means of the subjective ratings of the SO and SA in the in-phase movement condition (0.91±0.57 and 1.67±0.49, mean ± S. E.) were larger than means in the out-of-phase movement condition (-1.25±0.60 and 0.50±0.80), which is consistent with our results in wrist movement.

The preliminary results suggest that our methods are applicable not only in wrist movement but also in elbow movement. (COI: No)

1P-252

Hypoxia effect on daily activity is daily activity dependent wavelike response in mice

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Hypoxia of rest phase (nighttime) has strong impact on human physiology. In mice, rest phase (daytime) hypoxia reduced active phase activity and increased activity ending phase activity. This serial and bidirectional activity response (SBR) was also observed in molecular clock deficient, Cry1 and Cry2 double knockout, mice (CryDKO) expressing activity variation under 12h light 12h dark cycle (LD). SBR disappeared in arrhythmic CryDKO under constant darkness (DD). But, when daytime timing hypoxia was exposed at transition from LD to DD, ~6h interval down and up and down activity response appeared. This wavelike activity response in arrhythmic mice corresponded to SBR in rhythmic mice. Daytime hypoxia reduced forebrain adenosine and increased plasma corticosterone. Morning adenosine blockade by caffeine induced SBR and evening caffeine attenuated SBR by Daytime hypoxia. Glucocorticoid synthesis blockade enhanced reduction of active phase activity by Daytime hypoxia. Regulations of Daytime hypoxia induced SBR provide insights into pathophysiology of hypoxia. (COI: Properly Declared)

1P-253

Recency of pattern repetition degrades monkeys' performance in pattern recognition with visual noise

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The neural mechanisms of visual object recognition and short-term memory are often studied using old-world monkeys. Recently it has been reported that Rhesus monkeys and humans seem to use different strategies (Wittig et al., 2014, 2016). They reported that monkeys rely heavily on recency of stimulus repetition to solve the short-term memory task, whereas humans rely heavily on specific memorization.

Here, we analyzed the behavioral performance from 5 monkeys (2 Rhesus monkeys and 3 Japanese monkeys) to examine whether they also used recency in delayed match-to-sample tasks with visual noise. The monkeys were required to fixate a sequence of visual stimuli, and required to report whether the test (last) stimulus matched the first stimulus in the sequence. We previously reported that the behavioral performance of Rhesus monkeys declined when we added random dot visual noise on the test stimuli (Shidara et al., 2005; Kuboki et al., 2017), whereas that of Japanese monkeys was only slightly affected by the visual noise. Behavioral performance was also affected by the number of stimuli in the trial. All monkeys performed better in shorter trials. In addition, we found that the error rate increased when the matched stimulus in the previous trial was presented as the non-match stimulus (i.e. the distractor) in the current trial. These results suggest a recency effect in a visual recognition task in both species of monkey, an effect unaffected by visual noise on the test stimulus. (COI: Properly Declared)

1P-255

Lower c-Fos expressions in the posterior parietal cortex during rubber tail task in Caps2 KO mice

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We previously found that mice responded as if their own tails were being touched when the rubber tails were grasped after synchronous strokes to their tails and rubber tails (Rubber tail task; Wada et al., 2016), and found prominent activations of c-Fos in the posterior parietal cortex and primary somatosensory cortex during the task (Wada et al., 2017). In this study, we investigated the c-Fos expression during the task in Caps2 KO mice that did not show the rubber tail illusion (Wada et al., 2016).

After synchronously stroking the tails of mice and the rubber tails for more than 20 minutes, we perfused Caps2 KO mice (n= 8) and wild type (n= 6) mice with fixative. Subsequently, sections of the brain were immunostained with anti c-Fos antibody, and immunopositive cell densities at each 100μm square calculated in the whole sections. Then, we compared c-Fos positive cell densities among the groups.

We found that c-Fos positive cell densities were significantly lower in the posterior parietal cortex and primary somatosensory cortex in the Caps2 KO mice, compared to WT mice that received the same synchronous stroking ($p \leq 0.01$).

The regions were generally comparable to the regions that we previously found in the c-Fos imaging studies of the rubber tail illusion using WT mice. We speculate that the lower activation in the posterior parietal cortex in the KO mice reflects impairment of visuo - tactile integration. (COI: No)

1P-256

Interval timing of visual and auditory cues for duration discrimination in monkey prefrontal cortex

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To elucidate temporal information processing of different modes of sensory stimuli, neuronal activity of prefrontal cortex (PFC) was examined during a duration discrimination task with visual and auditory cues in two monkeys. In the task, two cues (the first cue, C1 and the second cue, C2) were successively presented for different duration ranging from 0.2 to 1.8 sec. Each cue was followed by a 1 sec-delay period (the first delay, D1 and the second delay, D2). Subjects were required to choose the longer presented cue after the D2 period. Cues were either visual or auditory and order of relative cue duration was long-short (LS) or short-long (SL). Out of 860 PFC neurons examined, 64, 35, 135 and 192 neurons were C1, D1, C2 and D2 responsive, respectively. The majority of C1 and C2 response neurons were modality-specific, responded to either the visual or auditory cue. However, the response neurons increased during the C2 period compared to the C1 period, suggesting that these neurons did not respond simply to sensory stimuli. Three-quarters of D2 response neurons exhibited differential responses between the LS and SL trials, showing discrimination results. The differential activity was affected by the C2 modality in one-third of D2 response neurons. These findings suggest that PFC integrates duration information on different modes of sensory stimuli and contributes to temporal discrimination processing, partially in parallel neural networks for each sensory cue. (COI: No)

1P-257

Haptic material perception in macaque monkeys, estimated by the material discrimination task

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Materials of surface provides powerful clues to object recognition by perceiving both visual texture information and secondary haptic information such as rough/smooth, hard/soft, hot/cold or dry/wet. To compare the haptic perception between human subjects and the model animals used for physiological studies, we have trained non-human primates with the material discrimination task and examined their material categorization. Two Japanese monkeys (*macaca fuscata*, female, 6.8&5.9 kg) has been trained over 18&12 months. After a target material, they chose one reference materials (metal, wood, carpet, soft-gel, fur) to indicate material categorization. We found that 1) their performance could be improved above 90% after long training procedures, 2) their errors depended on target materials, indicating that they learned properties of materials, 3) they were able to generalize material categorization for new objects. Their categorization was largely consistent with human subjects but showed peculiar differences for some materials. In conclusion, our material discrimination task was sufficient for examining the relationships between haptic properties and material perception in both human and non-human primate subjects. MI has no COI with regarding to the presentation. Following the Declaration of Helsinki (2013), institutional reviews of ethics (#2145) and animal experiments (#0160226A) of TMDU. Supported by TMDU and JSPS grant; KAKENHI ((C)17K07046). (COI: No)

1P-258

Physiological effects of two types of sitting positions on the brain and autonomic nerve activities

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This study aimed to investigate the physiological effects of a sitting position without back support (WBS) and a wheelchair sitting position (WC) in 18 healthy subjects. Subjects were maintained for 10 minutes in a resting supine position, then maintained in the WBS position (using a side-sitting position table "Sittan"; PARAMOUNT BED; Tokyo, Japan) or in the WC position for 15 minutes, and then again lay supine in bed for 10 minutes. The other sitting position was then adopted for 15 minutes. Electroencephalograms (EEG) and autonomic nerve activity were recorded during the experiments. EEG was analyzed at 4 sites (F3, F4, C3, and C4). The frequency of EEG was classified into 4 bands (δ, θ, α, and β bands), and the increase/decrease in the frequency band activity computed as ratio over controls. The protocol was approved by the Ethical Committee of Chiba University Graduate School of Nursing. There was no significant difference between the two groups in the α band (8–13 Hz) activity in the EEG. However, a significant increase in the β band (13–30 Hz) activity was observed in WBS compared to WC at 10 and 15 minutes in F3 and C4. There was no significant difference in the autonomic nervous activity. These results suggest that the brain activity was stimulated by WBS and that the use of WBS during nursing care may be effective in improving the level of consciousness of unconscious patients. This work was supported by JSPS KAKENHI Grant Number JP16K11910. (COI: No)

1P-259

Prefrontal-enriched *SLIT1* expression in primate cortex established during the postnatal development

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To elucidate the molecular basis of the specialization of cortical architectures, we searched for genes differentially expressed among neocortical areas of Old World monkeys by restriction landmark cDNA scanning . We found that mRNA of *SLIT1*, an axon guidance molecule, was enriched in the prefrontal cortex but with developmentally related changes. In situ hybridization analysis revealed that *SLIT1* mRNA was mainly distributed in the middle layers of most cortical areas, robustly in the prefrontal cortex and faintly in primary sensory areas. The lowest expression was in the primary visual area. Analyses of other *SLIT* (*SLIT2* and *SLIT3*) mRNAs showed preferential expression in the prefrontal cortex with a distinct laminar pattern. By contrast, the receptor *Roundabout* (*ROBO1* and *ROBO2*) mRNAs were widely distributed throughout the cortex. Perinatally, *SLIT1* mRNA was abundantly expressed in the cortex with modest area specificity. Down- regulation of expression initially occurred in early sensory areas around postnatal day 60 and followed in the association areas. The prefrontal area--enriched *SLIT1* mRNA expression results from a relatively greater attenuation of this expression in the other areas. These results suggest that its role is altered postnatally and that this is particularly important for prefrontal connectivity in the Old World monkey cortex. (COI: No)

1P-260

Response preference to artificial and environmental natural sounds in higher auditory cortices

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Experiments were performed in accordance with the Guidelines for Animal Experiments, University of Yamanashi, and the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan. The primary auditory cortex (A1) neurons exhibit the similar response time-courses to many kinds of artificial and natural sounds as long as the sound spectrum is located at the cell's frequency response field (FRF). Compared to the A1 neurons, higher auditory cortex neurons have wider range of the FRF, but show weaker responses to artificial sounds. In the previous studies we found neurons in the higher auditory cortex sensitive to direction of the amplitude change during amplitude-modulated noise. However, neural responses in higher auditory cortex to various kinds of sounds have seldom been reported and the sound preference is poorly understood. In this study we recorded unit activities from the secondary auditory cortex (A2) and posterior auditory field (PAF) and investigated peak responses to artificial sounds and natural sounds. Some neurons showed the response preference to both artificial and natural sounds with the similar amplitude change. Some neurons responded to the animal sounds and exhibited weak responses to other sounds. These results suggest that neurons in A2 and PAF might play an important role in processing complicated acoustic information such as direction of sound amplitude change and species-specific sound. (COI: No)

1P-261

Neural properties of macaque SII bimodal neurons and their functional role for self-body awareness

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Functional roles of macaque secondary somatosensory cortex (SII) have not been fully clarified though involvement of SII in the tactile object recognition and the sensorimotor integration for manipulating objects are suggested. Here we report that some of visual responsive neurons in SII showed discriminative neural responses to self-body touch or movement suggesting the presence of novel roles in macaque SII.

We recorded 822 single unit activities in the parietal operculum including SII from a Japanese monkey and found a total of 124 bimodal neurons (15%) that responded to both visual and somatic stimulation. The majority of those bimodal neurons (N=96, 77%) had visual receptive fields (v-RFs) in peri-personal space of the monkey. Among remaining 28 visual neurons, we found three types of neural activities that might be related to self vs others discrimination, that is, 1) neurons becoming active only when the monkey touched self-body (N=7), mostly the mouth or face (N=5) by own hands, 2) neurons showing less activities by self-body touch (N=5) rather than touching other objects including the experimenter's body, and 3) neurons being activated by the observation of self-body movements (n=2). These neurons showing specific responses to self-body touch or movement were found in rather caudal part of SII and its neighboring opercular regions, suggesting that these cortical areas are involved in the establishment of the self-consciousness of the monkey. (COI: Properly Declared)

1P-262

The relation between the NMDA receptor/NO/cGMP pathway and the antidepressant-like effects of GLP-2

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Our previous study showed that glucagon-like peptide-2 (GLP-2) exerted antidepressant-like effects in mice. The aim of the present study was to investigate a relation between N-methyl-$_D$-aspartate (NMDA) receptor-nitric oxide-cyclic guanosine monophosphate (NO-cGMP) synthesis and antidepressant-like effects of GLP-2 in forced-swim test (FST) in mice. Intracerebroventricularly administered GLP-2 (3 μg/mouse) decreased the immobility time in the FST. Pretreatment of mice with L-arginine (750 mg/kg, i.p.), a substrate for nitric oxide synthase, sildenafil (5 mg/kg, i.p.), a phosphodiesterase 5 inhibitor, or D-serine (300 mg/kg, i.p.), a NMDA receptor co-agonist, inhibited the antidepressant-like effects of GLP-2 (3 μg/mouse) in the FST. Meanwhile, L-nitroarginine methyl ester (L-NAME, 10 mg/kg, i.p.), a non-specific NOS inhibitor, 7-nitroindazole (7-NI, 30 mg/kg, i.p.), a neuronal NOS inhibitor, methylene blue (10 mg/kg, i.p.), an inhibitor of both NOS and soluble guanylate cyclase [sGC], ODQ (30 pmol/site, i.c.v.), a sGC inhibitor, or MK-801 (0.05 mg/kg, i.p.), an NMDA receptor antagonist, in combination with a sub-effective dose of GLP-2 (1.5 μg/mouse) decreased the immobility time in the FST. The present study provided evidence of the synergistic antidepressant-like effect of GLP-2 and an inhibition of the NMDA receptor-L-arginine-NO-cGMP pathway in the FST, contributing to the understanding of the mechanisms underlying the antidepressant-like effects of GLP-2. (COI: No)

1P-263

Systematic analysis on the seeding activity of familial mutant forms of α-synuclein

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Parkinson disease (PD) is one of the most common neurodegenerative diseases. PD is pathologically characterized by the deposition of aggregated α-synuclein proteins as Lewy bodies and Lewy neurites. Six missense mutations in the α-synuclein gene (i.e., A30P, E46K, H50Q, G51D, A53E, and A53T) have been identified in familial PD cases, implicating the pathological importance of α-synuclein. However, it remains unclear how pathogenic mutations in α-synuclein alter the propagation. In this study, we examined seeding and propagation activities of α-synuclein mutants in *in vitro* fibrillization assays, primary cultured neurons and wild-type mouse brain. First, the seeded aggregation with mouse α-synuclein monomer was measured by a thioflavin assay. We found that all the mutants, but not G51D, showed a seeding activity. Next, rat cortical primary neurons were treated with the α-synuclein seeds for 7 days, and all mutants were able to induce phosphorylation of endogenous α-synuclein. Finally, we tested the propagation of α-synuclein pathology in wild-type mouse brains unilaterally injected with the α-synuclein seeds into the striatum. One month after injection, we confirmed the induction and spreading of phosphorylated α-synuclein. These results will elucidate the difference between wild-type and mutant α-synuclein in terms of the seeding activity both *in vitro* and *in vivo*, which might explain the pathogenicity of the mutations. (COI: No)

1P-264

Olfactory impairment associated with left hippocampus volumes at earliest stages of schizophrenia

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Impaired olfactory ability has been reported as a first sign of schizophrenia during the earliest stages of illness, including before illness onset. Morphological brain changes in regions important for olfaction, including the amygdala and hippocampus have been observed across the stages of psychosis and schizophrenia. The aim of this study was to examine the relationship between structural volume changes and olfactory ability in three groups of subjects: healthy control subjects (Ctls), patients with first-episode psychosis who developed schizophrenia (FEP-Scz) and chronic schizophrenia (Scz). Olfactory ability and intelligent scale were decreased in FEP-Scz and Scz compared with controls. The results indicate that the decrease of olfactory ability was influenced by volume increases in the left hippocampus and rectus in the FEP-Scz stage. In Scz, reduction of the left and right hippocampal volume was associated with low olfactory ability, showing volume changes progressing in the opposite direction. Our findings suggest that in the early stage of schizophrenia, brain expansion occurs in the left hippocampus and rectus. We speculate that this expansion reflects swelling, potentially caused by an active neurochemical or immunological process, such as inflammation or neurotoxicity, that may lead to the declining olfactory abilities. (COI: No)

1P-265

Atypical Motility Patterns in Gut Preparation of LRRK2 Knockout Mice

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Background: Leucine-rich repeat kinase 2 (LRRK2) is a recently discovered molecule associated with familial and sporadic Parkinson's disease. It regulates many central neuronal functions such as cell proliferation, apoptosis, autophagy, and axonal extension. Our recent findings showed that LRRK2 is expressed in enteric neurons as well as brain neurons. In this study, we analyzed gut motility of LRRK2-knockout (KO) mice using ex vivo gut preparations and spatiotemporal mapping.

Methods: Gut preparations were dissected from LRRK2-KO and wild-type (WT) mice after cervical dislocation. Gut motility was recorded by a video camera following equilibration in organ bath. Data was converted to spatiotemporal maps via software package given by Dr. Bornstein (Melbourne University).

Results: Spatiotemporal maps showed that spontaneous motility patterns in LRRK2-KO mice were more fragmented and partial than that in WT mice. Interval between colonic migrating motor complexes (CMMC) were not constant in KO mice even though the total number of CMMCs was comparable to that of WT mice. NOS inhibition alleviated this aberrant CMMCs in KO mice, accompanied by increasing the number of CMMCs.

Conclusions: Analysis of spatiotemporal maps in gut motility provides a more comprehensive understanding of gastrointestinal motility. We showed that LRRK2 is associated with a periodic gut motility which seem to be caused by disrupted nitrergic neuronal regulation. (COI: Properly Declared)

1P-266

Withdrawn

1P-267

Hyperventilation test with indocyanine green kinetics predicts cerebral hyperperfusion after CAS

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Cerebral hyperperfusion syndrome (CHS) is a serious complication following carotid artery stenting (CAS), but definitive early prediction of CHS has not been established. Here, we evaluated whether indocyanine green kinetics and nearinfrared spectroscopy (ICG-NIRS) with hyperventilation (HV) and the breath-holding (BH) test can predict hyperperfusion phenomenon after CAS. The blood flow index (BFI) ratio during HV and BH was prospectively monitored using

ICG-NIRS in 66 patients scheduled to undergo CAS. Preoperative cerebrovascular reactivity (CVR) and the postoperative asymmetry index (AI) were also assessed with single-photon emission computed tomography before and after CAS and the correlation with the BFI HV/rest ratio, BFI BH/rest ratio was evaluated. Twelve cases (18%) showed hyperperfusion phenomenon, and one (1.5%) showed CHS after CAS. A significant linear correlation was observed between the BFI HV/rest ratio, BFI BH/rest ratio, and preoperative CVR. A significant linear correlation was observed between the BFI HV/rest ratio and postoperative AI ($r¼0.674$, $P<0.0001$). A BFI HV/rest ratio of 0.88 or more was the optimal cut-off point to predict hyperperfusion phenomenon according to receiver operating characteristic curve analyses. HV and BH test under ICG-NIRS is a useful tool for detection of hyperperfusion phenomenon in patients who underwent CAS. (COI: Properly Declared)

1P-268

Electrophysiological study of epilepticus recovering effect and mechanism of JBPOS0101 using MEA

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Status epilepticus (SE) is a single epileptic seizure lasting more than 5 minutes or two or more seizures within a five-minute period without the person returning to normal between them. Several chemicals are used to make SE model to test drugs that overcome SE. Pilocarpine used popularly to generate SE and also temporal lobe epilepsy. Therefore pilocarpine-induced SE model is used a potent antiepileptic drug screening test.

In this study, we investigated whether JBPOS0101 has effects on SE using cultured organotypic hippocampus by treating pilocarpine in multielectrode array (MEA) system. MEA has been widely used in extracellular recording of electrophysiological activity from neuronal networks and has served as a test for screening drugs. In addition, for the further study related with JBPOS0101's mechanism, we selected several targets (mGluR 1, 4, 5, 7) related with seizure and tested the functional role of JBPOS0101 as an agonist or an antagonist to the targets.

JBPOS0101 showed dramatically decreased spikes counts numbers in both low level and high level. Hereby, JBPOS0101 has a high possibility as a drug that overcome drug resistance of SE. Also, we figured out that JBPOS0101 functions as antagonist to mGluR1 but doesn't have any effects on mGluR5, and in case of type III mGluR, it functions as agonist to mGluR4 and antagonist to mGluR7. These results suggested JBPOS0101's specific target mechanism and play an important role as a clue to invent new drugs to overcome SE. (COI: No)

1P-269

ROS generation, Neuronal degeneration and Neurologic dysfunction after Ischemic Stroke in Mice

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In ischemic stroke, neurologic dysfunction is caused by neuronal damage via interruption of the blood supply associated with thrombosis in cerebral vessels. This interruption also causes the increase in blood-brain barrier permeability (BBBP). Recently, it has been reported that the facilitation of vascular permeability at the spinal cord induced reactive oxygen species (ROS) generation via microglial activation, which was resulted in spinal axon injury in murine multiple sclerosis model. Here, we investigated ROS generation, neurodegeneration and subsequent neurologic dysfunction on ischemic stroke, using a photochemical stroke model in mice. At 8 hours and 24 hours after ischemia onset, BBBP increased at the surrounding region of the damage, which was concurrent with fibrinogen leakage and ROS generation. This ROS generation was suppressed by treatment of an antagonist of fibrinogen receptor which is expressed in microglia. In addition, neuronal degeneration was also found at the same region where vascular permeability increased, associating with neuronal degeneration. The neuronal degeneration and neurological dysfunction was improved by treatment of a radical scavenger, Edaravone. These findings indicated that the increase in the BBBP at the surrounding region of damage on ischemic stroke caused ROS generation via activation of microglia, which resulted in the induction of neuronal degeneration and associating neuronal dysfunction in acute phase after ischemic stroke. (COI: No)

1P-270

Effect of orexin on the firing pattern of serotonergic dorsal raphe neurons

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Serotonergic (5-HT) dorsal raphe (DR) neurons regulate numerous brain functions. It has been well established that the wake-promoting neuropeptide orexin (hypocretin) depolarizes and increases the firing rate of 5-HT DR neurons. However, how orexins influence the response of 5-HT DR neurons to their inputs has not been well explored. This is important because 5-HT DR neurons are known to fire at slow tonic rates in relation to behavioral state but also to encode sensory/motor and reward events with phasic firing. To investigate this question, we used whole cell recording methods in mouse brain slices. We found that orexin-A strongly increased the amplitude and duration of the Ca^{2+}-dependent post-spike afterhyperpolarization (AHP). This orexin-enhanced AHP (oeAHP) had a potent impact on the firing properties of these neurons: The oeAHP reduced both the steady-state firing rate and firing fidelity for a given input, without attenuating the initial firing rate or firing fidelity. Additional experiments exploring the extracellular Ca^{2+} ($[Ca^{2+}]_o$) dependence of both orexin-induced excitation and the oeAHP revealed they are both nearly blocked by 10 mM $[Ca^{2+}]_o$ and are near-maximal at more physiological (~1 mM) $[Ca^{2+}]_o$. These results reveal new orexin actions on 5-HT DR neurons. Moreover, these findings are relevant to the pathophysiology of hypercalcemia which would attenuate these orexin actions and might contribute to the associated clinical sign of drowsiness. (COI: No)

1P-271

Would skin resistance be a novel neurophysiological marker for transcranial electrical stimulation?

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Purpose: Transcranial direct current stimulation (tDCS) is an increasingly adopted technology for promoting and maintaining brain function. However, neurophysiological indicators for evaluating the effectiveness of tDCS are very limited. This study sough to identify the predictive value of skin resistance for individuals with neurocognitive disorders.

Methods: A randomized clinical trial was conducted in older adults with mild neurocognitive disorder duo to Alzheimer's disease (NCD-AD). 40 cases with NCD-AD received 12 sessions of tDCS. Cognitive assessments were conducted at baseline (Pre-intervention) and 4th week (Post-intervention). Skin resistance was recorded and measured as the impedance during tDCS treatment. Working memory tests were as used to evaluate the cognitive ability.

Results: The tDCS showed positive cognitive effects in NCD-AD patients. Increased scores of memory were found at 4th week (Paired t-test: $t=-5.9$, $p<0.001$). The mean impedance across 12 sessions was 5.02 ± 1.32 kΩ and was associated with advanced age ($r=0.25$, $p=0.023$). Moreover, the impedance with the score of memory at 4th week (Digit span backward: $\beta=-0.38$, $t=-2.6$, $p=0.014$).

Conclusions: Skin resistance may serve as a neurophysiological indicator to predict the response in tDCS treatment trial. The findings may also provide in-depth understanding of the interaction between human body and brain stimulation techniques, which might be employed to promote the individualized healthcare in future. (COI: Properly Declared)

1P-272

Proposal for the classification sweating disorders based on lesion site

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Sweating disorders are classified; e.g. generalized or localized and hemilateral or segmental. We propose a classification of sweating disorders based on the anatomical lesion site according to the sweating disorders cases in our experience. 1. **Hypothalamic disorders** cause generalized anhidrosis with heat retention and dry skin. The patient may be vulnerable to cold, cause poikilothermia, and may respond to thyroid hormone, TSH/TRH, or *Pueraria* decoction therapies. 2. **Spinal cord disorders** present as ipsilateral anhidrosis inferior to the affected segment, with contralateral compensatory hyperhidrosis, and may include other autonomic symptoms or sensory/motor disorders. 3. **Cervical sympathetic trunk disorders** present as ipsilateral hemifacial anhidrosis superior to the lesion, with contralateral flushing and compensatory hyperhidrosis, and may be accompanied by ipsilateral Horner's syndrome. 4. **Sympathetic ganglionic/Postganglionic sudomotor disorders** present as patchy hypohidrosis. 5. **Sweat gland disorders**; E.g. idiopathic pure sudomotor failure which presents as anhidrosis, sometimes followed by sharp pain or cholinergic urticaria and high IgE level and respond to steroid therapy. 6. **Anhidrosis caused by an anomaly**. These classifications provide accurate strategies to treat sweating disorders, based on clinical manifestations including sweat distribution, laboratory examination, head and spinal cord magnetic resonance imaging, and a qualitative sudomotor axon reflex test. (COI: No)

1P-273

Reduced synaptic inputs in prefrontal cortex by lack of a mental disorder-related epigenetic factor

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Several epigenetic factors have been identified as potential regulators of many mental disorder-candidate genes based on the analysis of clinical cases. However, it remains unclear how abnormalities in epigenetic factors affect brain function. Here we focused on a mental disorder-associated gene that encodes one of the epigenetic factors. We performed RNA interference-mediated knockdown (KD) of the factor in layer 2/3 neurons of the medial prefrontal cortex (mPFC) by *in utero* electroporation at embryonic days 14. We prepared coronal brain slices including the mPFC from mice at postnatal days 16-25, performed whole-cell recordings from layer 2/3 pyramidal cells, and measured excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs). We recorded from both green fluorescent protein (GFP)-positive KD cells and GFP-negative control cells in the same mPFC slices. We found that both EPSCs and IPSCs were significantly reduced in the KD cells compared with control cells. To examine whether KD of the epigenetic factor in the mPFC causes any behavioral abnormality, we produced mice with KD of the factor in bilateral frontal cortices and examined their behaviors. The KD mice exhibited mild behavioral alteration relevant to representative mouse models of mental disorders. Overall, our results suggest that the lack of the epigenetic factor causes impairment of synaptic transmission in layer 2/3 neurons of the mPFC and mental disorder-like behavioral abnormality in mice. (COI: No)

1P-274

Common behavioral characteristics in the mice maternally exposed to different types of dioxins

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Developmental exposure to dioxins has been reported to impair brain functions in epidemiological studies. Empirical approaches using laboratory animals support the notion by revealing that a certain amount of developmental exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a representative chlorinated congener, altered specific social and cognitive phenotypes. Since TCDD binds to the dioxin receptor called aryl hydrocarbon receptor (AhR), these behavioral alterations are thought to be mediated through AhR activation. Although other types of dioxins such as brominated dioxins released into the environment have been reported to possess potential to activate AhR in *in vitro* assays, behavioral evaluations of the brominated dioxins were not fully applied. In this study, we tried to find behavioral impairments in mouse offspring born to dams treated with 2,3,7,8-tetrabromodibenzofuran (TBDF), or TCDD as a positive control. In neonatal period, we found that the maternal exposure to either TCDD or TBDF significantly suppressed ultrasonic vocalization (USV) in offspring. In adult period, by using automated behavior recording system called IntelliCage, we found that the adaptivity to a novel environment was significantly suppressed in the mice maternally exposed to either TCDD or TBDF. These results suggest that USV in neonate and the adaptivity in adult might be universal behavioral makers for evaluating a wide variety of dioxins in the environment. (COI: No)

1P-275

TSPO-targeting compound ameliorates the abnormal behaviors of mice received social defeat stress

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Translocator protein 18kDa (TSPO) is a mitochondrial protein expressed on glial cells in the central nervous system, and is proposed as a useful biomarker of brain injury and inflammation. Chronic social defeat stress (CSDS), an animal model for depression-related behaviors, is known to induce the activation of microglial cells. In this study, we found that CSDS increased the expression of TSPO within microglia, with accompanying increase in cytokines production in brain regions implicated in the pathophysiology of depression.

We hypothesized that ONO-2952, a compound targeting TSPO, would inhibit the microglial activation, and improve the abnormal behaviors in CSDS mice. Interestingly, orally administration of ONO-2952 ameliorated the social avoidance and anxiety-like behaviors with suppression of inflammatory cytokines production. Conversely, ONO-2952 has no effect on their innate behaviors of non-defeated mice. Besides, analysis *in vitro* showed that ONO-2952 inhibited release of cytokines and mitochondrial reactive oxygen species (ROS) in cultured microglia stimulated by lipopolysaccharide. These data suggested that ONO-2952 had an ameliorative effect on the abnormal behaviors in CSDS mice, through the regulation of microglial activities. Since TSPO plays pivotal roles in microglial activities to modulate the neural network, we believe that ONO-2952 can be useful tool to elucidate the pathophysiology underlying psychiatric disorders. (COI: No)

1P-276

Investigation of the effect of seaweed on the metabolic dysfunction-associated neurodegeneration

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[Purpose] Recent clinical and experimental study indicates that many neurodegenerative disorders often display a metabolic dysfunction. Recently, we found that intake of seaweed (*Undaria pinnatifida*, wakame) improve the glucose intolerance in high-fat diet (HFD) fed mice. Therefore, we investigated the effect of wakame on the abnormal protein phosphorylation and neuronal cell death induced by HFD.

[Methods] The mice were fed the normal and high-fat diet with or without wakame. The glucose tolerance of these mice was determined by oral glucose tolerance test. The expression and phosphorylation level of Tau, α-synuclein and cleaved-caspase3 in frontal cortex and olfactory bulb of these mice were analyzed by western blotting. The effect of wakame components on the phosphorylation of Tau and AKT at cellular level was determined using human tau stably-expressing HEK293 cell.

[Results and Discussion] We found that high-fat diet (HFD)-induced glucose intolerance was improved in wakame fed mice. Furthermore, expression of cleaved-caspase-3 in the frontal cortex of mice fed HFD with wakame (HFD+W) was significantly lower than the HFD mice. In contrast, the phosphorylation of AKT was significantly increased in HFD+W group. Furthermore, seaweed components stimulated the AKT phosphorylation and suppressed the phosphorylation of Tau in HEK293 cells. These results suggest that wakame may have the preventive effect on the metabolic dysfunction-associated neuronal cell death. (COI: No)

1P-277

The expression and activation of Smad in the rat hippocampus following global cerebral ischemia

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Smad proteins are known to transduce the action of TGF-β superfamily proteins including TGF-βs, activins, and bone morphogenetic proteins (BMPs). Recently, several *in vitro* studies have reported that the Smad signaling might play an important role for homeostasis in glial cells. In this study, we examined the expression and activation of Smad proteins in glial cells of hippocampus after ischemia using a global cerebral ischemia model of rat. Furthermore, we examined the changes in the expression level of TGF-βs, activins, and BMPs mRNA by RT-PCR to explore a candidate factor that induces Smad activation. This study is a fundamental research to elucidate how Smad signaling modifies the brain inflammation following the global ischemia. Nine-week-old male Sprague-Dawley rats were used. Ischemia for 5 min induced neuronal cell death in the hippocampal CA1 region. The neuronal cell death was obviously observed at 3 days after ischemia and most died within 7 days after ischemia. On the other hand, astrocytes and microglia increase from 3 days after ischemia. The immunoreactivity for Smad1/5/8, Smad2, and Smad3 was detected in the astrocytes or microglia. The phosphorylation of Smad1/5/8 and Smad3 was detected immunohistochemically in the astrocytes or microglia from 3 to 7 days after ischemia. RT-PCR analysis revealed the increase in the expression level of TGF-β1 mRNA. These results suggest that TGF-β1-Smad signaling is activated in the hippocampus after ischemia. (COI: No)

1P-278

Abnormalities in synaptic structure and function in valproate-induced autism model marmosets

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Autism spectrum disorder (ASD) is characterized by impaired social interaction and communication, restricted interests and repetitive behaviors. ASD patients often show abnormalities in the number and structure of dendritic spines in cortical neurons. How these abnormalities appear during the postnatal development, or how these are related to circuit functions has been poorly understood. To clarify these issues, we used the common marmoset, which shows similar cortical development to humans. We have reported an ASD model marmoset with exposure to valproic acid (VPA) in utero (Yasue et al. Behav. Brain Res. 2015). We made acute slices of the prefrontal cortex (areas 8 and 9) of VPA-exposed and unexposed (UE) neonates and infants (3 and 6 months), and performed electrophysiological and structural analyses in layer 3 pyramidal neurons. In UE animals, the density of dendritic spines initially increased toward 3 months and then decreased. In VPA-exposed neonates, the spine density was lower than in UE neonates. In VPA-exposed infants at 6 months, in contrast, the spine density was higher than in UE infants. The size of spines, mEPSC frequency and the ratio of evoked EPSC to IPSC also showed stage-dependent alterations. These results suggest that synaptic structure and function are modulated in a specific time window during the postnatal development. Temporally regulated targeting of molecules underlying these modulations may lead to a novel therapeutic strategy for ASD. (COI: No)

1P-279

Neonatal dexamethasone treatment suppresses hippocampal ERa expression in adolescent female rats

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Purpose: This study was aimed to investigate the long-term adverse effect of neonatal dexamethasone treatment (NDT) on the hippocampal function of female rats.

Methods: Wistar female pups were subjected to tapering dose of DEX (0.5 mg kg-1, 0.3 mg kg-1 and 0.1 mg kg-1, subcutaneously) from postnatal day 1 to 3 and subjected to experiments at the age of 6 weeks (adolescent). Brain slices extracellular recording and inhibitory avoidance (IA) test were used to evaluate the NDT effects on hippocampal function. The expression of ERa was determined using western blotting.

Results: Results showed NDT completely blocked the hippocampal long-term potentiation (LTP) formation and IA learning of adolescent. The expression level of hippocampal estrogen receptor alpha (ERa) was attenuated in NDT subjects. Reduction of histone acetylation of the ERa gene was found which may account for the reduction of hippocampal ERa in NDT female rats. Suprafusion of estradiol (E2) partially restored the hippocampal LTP formation in adolescent NDT female rats. Co-administration of histone deacetylases inhibitor trichostatin-A restored the hippocampal ERa expression, hippocampal LTP formation and IA learning in adolescence NDT female rats.

Conclusions: Collectively, these results suggested NDT has epigenetic modulation effect on the expression of hippocampal ERa which is responsible for its adverse effect on hippocampal function. (COI: No)

1P-280

Rosmarinic acid protects against MPTP-induced toxicity and inhibits iron-induced α-syn aggregation

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Rosmarinic acid (RA) is a naturally occurring polyphenolic compound. In this study, we demonstrated that RA could protect against the degeneration of the nigrostriatal dopaminergic system in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson's disease (PD). In addition, RA could inhibit MPTP-induced decrease of superoxide dismutase (SOD) and tyrosine hydroxylase (TH) and increase innigral iron content. Further studies elucidated the effects of RA on iron-induced neurotoxicity and the possible underlying mechanisms in the SK-N-SH cells. Results showed that iron could induce a decrease in the mitochondrial transmembrane potential (△Ψm) and α-synuclein aggregation in the SK-N-SH cells, which could be restored by RA pretreatment. Further results showed RA pretreatment could inhibit iron induced α-synuclein aggregation by up-regulating hemeoxygenase-1 (HO-1). In addition, iron could increase the mRNA levels of α-synuclein via iron responsive element/iron regulatory protein (IRE/IRP) system. RA pretreatment could decrease the mRNA levels of α-synuclein via decreasing the protein levels of IRP1. These results indicated that RA protected against iron-induced α-synuclein aggregation by up-regulating HO-1 and inhibiting α-synuclein expression by IRE/IRP system. (COI: No)

1P-281

Automated, closed-loop stimulation of the medial septum alleviates temporal lobe epilepsy in rats

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Temporal lobe epilepsy (TLE) is frequently drug-resistant and inoperable due to their distributed seizure generation network. The medial septum (MS) has diffuse anatomical connections to the hippocampi exerting strong gating of the highly-synchronous hippocampal oscillations. Here we study the anti-seizure effects of precisely timed MS stimulations with mechanistic underpinning. Long-Evans rats were kindled by electrical stimulations to generate triggerable grand-mal TLEs. Seizure patterns were automatically detected, and the MS was stimulated either in an open-loop or closed-loop manner and either electrically or optogenetically with channerhodopsin-2 in a cell-type specific manner. Behavior and local field potentials of the unrestrained rats were evaluated. Closed-loop electrical stimulation alleviated motor and electrographic seizures more effectively than that in open-loop manner. Closed-loop activation of the MS glutamatergic neurons alleviated the seizures when excited in synchrony with the hippocampal population bursts (spikes), whereas that of the GABAergic neurons did with a delay (20-60 ms), coinciding with the hyperpolarization blocks (waves). Closed-loop activation of the MS cholinergic neurons did not affect the seizures, but cholinergic preconditioning reduced seizure susceptibility. We conclude that the proxy-stimulation of the distributed seizure generator cortical networks through MS could be a potent therapeutic approach for intractable TLE. (COI: Properly Declared)

1P-282

The effect of anti-arrhythmic drugs on glioma stem cells

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[Background]

Glioma aggressively invade into normal brain tissue, and it is very difficult to resect it completely. The cancer stem cells are very important for the treatment of recurrent glioma. Recent studies reported that ion channels which are transmembrane proteins play an important role in cell proliferation, metastasis, and invasion of cancer.

[Aim]

We aimed to identify ion channels that are expressed in glioma stem cells and find a novel drug in the treatment of glioma.

[Method]

Cancer stem cell lines were established from specimens of glioma patients by neurosphere assay. We measured whole-cell current of glioma stem cells using patch clamp technique. We evaluated the effect of inhibitors on the proliferation of glioma stem cells using WST assay. We detected the localization of ion channel proteins in glioma stem cells using immunostaining.

[Result]

We established five cancer stem cells from specimens of glioma patients. We observed TRP currents in glioma stem cells. We tried to inhibit TRP currents using approximate thirty kinds of inhibitors and confirmed that anti-arrhythmic drugs reduced TRP current. They suppressed the proliferation of glioma stem cells. Using immunostaining, we identified the expression of TRPML1 and TRPML3 proteins in glioma stem cells.

[Conclusion]

The results suggested that the anti-arrhythmic drugs inhibit TRPML channels and suppress the proliferation of glioma stem cells. The anti-arrhythmic drugs may be a novel treatment for gliomas. (COI: No)

1P-283

TRPV4 is critical to brain edema after traumatic brain injury

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Purpose: The transient receptor potential vanilloid type 4 (TRPV4) channel participates in neurogenic inflammation, pain transmission, and edema Our previous study found NKCC1 plays an important role in traumatic brain injury (TBI)-induced brain edema. In this study, we investigated the relationship between NKCC1 and TRPV4 and the related signaling pathways in TBI-induced brain edema and neuronal damage.

Methods: TBI was induced by the calibrated weight-drop device. Adult male Wistar rats were randomly assigned into sham and experimental groups for time-course studies of TRPV4 expression after TBI. Hippocampal TRPV4, NKCC1, MAPK, and PI-3K cascades were analyzed by western blot, and brain edema was also evaluated among the different groups.

Results: Expression of hippocampal TRPV4 peaked at 8 h after TBI, and phosphorylation of the MAPK cascade and Akt was significantly elevated. Administration of either the TRPV4 antagonist, RN1734, or NKCC1 antagonist, bumetanide, significantly attenuated TBI-induced brain edema through decreasing the phosphorylation of MEK, ERK, and Akt proteins. Bumetanide injection inhibited TRPV4 expression, which suggests NKCC1 activation is critical to TRPV4 activation.

Conclusion: Our results showed that hippocampal NKCC1 activation increased TRPV4 expression after TBI and then induced severe brain edema and neuronal damage through activation of the MAPK cascade and Akt-related signaling pathway. (COI: No)

1P-284

Three-dimensional kinematical gait analysis of hindlimbs in rats with focal cerebral infarction

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To understand the neurological role of rehabilitation after cerebral infarction, it is required to determine the precise relationship between dysfunction of motor behavior and local neuronal circuits damaged by ischemia. Although rodent motor cortex is known to be involved in skill learning or fine movement, it remains controversial if this region can impact motor coordination during locomotion. Three-dimensional (3D) kinetic analysis enables us to quantify the motor behavior in spatio-temporal manner and detect its deficit in detail. To this end, we developed rats with focal motor cortex infarction by photochemically induced thrombosis (PIT) method, and employed the KinemaTracer system (KISSEI COMTEC) with some modifications to evaluate the movement of hindlimbs of these rats during locomotion on treadmill at pre- or post 1 day PIT operation. There were no significant differences in gait parameters (gait cycle, stance phase, swing phase, step length and step width) between pre- and post-operated rats, which were also confirmed by foot-printing tests. However, kinematical analysis of the ankle, knee and hip joints on different planes revealed that there were significant differences in certain parameters in PIT operated rats compared to pre-operated ones. These results suggest that more precise evaluation of the motor behavior by 3D motor analysis should unmask the motor deficits in focal motor infarction. We would like to discuss these motor deficits in the meeting. (COI: No)

1P-285

TrkB activation promotes neuronal survival via Akt-ASK1 signaling after intracerebral hemorrhage

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Apoptosis is involved in the pathogenesis of delayed neuronal death following intracerebral hemorrhage (ICH). Activation of TrkB reduces apoptosis and promotes neuronal survival via PI3K/Akt and Erk pathways. The present study investigated the effects and underlying mechanisms of TrkB activation by TrkB agonist 7,8-dihydroxyflavone (7,8-DHF) on ICH. Mice subjected to collagenase-induced ICH received 7,8-DHF or vehicle by ip injection immediately post-injury and subsequently daily for 3 days. The protective effect of 7,8-DHF was also examined in hemin-stimulated cultured neurons.

The results showed that treatment with 40 mg/kg 7,8-DHF reduced functional deficits and brain damage up to post-injury day 28. 7,8-DHF also reduced brain edema, neuronal death, and apoptosis at day 3. Mechanistically, 7,8-DHF enhanced activation of TrkB and Akt on both Ser473 and Thr308, but had no effect on Erk activation. Moreover, mitochondrial Bcl-2/Bax ratio, cytosolic Cyto C and Smac/DIABLO levels did not change after 7,8-DHF treatment. However, 7,8-DHF induced activation of ASK1 and FOXO1. Double immunostaining indicated that TrkB activation was mainly localized in neurons, but rarely in astrocytes or microglia. 7,8-DHF also promoted survival and reduced apoptosis in hemin-stimulated cortical neurons.

Our study indicates that activation of TrkB signaling by 7,8-DHF protects against ICH via Akt but not Erk pathway, and this protective effect may not be mediated via mitochondria-regulated pathway. (COI: No)

1P-286

Neuroprotective effects of COPPIX against dopaminergic neurons degeneration in MPTP-intoxicated mice

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Purpose: Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) with accompanying evidence of increased oxidative damage. Heme oxygenase-1 (HO-1) is crucial to the response to oxidative stress via the catabolism of heme into carbon monoxide, biliverdin and iron. The present study aims to investigate neuroprotective effects of HO-1 activation induced by cobalt protoporphyrin IX (CoPPIX) MPTP intoxicated mice.

Methods: Double immunefluorescence staining, western blots, high performance liquid chromatography and flow cytometry.

Results: MPTP triggered a robust HO-1 activation in the astrocytes of striatum after 1d treatment, and then dropped dramatically. Intraventricular administration of CoppIX for 8 days could preferentially activate HO-1 in astrocytes in striatum rather than SNpc. The loss of dopaminergic neurons was blocked and striatal dopamine content were restored in MPTP models with CoPPIX administration. We then analyzed HO-1 response in primary cultured ventral mesencephalic astrocytes and neurons treated by MPP+. The results showed HO-1 up-regulation in astrocytes appeared much earlier than that in neurons. Although HO-1 activation induced by CoppIX might be double-edged in neurons, it always showed cytoprotective effects in astrocytes.

Conclusion: These results indicated that preferential HO-1 activation in striatal astrocytes might convey neuroprotective effects on dopaminergic neurons in PD. (COI: No)

1P-287

Investigation of the antidepressant agomelatine and ketamine on the synaptic plasticity in mice

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Major depressive disorder is a major public issue which impaired health-related life. The impairment of synaptic plasticity such as long-term potentiation (LTP) in the prefrontal cortex (PFC) resulted from depression have been demonstrated. There are several antidepressants are currently used, the delayed onset of antidepressants action is still the major limitations of antidepressant therapy; however, there were some ketamine studies supported the progress of the antidepressant. The low-dose ketamine, a N-methyl-D-aspartate (NMDA) receptor antagonist, had rapid treatment effect in the depression, and which could improve the LTP impairment. Recent study reported that treatment with agomelatine, a melatonin receptors agonist and a 5-hydroxytryptamine (5-HT) 2C antagonist, for 1 week also has rapid antidepressant effect. Until now, the effect of agomelatine whether improve the LTP is still unclear. In present study, we used the chronic social defeat stress (CSDS) as a depression animal model to compare the effect after agomelatine and ketamine treatment. The results showed that the depressive-like behaviors were reversed by ketamine treatment. Moreover, the depressive-like behaviors also improved by 1-week agomelatine treatment. Finally, the impairment of LTP was reversed by agomelatine and ketamine treatment in PFC. Taken together, the data demonstrated that the agomelatine had rapid antidepressant effect and improved the LTP impairment which resulted from the depression. (COI: No)

1P-288

Prenatal stress on *Gad1*-heterozygotes perturbs development of GABAergic networks affecting behavior

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Exposure to prenatal stress (PS) and mutations in *GAD1*, which encodes the GABA synthesizing enzyme glutamate decarboxylase (GAD) 67, are both risk factors for psychiatric disorders. Using GAD67-GFP knock-in heterozygous mice (HT) subjected to PS from embryonic day 15.0 to 17.5, we previously reported disruption of GABAergic neurogenesis in the MGE and loss of PV neurons in the mPFC of HT-PS mice. In this study, we showed multiple differentially expressed genes (DEGs) were functionally associated with neurogenesis, behavior, cell proliferation and differentiation. Some of DEGs related to neurogenesis and/or neural migration were differentially methylated. Behavioral evaluation showed lack of social novelty recognition and impairments in prepulse inhibition of startle response in HT-PS mice. We further determined the functional implications on inhibitory synaptic inputs and post synaptic changes. We found mIPSCs frequency was decreased without significant changes in amplitude and decay time constant in layer V pyramidal neuron of mPFC in HT-PS mice. The amplitude, frequency and decay time constant of sIPSC as well as $GABA_A$R-mediated tonic current were significantly altered in HT-PS mice. Finally, EEG recording showed reduction in power spectrum density at gamma-frequency range in mPFC of HT-PS mice, indicating GABAergic dysfunction underlying the behavioral deficit. These findings may provide new insights into mechanisms of the pathogenesis of psychiatric disorders. (COI: No)

1P-289

Suppression of FoxO1 by leptin enhances tyrosine hydroxylase and leads to anxiolytic behavior

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Leptin has been linked to psycho-physiological functions. However, the molecular network in leptin-induced mood regulation is poorly understood. Here, we show that administration of leptin induces anxiolytic-like phenotype through the activation of signal transducer and activator of transcription 3 (STAT3) and the inhibition of forkhead box protein O1 (FoxO1) in dopaminergic (DA) neurons of the midbrain. Specifically, STAT3 and FoxO1 directly bind to and exert opposing effects on tyrosine hydroxylase (TH) expression, where STAT3 acts as an enhancer and FoxO1 acts as a prominent repressor. Accordingly, suppression of the prominent suppressor FoxO1 by leptin strongly increased TH expression. Furthermore, specific deletion of FoxO1 in DA neurons (FoxO1 KO^{DAT}) led to a profound elevation of TH activity and dopamine contents. Finally, FoxO1 KO^{DAT} mice exhibited enhanced leptin sensitivity as well as displayed reduced anxiety- and depression-like behaviors. Altogether, this work establishes a novel molecular mechanism of mood regulation by leptin and suggests TH activation through FoxO1 suppression might be a key for leptin-mediated behavioral manifestation in DA neurons. (COI: Properly Declared)

1P-290

Nociceptor-mediated outcomes under hydroxyphenyl octanediamide exposure via TRPV4 modulation

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Biological effects of hydroxyphenyl octanediamide have been explored in the context of tumor suppression via its inhibitory effect on histone deacetylases, but other potential outcomes from its use have also been proposed in different fields such as pain control. Although the underlying mechanisms are still unclear, its off-target actions that are unrelated to epigenetic modulation have been raised. We hypothesized and examined whether the compound may alter pain states by a mechanism not limited to the epigenetic mechanisms. Its localized exposure acutely relieved inflammatory and chemical-induced pain in a modality-specific manner in in-vivo models. It is likely that its inhibitory effect on the activity of sensory neuronal transient receptor potential vanilloid subtype 4 (TRPV4), despite being unstable, can explain the non-epigenetic anti-nociceptive mechanism. Thus, this study provides evidence for a novel off-target action of hydroxyphenyl octanediamide in modality-specific anti-nociceptions and suggests a TRPV4-related mechanism, and raises the utility of this compound for pharmacological modulation of pain. (COI: No)

1P-291

Withdrawn

1P-292

Increase of histone acetylation in the RVM in the rat with stress-induced hyperalgesia

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(Purpose) The RVM and LC are core elements of the descending pain modulatory system. In the present study we examined the acetylation of histone H3 in the RVM and LC after repeated restraint stress for 3 weeks to clarify changes in the descending pain modulatory system in the rat with stress-induced hyperalgesia.(Methods) Rats were divided into the 2 groups: 1) The control group did not receive any stress procedure. 2) The repeated restraint group received restraint stress (daily 6h for 3weeks). Von Frey tests were conducted in the rats. In immunohistochemical analysis, acetylated histone H3-IR cells in the RVM and LC were counted. We also performed double immunohistochemistry of acetylated histone H3 with 5-HT or GABA. In western blot analysis, GAD67 levels in the RVM were examined.(Results) The repeated restraint stress induced mechanical hypersensitivity in the hindpaw and an increase in acetylation of histone H3 in the RVM but not the LC. Furthermore, the repeated restraint stress increased acetylation of histone H3 in the RVM GABAergic neurons but not the RVM serotonergic neurons. The GAD67 protein level in the RVM was significantly higher in repeated restraint group than that in the control group.(Conclusions) These findings suggest the possibility that the stress-induced neuroplasticity in the RVM GABAergic neurons is involved in the mechanical hypersensitivity due to the dysfunction of the descending pain modulatory system. (COI: No)

1P-293

Psychological stress modulates On- and Off-cell activity in the rostral ventromedial medulla

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Neural change in the rostral ventromedial medulla (RVM) is well documented to increase orofacial nociception under psychological stress conditions. Functionally, RVM neurons are classified into 3 types such as On-, Off- and Neutral-cell, which regulate nociception in the trigeminal caudalis; however it remains unclear if neural properties for these neurons are altered under psychological stress conditions.

Male SD rats were subjected to repeated forced swim stress (FST). After 3 day FST, 3 types of units were characterized by the responsiveness to facial pinch stimulation. ON- cells and Off-cells were defined by increases in ongoing activity, and exhibited a pause in ongoing activity, respectively. Neurons whose ongoing activity did not change during facial stimulation were defined as Neutral-cells. Neural activities evoked by noxious heat stimulation to the facial skin for these neurons are quantified in FST and sham rats.

Heat stimulation caused typical response patterns in ON- and OFF-cells. FST increased neural activities of ON-cells evoked by heat stimulation, which was due to increases in prolonged after-discharges. FST had modulatory effects on OFF-cell activity indicated by prolonged the duration of a pause in spontaneous activity. FST had no effects on neural activity in Neutral-cells.

The basis for dysfunction of descending pain controls under psychophysical stress conditions could be explained by neural changes in On- and Off-cell activity in the RVM. (COI: No)

1P-294

Descending orexinergic inhibition contributes to the linalool odor-induced analgesia in mice

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Previously we showed that odor exposure of linalool induced significant analgesic effects in mice, and hypothalamic orexin-containing neurons played a pivotal role for the analgesia. In this study we further examined the contribution of descending orexinergic pain inhibitory pathway to the analgesic effects. Linalool was vaporized in an odor chamber at room temperature and the odorized air was ventilated into an observation chamber at constant rate (1 L/ minute). A mouse was exposed to odorized air for 5 minutes in the observation chamber and then was performed a tail pincher test. The contribution of orexinergic transmission in spinal cord to the linalool odor-induced analgesia was assessed by intrathecal injection of selective orexin receptor antagonists (SB334867 for orexin-1 receptor, and TCS OX2 29 for orexin-2 receptor) 10 minutes before the tail pincher test. The intrathecal injection of SB334867 completely suppressed the linalool odor-induced analgesia, whereas compound 29 did not show any significant effects, indicating the contribution of orexin-1 receptors for the analgesia in the spinal cord. Because the orexinergic neurons were localized in the hypothalamus, our results indicated that the descending orexinergic pain inhibitory pathway via orexin-1 receptor in spinal cord was activated by linalool odor stimulation.there is no state of conflict of interest requiring disclosure. (COI: No)

1P-295

Modulation of nociception via Endothelin-1 signaling in early-stage tongue cancer in rats

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[Purpose] Patients with early-stage tongue cancer do not frequently complain of tongue pain. Endothelin-1 signaling is up-regulated in the cancerous tongue at the early stage. We tested the hypothesis that endothelin-1 signaling contributes to the modulation of tongue nociception.

[Methods] Squamous cell carcinoma cells were inoculated into the tongue under general anesthesia. Lingual mechanical sensitivity under light anesthesia using forceps and the amounts of endothelin-1 and β-endorphin in the tongue were examined after the inoculation. The effect of endothelin-A or μ-opioid receptor antagonism on the mechanical sensitivity was examined.

[Results] Lingual mechanical sensitivity did not change at the early stage but increased at the late stage. The amount of endothelin-1 increased, and endothelin-A receptor antagonism in the tongue induced mechanical hypersensitivity at the early stage. The μ-opioid receptor antagonism enhanced mechanical hypersensitivity, and the amount of β-endorphin increased at the early stage. Moreover, endothelin-A receptor antagonism in the tongue depressed the increase of β-endorphin amount in the tongue at the early stage.

[Conclusions] These results indicate that β-Endorphin released from the cancer cells via endothelin-1 signaling is involved in the analgesic action in mechanical hypersensitivity at the early stage. (COI: No)

1P-296

TRPV1 Expression in the TG and Spinal Trigeminal Nucleus Following Dental Pulp Inflammation

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Current evidence suggests that the pulpal inflammation produces significant changes in peripheral and central nervous system, which induces hyperalgesia. However, the activation of TRPV1 and c-Fos following pulpal noxious stimulation has not been investigated yet. The aim of the present study was to verify whether pulp inflammation activates the expression of TRPV1 and c-Fos. Acute pulpitis was assigned to SD rats through pulp exposure and application of CFA or saline. Three days post application, grooming behaviors were analyzed then rats were sacrificed in order to conduct histological analysis on teeth, trigeminal ganglion and spinal trigeminal nucleus levels. At the results, we observed significantly increased nociceptive behaviors in CFA-treated animals with histological evidence of a severe pulp inflammation. In trigeminal ganglions belonging to CFA group, neuronal activity was significantly higher as compared to saline group and higher immunoreactivity with TRPV1. In the spinal trigeminal nucleus, the lack of c-Fos immunoreactivity indicated the absence of neuronal activity in the intermediate region in all animals with a comparable expression of TRPV1 between all groups in early stage. These findings indicated that acute pulp inflammation triggered peripherally a robust neuronal activation in trigeminal ganglion with a significant involvement of TRPV1 in nociceptive signal processing. (COI: No)

1P-297

TRPV1 inhibition by α_2 adrenergic receptors on peripheral sensory neurons causes analgesia

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Adrenergic nervous systems are known to modulate the pain. Noradrenaline (NA) is known to inhibit pain transmission through α_2 adrenergic receptors within the descending antinociceptive system. TRPV1 channels are predominantly expressed in nociceptive sensory neurons and activated by various noxious stimuli. Although TRPV1 activation is modulated by various neurotransmitters or hormones, the functional association between NA and TRPV1 in peripheral nervous systems has not been well studied. In the present study, we examined effects of NA on capsaicin-evoked TRPV1 currents in rat cultured dorsal root ganglion neurons and on capsaicin-induces pain behaviors of rats. NA significantly reduced amplitudes of capsaicin currents recorded at -60 mV by the whole-cell recording. The α_2 agonists, clonidine and dexmedetomidine also decreased capsaicin currents, and yohimbine (an α_2 antagonist) reversed the effects of NA and clonidine. Pain responses to the capsaicin injection into the plantar of the hind paw was reduced by injection of clonidine at the same site and yohimbine reversed the inhibition by clonidine. The prior injection of clonidine into the contralateral hind paw or combination of clonidine and yohimbine did not inhibit pain responses to capsaicin. These results suggest that activation of α_2 adrenergic receptors inhibits the TRPV1 activity in primary sensory neurons and this inhibitory action may cause peripheral analgesia. (COI: No)

1P-299

Effects of QX314 / Flagellin (Q/F) on the conduction of the peripheral nerve in rats

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The purpose of this study clarify the effects of Q+F on the conduction of the sciatic nerve in rats. QX-314 is a derivative of lidocaine and block the conduction of C-fibers by binding Na^+ channel from the inside after entrance to the inside of nerve fiber through the TRPV1 channel following the capsaicin application. Flagelline is a ligand of the Toll-like receptor 5. Recently, it has been reported that Q+F solution selectively blocked Ab fibers in mice (Nature 2015). But detail effects on the peripheral nerve fibers in rats are unknown. In this study, compound action potentials were recorded from the dorsal roots of lumber 4 - 6 by the electrical stimulation of sciatic nerve at the level of the distal of the mid-thigh under gas anesthesia (2 % isoflurane with 2 l/min O_2). We investigated effects of Q+F solution on conductions of Group I to IV after application of Q+F solution to sciatic nerve at the level of hips. Q+F solution most effectively blocked Group II fibers in dose dependent manner. But the solution also finally blocked other types of fiber and response did not recover over 4 hours after washout of the solution. These results suggest that Q+F solution may be required any ingenuities for the application. (COI: No)

1P-300

Investigation of the antipruritic mechanisms of nalfurafine in the murine spinal cord

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Itch is an unpleasant sensation that evokes scratching behavior which often damages the skin. Nalfurafine (Nal.) is a kappa opioid receptor (KOR) agonist known as an effective drug to control intractable itch. The spinal cord is a target of Nal., however little is known about specific sites important to the antipruritic effects of Nal.. Our purpose was to identify the action points of Nal. in the spinal neural pathway of itch.

To investigate the antipruritic action of Nal. in murine spinal dorsal horn, we analyzed *in vivo* electrophysiology, behavioral experiments, and high-sensitive *in situ* hybridization (ISH) using normal C57BL/6J mice.

Results of behavioral experiments showed intrathecal (i.t.) injection of Nal. reduced but not eliminated gastrin-releasing peptide (GRP)-evoked scratching behavior. *In vivo* electrophysiological recordings revealed Nal. administration suppressed chloroquine-responsive dorsal horn neurons in 15.8% (3/19) of mice. Only one of three nalfurafine-suppressed mice responded to GRP. ISH in three sections of the spinal cord showed that 24.6% (154/777) were double positive for GRP and KOR, and 13.6% (68/567) for GRP receptor (GRPR) and KOR in total KOR^+ cells. Most KOR^+ cells were negative for GRP and GRPR.

In conclusion, Nal. targets both GRP^+ KOR^+ and $GRPR^+$ KOR^+ cells which are present in a 2:1 ratio, in the spinal dorsal horn. These findings suggest that GRP^+ KOR^- or $GRPR^+$ KOR^- cells may function as interneurons in the spinal neural pathway of itch. (COI: No)

1P-301

Enhanced basal pain sensitivities observed in mice lacking interleukin-27

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[Purpose] Interleukin (IL)-27 has an anti-inflammatory effect through reduction of IL-17 and induction of IL-10. Recent studies have shown that IL-17 promotes pain behavior, but IL-10 suppresses it. Thus, we hypothesized that IL-27 would play a role in pain sensation and tested the hypothesis by using mouse strains lacking IL-27 subunits (p28 and EBI3: Epstein-Barr virus–induced gene 3) or its specific receptor (WSX-1). [Methods] Adult male mice were used to evaluate pain behavior. Mice were received an intraperitoneal injection of recombinant IL-27 (rIL-27). Chronic pain models were also applied to these mice. Skin-nerve preparations were also used to evaluate activities of nociceptors. [Results] All knockout (KO) strains spontaneously showed chronic pain-like hypersensitivity. An injection of rIL-27 rapidly reversed this phenotype in mice lacking p28 or EBI3 but not WSX-1. This phenotype was also observed in C-fiber nociceptors recorded by using the skin-nerve preparation. These KO mice showed additional hypersensitivity when subjected to chronic pain models. [Conclusions] The rapid effect of rIL-27 suggest that the phenotype is not the result from developmental abnormality and not mediated by T cell differentiation. Our results suggest that the mechanisms underlying hypersensitivities in these KO mice are different from those underlying chronic pain models. This novel pain control mechanism might indicate a new mechanism for the chronic pain hypersensitivity. (COI: No)

1P-302

Withdrawn

1P-303

Astrocytes are a novel target for treatment of the chronic pain

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Background and objectives: Clinical management of chronic pain is insufficient so far, patients afflicted with unpleasant pain after the pathological conditions were removed. Recently, astrocytes play an important role in rewiring the neural circuits even in somatosensory cortex. Therefore, we carried out to find a new approach to modulate astrocyte activation for remodeling noxious circuits to innocuous circuits in chronic pain.
Methods: Mechanical sensitivity or response to pressure stimuli was measured using classical von Frey filaments in chronic pain model mice performed partial sciatic ligation (PSL) on day0. Astrocytes were modulated by DREADD (hM3Dq), which is activated by the pharmacological ligand clozapine-N-oxide (CNO). CNO (1 mg/kg, CNO group) or saline (control) were intraperitoneally administrated from day14 to day21.
Result: After hind withdrawal thresholds were decreased on day15, CNO group maintained lower hind withdrawal thresholds until day28 but not control group (1.580±0.4562, 0.3563±0.1782 g for CNO group and control, respectively, on day28).
Conclusion: Reactive astrocytes have the potential to remove chronic pain as a result of modulation in somatosensory cortex. (COI: No)

1P-304

IFN-γ signaling in trigeminal spinal subnucleus caudalis is involved in orofacial neuropathic pain

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Purpose:It is well known that trigeminal nerve injury sometimes causes orofacial persistent pain. To develop the appropriate treatment for these patients, it's important to know the mechanism underlying this pathological pain. However, the exact mechanism is still unknown. Here, we examined the involvement of Interferon gamma (IFN-γ) signaling in trigeminal spinal subnucleus caudalis (Vc) in orofacial pain associated with trigeminal nerve injury.
Methods:Male Sprague Dawley rats were used in this study. infraorbital nerve injury (IONI) was established by partial ION ligation. The head-withdrawal reflex threshold (HWT) to mechanical stimulation of the whisker pad skin was measured before and on day 3 after IONI or sham treatment. The HWTs were also measured on day 3 following the continuous intra cisterna magna (i.c.m) administration of IFN-γ antagonist in IONI rats or IFN-γ in naive rats. Moreover, localization of IFN-γ receptor and quantification of IFN-γ in Vc was evaluated on day 3 after IONI or sham treatment.
Results: The HWT was decreased on day 3 after IONI. IFN-γ antagonism in Vc recovered from the decrement of the HWT after IONI rats, and i.c.m administration of IFN-γ decreased the HWT in naive rats. In Vc, IFN-γ receptor was expressed in microglia and its expression level was increased on day 3 after IONI. Conclusions:The present findings suggest that expression of IFN-γ signal in activated microglia is a key mechanism underlying orofacial neuropathic pain. (COI: No)

1P-305

Analgesic effects of calcitonin on radicular pain in rats

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Purpose: Radicular pain is a frequently observed symptom of lumbar disk herniation or lumbar spinal canal stenosis. Calcitonin (elcatonin, eCT) has been mainly used for hypercalcemia and pain associated with osteoporosis. The purpose of this study was to investigate analgesic effects of repeated eCT administration on radicular pain in rats and changes in mRNA expression levels of voltage-dependent sodium channels in the dorsal root ganglion (DRG).
Materials and methods: Seventy male Sprague–Dawley rats were used. Under a microscope, the extradural and proximal parts of DRG were tightly ligated with 8-0 nylon suture to cause radicular pain in rats. Mechanical hyperalgesia, thermal hyperalgesia, and analgesic effects of eCT were compared between rats with radicular pain who received eCT, those who received the vehicle, and sham rats who received the vehicle. Real-time RT-PCR was performed to measure mRNA expression levels of tetrodotoxin (TTX)-sensitive (Nav1.3 and Nav1.6) and TTX-resistant (Nav1.8 and Nav1.9) sodium channels in DRGs.
Results: Mechanical and thermal hyperalgesic reactions occurring in rats with radicular pain significantly improved on days 5 and 9 of eCT administration, respectively. In rats with radicular pain, mRNA expression levels of Nav1.3, Nav1.8, and Nav1.9 increased. After repeated eCT administration, mRNA expression levels of these sodium channels in rats with radicular pain improved to the same levels as in sham rats. (COI: No)

1P-306

Effect of intraarticular hyaluronic acid in a rat monoiodoacetate-induced ankle osteoarthritis model

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PURPOSE: Hyaluronic acid (HA) is reported to alleviate osteoarthritis (OA) pain clinically, but the mechanism of pain relief is still unknown. We examined effects of HA using a rat monoiodoacetate (MIA)-induced ankle OA model. **METHODS:** 45 male SD rats were assigned to three groups: 1) MIA rats injected with MIA in the right ankle joint; 2) Sham rats injected with saline; and 3) MIA-HA rats injected with HA on the 7th, 14th and 21st day after MIA injection. Ankle swelling, range of motion (ROM), stride length and pain-related behaviors (mechanical allodynia and thermal hyperalgesia) were evaluated. Pathological changes in the ankle joint and bilateral L5 DRGs were assessed on the 28th day after the injection. Ankle sections were stained with safranin-O fast green. DRGs were stained with antibodies to tyrosine hydroxylase to estimate the sprouting of sympathetic nerves. **RESULTS:** Ankle swelling, restriction of ROM and pain-related behaviors were seen in MIA rats. No improvement of swelling and ROM were observed in MIA-HA rats compared MIA rats. On the other hand, pain-related behaviors and stride length were improved and cartilage degeneration was significantly suppressed in MIA-HA rats. Sympathetic nerve sprouting was observed in the ipsilateral L5 DRG of MIA rats. That was not seen in the DRG of MIA-HA and sham rats. **Discussions:** This study indicates HA has effects on pain relief of ankle OA. Peripheral sensitization may be improved after HA treatment. (COI: No)

1P-307

Chronic pain model alters GABAergic synaptic transmission in the mice anterior cingulate cortex

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Chronic pain is a persistent unpleasant sensation that produces pathological synaptic plasticity in the central nervous system. Both human imaging study and animal studies consistently demonstrate that the anterior cingulate cortex (ACC) is a critical cortical area for nociceptive and chronic pain processing. Thus far, the mechanisms of excitatory synaptic transmission and plasticity have been well characterized in the ACC for various models of chronic pain. By contrast, the potential contribution of inhibitory synaptic transmission in the ACC, in models of chronic pain, is not fully understood.
Here, we used a mouse model of chronic inflammation induced by Complete Freund Adjuvant (CFA). Then, we performed *in vitro* whole-cell patch-clamp recordings from layer II/III pyramidal neurons in 2 to 3 days after the CFA injection, and examined if the model could cause plastic changes, including transient and tonic type A g-aminobutyric acid ($GABA_A$) receptor-mediated inhibitory synaptic transmission, in the ACC. We analyzed miniature/spontaneous inhibitory postsynaptic currents (IPSCs), $GABA_A$ receptor-mediated tonic currents and evoked IPSCs. Furthermore, we studied if GABAergic transmission-related proteins such as vesicular GABA transporter and $GABA_A$ receptors subunits in the presynapse and postsynapse of the ACC were altered. (COI: Properly Declared)

1P-308

NGF induces constitutive activity of TRPV1 triggering spontaneous firing in sensory neurons

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Nerve growth factor (NGF) is considered to be one of the pathogenic mediators of neuropathic pain. We reported that rat dorsal root ganglion (DRG) neurons cultured in the presence of NGF often show a spontaneous action potential. However, underlying ionic mechanisms of the spontaneous firing were unclear. Therefore, we examined them using the patch clamp recording in rat DRG neurons.
The spontaneous firing in the on-cell configuration was abolished by a decrease in the $Na^+$and by the TRPV1 antagonists capsazepine and BCTC. These responses were accompanied by hyperpolarization of the resting potential. The holding current observed in neurons voltage clamped at –60 mV in the whole-cell configuration was significantly larger in the neurons that fired spontaneously, indicating that these neurons had an additional cation conductance. The holding current in the firing neurons was decreased by extracellular Na^+reduction, capsazepine and BCTC. The amplitudes of the capsazepine- or BCTC-sensitive component of the holding current in the spontaneously firing neurons were ten times as large as those recorded in the other neurons showing no spontaneous firing.
These results indicate that chronic NGF treatment of DRG neurons in rats induces a constitutively active cation conductance through TRPV1, which depolarizes the neurons and triggers spontaneous action potentials in the absence of any stimuli. This NGF-induced constitutive activity of TRPV1 may be a cause of neuropathic pain. (COI: No)

1P-309

Characterization of mechanically-insensitive afferents and sympathetic efferents in skeletal muscle

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Afferent and efferent fibers innervating skeletal muscle play crucial physiological functions such as pain perception and hemodynamics, but the peripheral neural mechanisms have not been fully understood. In this study, we tried to identify and characterize mechanically-insensitive afferents (MIAs) and sympathetic efferents in the muscle using the single-fiber recording *in vivo*. The axonal and receptive properties to noxious stimulus were systematically examined. Of 187 C-fibers electrically identified by centripetal recordings of the gastrocnemius nerves, 143 fibers did not respond to mechanical stimulus. Seventeen of 187 (9.1%), which exhibited large activity-dependent slowing of conduction velocity (ADSCV, > 10%), were presumed to be MIAs. Three of the 17 MIAs have acquired the responsiveness to mechanical stimulation 3–10 min after a cocktail injection of inflammatory substances. The ADSCV of 108 mechanically-insensitive C-fibers (57.8%) showed small ADSCV (< 5%). In centrifugal recordings, almost all sympathetic efferents identified by electrically stimulating lumbar sympathetic trunk had a similar ADSCV pattern (2.1±0.3%, n = 24). These results demonstrate that MIAs do exist in skeletal muscle, and that the modal shift of MIAs from silent to active may contribute to peripheral mechanisms of muscular mechanical hyperalgesia. In addition, the present results show that the most of mechanically-insensitive fibers in the gastrocnemius nerves are sympathetic efferents. (COI: No)

1P-310

An alteration of gut microbiota is associated with pain in fibromyalgia patients: a pilot study

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Purpose: Fibromyalgia (FM) patients report a high prevalence of gastrointestinal symptoms. In addition, the alterations of the intestinal microbial flora have been found to be associated with visceral pain. However, the gut microbiota in FM patients has not been investigated. We hypothesize that the alterations of gut microbiota are associated with pain, anxiety, and quality of life in FM patients.
Methods: Five FM patients were included. All patients gave written informed consent for research participation. Body mass index (BMI), pain visual analogue scale (VAS), anxiety score, and FM impact questionnaire (FIQ) were measured. A stool of all patients were collected, bacterial DNA were extracted. The levels of gut microbiota including Clostridial, Lactobacillus, Bacteroidetes, and Proteobacteria were determined using a real time polymerase chain reaction (PCR). Moreover, the ratio of Fermicutes and Bacteroidetes (F/B) was calculated.
Results: Our results demonstrated that BMI had a positive correlation with the Clostridiale levels. Pain and anxiety scores were negatively associated with F/B ratio and the level of Proteobacteria.
Conclusion: Pain severity in FM patients is associated with the alteration of gut microbiota, including F/B ratio and Proteobacteria. (COI: No)

1P-311

In vivo two-photon imaging of thermo-sensing at the skin of living rats

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Endothermic animals have the thermoregulation system that includes sensing of temperatures at the body surface (i.e., skin) and within the body. Regarding the thermo-sensing at the skin, earlier in-vitro studies have suggested two hypotheses. One hypothesis is that skin sensory neurons directly sense temperature. The other hypothesis is that skin keratinocytes directly sense temperature and release ATP to secondly stimulate sensory nerves. However, it remains unclear whether one or both of these hypotheses derived from in-vitro studies would hold true for living animals in their thermo-sensing at the skin. In the present study, we have developed transgenic Nav-Cre/Floxed-GCaMP6f rats, load thermal stress (15-43 degree) to the sole and in-vivo imaged Ca activity of sensory nerves and keratinocytes at the skin of sole by two-photon microscopy. The results show that 1) the skin keratinocytes Ca activities peaked at 37 degree, 2) the skin sensory neurons Ca activities had 5 subpopulations of hot, warm warm, cool and cold sensitive types, and 3) ATP receptor antagonists decreased the Ca activities of warm sensitive-type skin sensory neurons but did not affected them of the remaining types. These results suggest that the skin sensory nerves directly sense hot, cool and cold temperatures, while the skin keratinocytes sense warm temperature and secondly stimulate the skin sensory nerves by ATP. (COI: No)

1P-312

Cisplatin-induced intraoral neuropathy due to TRPA1 sensitization in rats

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Chemotherapy-induced peripheral neuropathy (CIPN) with platinum compounds is one of the most common dose-limiting side effects in cancer patients. Cisplatin-induced CIPN usually appear in hands and/or feet, but no study has examined intraoral CIPN development. Here, we demonstrate TRPA1-mediated intraoral neuropathy following cisplatin, using our proprietary intraoral pain assay system for conscious rats. As previously reported, cisplatin administration induced TRPA1-mediated CIPN in the hind paw, in addition to anorexia and enhancement of leukocyte phagocytosis. Similar to the hind paw, the oral mucosa in the cisplatin-treated rats demonstrated TRPA1-mediated CIPN and hypersensitivity to the TRPA1 agonist allyl isothiocyanate, with ultrastructural damages of trigeminal ganglion neurons. Topical anti-oxidative treatment with α-lipoic acid suppressed the cisplatin-induced CIPN. These results indicate that CIPN is caused in the oral mucosa by TRPA1 sensitization following oxidative stress. In cancer patients receiving chemotherapy, CIPN is likely observed in the whole body including intraoral region. The mechanism underlying the CIPN supply new insight for CIPN treatment in cancer patients. (COI: No)

1P-313

Amitriptyline-induced suppression of spinal dorsal horn neurons in a rat model of fibromyalgia

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Amitriptyline, an antidepressant drug, is often used for the treatment of chronic widespread pain like fibromyalgia (FM), yet the antinociceptive mechanisms are poorly understood. This study was aimed to examine whether administration of amitriptyline could affect response behaviors of superficial dorsal horn (SDH) neurons in the spinal cord using a rat model of FM. Reserpine, a depleter of biogenic amines in the nervous system, was subcutaneously injected to make the FM model. Under urethane anesthesia, activities of the SDH neurons was extracellularly recorded *in vivo* at the spinal segments L4 and L5. The SDH neurons showed the higher spontaneous firings in the FM group compared with the control group. Mechanical stimulation with calibrated von Frey filaments applied to the neurons' receptive field induced stimulus intensity-dependent increases in the firing rate, and the rate was significantly greater in the FM group compared with the control. Bath application of amitriptyline on the surface of the lumbar spinal cord remarkably suppressed the increased spontaneous and mechanical firings. These results suggest that facilitated spontaneous activity and mechanical sensitivity of the SDH neurons is involved in the pathogenesis of FM, and that the SDH neurons could be the action site of the antidepressant drugs for FM. (COI: No)

1P-314

Presynaptic inhibition of muscle afferent in awake, behaving monkeys: task-dependent modulation

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In the previous studies, we reported increasing presynaptic inhibition (PSI) on the input from cutaneous afferent in monkey spinal cord during voluntary movement. We developed the method to estimate the size of primary afferent depolarization by evoking antidromic volleys in the cutaneous nerve by applying the microstimulation (MS) to their intraspinal (IS) terminals.
In the present study, we applied the technique to a muscle afferent in two monkeys performing wrist flexion-extension task. We implanted a nerve cuff electrode to the deep radial nerve (DR) innervating wrist extensor muscles and attached a chamber to the cervical vertebrae (C4-T1) of the trained monkeys. Then we applied ISMS (10 Hz, 1-50 μA) to activate the terminal of DR and recorded 64 volleys.
We observed growing PSI after the instruction of movement direction as the cutaneous nerve; however, it occurred only for flexion. Moreover, we found transient and non-directional specific decrease of PSI at wrist movements. In total, a significant decrease in PSI was observed only during the extension movement in the DR ($p < 0.05$).
These results suggested the level of PSI on the peripheral nerve is modulated by at least two discrete modality-specific processes. Such modulations might allow the spinal cord to use the information from afferents in an efficient way for the control of ongoing movement. Therefore, we can conclude that task-relevant peripheral inputs are highlighted by the PSI in a highly selective way. (COI: No)

1P-315

Tentonin 3/TMEM150c, a mechanotransduction channel for Arterial-pressure sensing baroreceptors

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Arterial baroreceptors are end organs in the adventitia of carotid sinus or aortic arch detecting arterial pressure (AP) changes. Mechanosensitive channels are thought to transduce the beat to beat change of AP to electrical activity in big arteries. Here we show that Tentonin 3 (TTN3/TMEM150C), a cation channel activated by mechanical strokes with slow inactivation kinetics, is expressed in nerve terminals of aortic depressor nerves or in nodose ganglion (NG) neurons. Ttn3-/- mice exhibited ambient hypertension, tachycardia, greater fluctuation of APs, and lower baroreflex sensitivity than those of wild-type mice. More importantly, the overexpression of Ttn3 in NG of Ttn3-/- mice rescued those changes in AP, heart rate, and the baroreflex sensitivity observed in Ttn3-/- mice. These results clearly conclude that TTN3 plays an essential role in sensing dynamic AP change in the aorta and carotid sinus. (COI: No)

1P-316

The role of Cdkal1-mediated tRNA modification in peripheral neuropthy

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Neuropathy is a major diabetic complication, but the molecule mechanism has been largely unknown. Recently, genetic variations in CDKAL1 gene have been associated with the development of type 2 diabetes. CDKAL1 is a methylthiotransferase that catalyzes 2-methylthio (ms^2) modification of the adenosine at position 37 of $tRNA^{Lys(UUU)}$. The ms^2modification is important for accurate decoding of Lys codon in Proinsulin, and thus contributes to the glucose metabolism. In addition to Proinsulin, Lys is also critical for the processing of various neurotropic factors. Because dysregulation of neurotropic factors has been implicated in the development of neuropathy, we hypothesized that the deficiency of ms^2modification might cause aberrant translation of neurotropic factors, which subsequently induces the development of neuropathy. To test this hypothesis, we investigated the sensory functions in Cdkal1-knockout mice. (COI: No)

1P-317

Mild traumatic brain injury induce sensitization of neurovascular system: Relevance for migraine

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Purpose**:** Migraine-like headache is the most symptom following mild traumatic brain injury (mTBI). Previously we reported that laser-induced shock wave (LISW) lead to mTBI and nociceptive and light-aversive behavior increased (migraine-like phenotype). This study determine the effect of mTBI on trigeminal-parasympathetic circuit, a major systems for dural vasodilation induced headache.

Methods: Male rats were irradiated with LISW under barbiturate anesthesia and tested 7-10days (TBI rat). Under isoflurane anesthesia, light-evoked blood flow change was monitored in arteries of the dura mater in naive and TBI rats. Involvement of the trigeminal subnucleus caudalis (Vc) was assessed by the synaptic blocker $CoCl_2$. In separate experiment, under isoflurane anesthesia dura/cornea responsive unit was recorded in the Vc and activated by the light stimulus. Vasoactive drugs (phenylephrine) was given onto the dura 10 min after light stimulus.

Results: In TBI rats, bright light enhanced the magnitude of blood flow but not naive rats. Light-evoked blood flow increase was markedly inhibited by $CoCl_2$ injection into the trigeminal subnucleus interpolaris /caudalis transition in TBI rat. Phenylephrine inhibited afterdischarge of light evoked unit activity and partially recovered after wash out.

Conclusions: mTBI produces light evoked vessels dilation that is reflected in the sensitization of neurovascular systemand induce migraine. This model may be suitable for future studies of migraine. (COI: No)

1P-318

Mechanical and reactive oxygen species-sensitive TRP channels mediate tooth movement-induced pain

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In dental orthodontic patients, tooth movement causes pain transiently due to mechanical pressure in the periodontal ligament (PDL). The PDL contains many kinds of tissue structures, for example neuronal fibers, vasculars and fibroblasts. Since mechanical pressure applies direct mechanical stimulation to cells and produces reactive oxygen species (ROS) in the PDL during orthodontic treatments, the mechanical and ROS-sensitive ion channels, TRPA1 and TRPV4 may mediate tooth movement-induced pain. In this study we investigated involvements of TRPA1 and TRPV4 in using experimental tooth movement (ETM) models of rats. In the PDL, some PGP9.5-immunopositive nerve terminals were immunopositive to anti-TRPA1 and anti-TRPV4 antibodies. Under anesthesia, male Wistar rats were connected with a closed-coil spring (50g force) between the right maxillary first molar and the ipsilateral upper incisor. ETM increased facial grooming-like behavior, which a sign of intraoral nociception, on day 1 after coil setting. TRPA1 and TRPV4 antagonists and ROS scavengers reduced the prolonged facial grooming-like behavior compared with vehicle. In qRT-PCR study, expression levels of TRPA1 mRNA in the human PDL samples were equivalent or very lower than that of the human dorsal root ganglion (DRG). The expression level of TRPV4 mRNA was expressed higher in the human PDL than the human (DRG). These results suggest that the mechanical and ROS-sensitive TRP channels mediate tooth movement-induced pain. (COI: No)

1P-319

Therapeutic effects of highly-residual ointments on oral ulcerative mucositis

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Efficacy and effective mechanism of ointment treatment for oral ulcerative mucositis (OUM) are unclear because there are differences in adhesiveness and residual property in the oral cavity among ointments. In this study, we compared the physical properties of various ointment bases and investigated effects of the highly-residual ointment on pain and OUM severity. In vaseline, plastibase, traful ointment (TO) base and traful ointment pro-quick (TOPQ) base, adhesiveness and persistence were measured by a rheometer and evaluated in human sensory evaluation. TO and TOPQ showed superior property, in relative to vaseline and plastibase. Therefore, we selected the TO base as highly-residual ointment for the oral cavity and prepared TO ointment with triamcinolone acetonide (Tmc) or cetylpyridinium chloride (CPC). In rats, OUM was developed in the inferior labial fornix region by soaking in 50% acetic acid under anesthesia. Ointments were applied twice a day. On day 2, prolonged facial grooming behavior (a sign of spontaneous pain) was shortened by Tmc-containing TO ointment, compared with non-treatment group. In the model on days 2-4, there were no differences in OUM severity among treatment and non-treatment groups. On day 5, CPC-containing TO group accelerated healing. These results suggest that the steroid (Tmc) and antibacterial agent (CPC) are effective for pain relief and healing, respectively. (COI: Properly Declared)

1P-320

mGluR5 in the dysgranular zone of primary somatosensory cortex mediates neuropathic pain in the rat

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Primary somatosensory cortex (S1) receives information from the body and processes somatic sensation including nociception. Within subdivisions in S1, the dysgranular zone of rodent S1 (S1DZ), which is homologous to Brodmann's area 3a of primate S1, is known to be involved in the processing of prolonged noxious signals from deep somatic tissues. However, the molecular alteration of the region in the chronic pain states and its role in the pathological pain have never been studied. Here we report that the metabotropic glutamate receptor 5 (mGluR5) level in the S1DZ is involved in the pain amplification of chronic neuropathic pain model rats. Analysis of PET scan images acquired with mGluR5 specific radiotracer [11C] ABP688 revealed that the mGluR5 availability level was increased in the S1DZ of neuropathic pain model rats compared to the control group. The pharmacological blockade of mGluR5 in the S1DZ ameliorated the mechanical allodynia of the nerve-injured hind paw. Using a conditioned place-preference experiment, we could confirm that the blockade of S1DZ-mGluR5 induced a relief from the tonic-aversive state in the neuropathic pain rats, but not in control rats. Our study reveals a previously unknown molecular alteration of the somatosensory circuits in chronic neuropathic pain condition and highlights the S1DZ-mGluR5 as a future therapeutic target. (COI: No)

1P-321

Thermosensory processing in excitatory and inhibitory neurons of the primary somatosensory cortex

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Thermal perception is critical for adapting to new environments. Cutaneous thermal information is transmitted to cortical regions, including the primary somatosensory cortex (S1) via the spinal cord and the thalamus. Electrical stimulations of the S1 cause thermal sensation in human and lesion of this area causes loss of thermal sensation. Thus, the S1 has critical roles in thermal sensation. In the S1, excitatory neurons transmit sensory information to other brain regions, and inhibitory neurons regulate excitatory neuronal activity, and the balance between excitation and inhibition is critical for sensory processing. However, it remains elusive how these neurons process cutaneous temperature and whether there are different neuronal populations responsive to warm and/or cold sensation in the S1. To reveal this, we observed excitatory and inhibitory neuronal activities of layer 2/3 in the S1 in response to thermal stimulations using a two-photon microscope. Neuronal activities were monitored with a genetically encoded calcium indicator infected by adeno-associated virus three weeks prior to experiments. Thermal stimulation evoked calcium responses in S1 excitatory and inhibitory neurons. In addition, we also found both types of neurons responded to warm stimulation, cooling stimulation, or both. These results provide a comprehensive explanation of coding of cutaneous temperature in the excitatory and inhibitory neurons in the S1. (COI: No)

1P-322

Electrophysiological characterization of bradykinin B_2 receptors in rat intracardiac neurons

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PURPOSE: The heart is under control of autonomic nervous system. The neurons of intracardiac ganglia (ICG) located outer surface of atria innervate myocardial tissue. Bradykinin (BK) is a member of kinin-system and physiologically relevant peptide to tissue injury. The previous studies suggested the importance of intrinsic cardiac nervous system in mediating cardiac effects of BK. We recently found that activation of B_2 receptors excites the ICG neurons. However, the mechanisms underlying the BK action are not clarified. Therefore, we investigated the effect of BK on ICG neurons. METHODS: Experiments were performed on ICG neurons freshly isolated from Wistar rats. Membrane currents and potentials were recorded using perforated patch-clamp configuration with amphotericin B. RESULTS: BK activated non-selective cation channels via an activation of PLC and intracellular Ca^{2+} release. BK also inhibited M-currents. The BK-induced depolarization was markedly inhibited the cation channel inhibitor ML204, while the M-current inhibitor XE991 had no effect on the resting membrane potential. CONCLUSION: BK excites ICG neurons via an activation of non-selective cation channels. The inhibition of M-type K^+ channels might modulate firing rate during the BK excitation.

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1P-323

Cell type-based activation timing and order in the sequence in the preBötzinger Complex

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Spontaneous inspiratory rhythm is generated in the preBötzinger Complex (preBötC). In the rodent medullary transverse slices, the activation sequence of inspiratory neurons in the preBötC changes at every respiratory cycles. Here, we studied activation timing and order in the sequence during rhythmic bursts using Ca2+ imaging to transgenic mice expressing EGFP in GlyT2+ neurons and tdTomato in GAD65+ neurons. Based on the maximum cross-correlation coefficient between Ca2+ fluctuation and field potential, we classified inspiratory neurons into 2 types, regular type and irregular type. At earlier timing, the cycles with one and more activated irregular types of excitatory neurons (Irr-Ex) was more often detected than the cycles with the regular types of excitatory neurons (R-Ex). The percentages of activated Irr-Ex were high at the initial phase but decreased later in the cycles, while R-Ex were very low first but increased later. R-Ex showed smaller increase of Ca2+ signal at smaller-amplitude rhythmic burst, potential low-amplitude preinspiratory component recorded as burstlet. We assume that Irr-Ex may activate R-Ex and sufficient firing of R-Ex may percolate to generate the high-amplitude inspiratory bursts. Irregular and regular type of glycinergic inspiratory neurons indicated similar results. Thus, glycinergic inspiratory neurons might gain control of excitatory inspiratory neurons. We propose inspiratory neuronal network model in the preBötC during rhythmic bursts. (COI: No)

1P-324

Respiratory fluctuations in pupil diameter are not maintained during cognitive tasks

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Pupil diameter fluctuation throughout the respiratory cycle is autonomically controlled in the resting state, as pupils dilate during inspiration and constrict during expiration. Furthermore, pupil size is differentially modulated by cognitive states between task engagement and disengagement. To determine whether respiratory-dependent fluctuations in pupil size are maintained during a cognitive task, we employed healthy human subjects performing a delayed matching-to-sample task with a short delay and measured their pupil sizes and R wave-to-R wave intervals (RRIs). We detected respiratory fluctuations in pupil size and the RRI during the delay period immediately before the discrimination stage of the task. During the discrimination stage, the cognitive state with the higher task engagement yielded more pupil dilation. However, respiratory fluctuations in pupil size were abolished, whereas those in the RRI were still discernible during the discrimination stage. Our results suggest that an alternative control mechanism involving the cognitive state associated with task engagement overrides the respiratory-related autonomic control of pupil diameter. (COI: No)

1P-325

Morphology and vanilloid-susceptibility of sensory neurons innervating perirenal adipose tissue

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Perirenal adipose tissue (PrAT) is a visceral fat pad involved in the pathogenesis of obesity and cardiovascular diseases via neural pathways. However, the origin, morphological characterization, and resiniferatoxin (RTX)-susceptibility of sensory neurons that innervate rat PrAT is unknown. Injection of a neural tracer named DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) into rat PrAT revealed that sensory neurons that innervate PrAT resided in T9-L3 dorsal root ganglia (DRG). The peak labeling occurred in T13 and L1 DRGs. There were two distinct peaks in the cross-sectional areas of the labeled soma, and 79.0% of the labeled soma were below 800 μm^2. The mean cross-sectional area was 717.1 ± 27.7 μm^2. Immunofluorescence staining for vanilloid receptor 1 (VR1) showed that there were two distinct peaks in the expression level of VR1. Cross-sectional areas of neurons with a high level of VR1 were below 800 μm^2, therefore separating the DiI-positive neurons into three subpopulations: small VR1-negative, small VR1-positive, and large VR1-negative. Furthermore, local injection of RTX into PrAT reduced the labeled cells by 30.2%, where small VR1-positive cells were the main target of RTX denervation. These novel findings provide the structural basis for future VR1-dependent and VR1-independent studies on sensory innervation of PrAT, which may be of interest for future therapeutic obesity and interventions. (COI: No)

1P-326

Withdrawn

1P-327

Involvement of PVN neurons projecting to the RVLM in sympathetic dysfunction in heart failure

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We recently reported that excitation of rat hypothalamic paraventricular nucleus neurons projecting to the rostral ventrolateral medulla (PVN-RVLM neurons) caused sympathoexcitation. This monosynaptic pathway may mediate sympathetic hyperactivation in congestive heart failure (CHF), a hallmark of this disease. Here, via optogenetics, we tested if the effect of the inhibition of the PVN-RVLM neuronal activity to suppress sympathetic nerve activity (SNA) becomes augmented in CHF. In male rats (n = 9), left coronary artery was ligated to induce chronic myocardial infarction (MI) [48 ± 6% of infarct size, vs. 2 ± 1% in sham-operated control rats (n = 8)]. A retrograde adeno–associated virus vector, that encoded the red light–sensitive optogenetic inhibitor Jaws with GFP, was bilaterally microinjected into the rat RVLM. More than 6 weeks after ligation surgery, in anesthetized rats, optogenetic inhibition of the cell bodies of PVN-RVLM neurons was achieved by laser illumination (643 nm wavelength, 10 mW) intermittently (2-sec on/13-sec off, 20 times) provided to the PVN. In MI rats, a decrease in renal SNA during a 2-sec bout of illumination was followed by the rebound increase immediately after the offset of illumination. The integrated decrease in renal SNA elicited by laser illumination was significantly greater in MI rats than in sham rats (-6.7 ± 2.2 vs. 2.7 ± 1.7 arbitrary units). The results suggest that PVN-RVLM neurons are involved in sympathetic dysfunction in CHF. (COI: No)

1P-328

Responses to hypercapnia and hypoxia of Phox2b-positive cells in the ventral medulla of newborn rats

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Phox2b is a key genetic marker for pFRG/RTN neurons in the rostral ventrolateral medulla (VLM). Phox2b-positive neurons consist of tyrosine hydroxylase (TH)-negative and -positive cells (C1 adrenergic neurons). Phox2b-positive/ TH-negative pFRG/RTN neurons are intrinsically CO_2 sensitive. Here, we report effects of hypercapnia and hypoxia on Phox2b-positive (TH-positive or negative) neurons in transgenic (Tg) newborn rats in which Phox2b-positive cells expressed one of channelrhodopsin variants; ChRFR(C167A). This research was approved by the Animal Res. Committee of Showa Univ. Brainstem-spinal cord preparations were isolated from 0-3 day old Tg rats and were superfused with ACSF, equilibrated with 95% O_2 and 5% CO_2, pH 7.4 at 25-26˚C. Membrane potentials were recorded from neurons excited by photo-stimulation with blue LED light to the VLM. Responses to hypercapnia and/or hypoxia were examined in the presence of TTX. We found that at least some of Phox2b-positive/TH-positive neurons were intrinsically sensitive to hypoxia but less sensitive to hypercapnia, whereas Phox2b-positive/TH-negative neurons are intrinsically CO_2 sensitive. We hypothesize that the Phox2b expression provides fundamental intrinsic chemosensing properties in cells and further expression of TH (one of the downstream targets of Phox2b) is closely associated with O_2 sensing. Supported by KAKENHI on Innovative Areas (Comprehensive Brain Science Network) from MEXT and KAKENNHI, 16K07003, 25430012. COI: No. (COI: No)

1P-329

Involvement of the lateral parabrachial nucleus in the pressor responses to pinching of the hindpaw

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The arterial pressure reflexly increases in response to noxious mechanical stimulation (pinching) of the unilateral hindpaw in anesthetized rats. The pressor reflex is mediated via the supraspinal structure; however the precise brain mechanisms have not yet been elucidated. On the other hand, it is known that more than 90% of the projection neurons from spinal lamina I terminate in the contralateral side of the lateral parabrachial nucleus (LPBN) in rats. The present study aimed to clarify the involvement of the LPBN in the pressor reflex responses to pinching of the hindpaw in anesthetized rats. Arterial pressure was recorded via a catheter inserted into the carotid artery. Muscimol, a widely used neuronal inhibitor, was nanoinjected into the unilateral LPBN. Pinching was applied with a surgical clamp at a force of 3–5 kg to the unilateral hindpaw for 20 s. Administration of muscimol into the LPBN had no influence on the tonic arterial pressure. On the other hand, the pressor responses to pinching of the hindpaw were significantly attenuated after the administration of muscimol when pinching was applied to the hindpaw contralateral to the site of muscimol injection. The pressor responses to pinching of the ipsilateral hindpaw were slightly attenuated, but the attenuation was not statistically significant. The present results demonstrate that the LPBN is involved in the pressor reflex responses elicited by pinching of the conralateral hindpaw. (COI: No)

1P-330

Raphe-projecting oxytocinergic hypothalamic neurons stimulate brown adipose tissue thermogenesis

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Oxytocin (OXY), a neuropeptide synthesized in the paraventricular hypothalamic nucleus (PVN) and the supraoptic nucleus (SON), contributes to many brain functions, including maternal, social and sexual behaviors and stress responses, which all involve autonomic responses. In this study, by using immunohistochemical staining and *in vivo* physiological recordings, we examined the role of OXY in the central sympathetic regulation of brown adipose tissue (BAT) thermogenesis. We found that OXY-containing axon terminals were closely associated with serotonergic neurons in the rostral medullary raphe region (rMR), potentially BAT sympathetic premotor neurons. Our retrograde tracing from the rMR labeled a portion of OXY neurons in the caudal PVN, but not in the SON. In anesthetized rats, optogenetic stimulation of OXY terminals in the rMR elicited thermogenic and cardiac sympathetic responses: increases in BAT sympathetic nerve activity and temperature, expired CO_2, and heart rate. Nanoinjection of OXY into the rMR also evoked similar, but weak, sympathetic responses, which were blocked by the OXY receptor antagonist, L-368,899. OXY in the rMR was found to potentiate glutamatergic excitation of BAT sympathetic premotor neurons. These findings indicate that OXY transmission from the PVN to the rMR stimulates thermogenic sympathetic outflow to BAT, and this OXY pathway may be involved in emotion-related metabolic and thermal responses. (COI: Properly Declared)

1P-331

Strychnine enhances inspiratory-related calcium rise in the thoracic inspiratory interneuron

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Previously, we have shown that bath application of 10 μM strychnine, a broad antagonist of glycine and $GABA_A$ receptors, enhances the inspiratory motor activities in the thoracic ventral root in the isolated brainstem-spinal cord preparation from neonatal rats. To examine our hypothesis that this enhancement in part would come from enhancement of the activity in the inspiratory interneurons those give excitatory synaptic inputs to the motoneruon, calcium imaging was used in the present study. The thoracic cord was hemisected, and the left or right side was removed. Then 200 μM Oregon Green 488 BAPTA-1 AM, a calcium indicator, was injected into the ventro-medial surface of the thoracic cord using a fine glass pipette. Under control, many cells in the ventral side showed rhythmic calcium rise in phase with the C4 inspiratory activity. Strychnine caused the seizure-like motor activity in the C4 ventral root. Synchronized to this activity, large calcium rise was observed in these inspiratory cells. The inspiratory-related calcium rise was also increased. Most of non-inspiratory silent cells showed large calcium rise during the seizure-like activity, but did not become to show the inspiratory-related calcium rise under strychnine. These results suggest that the enhancement of the thoracic inspiratory motor activity induced by strychnine would come from enhancement of firing in the inspiratory interneurons. (COI: No)

1P-332

Effects of feeding-promoting peptides on excitability of the superior salivatory nucleus neurons

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The lateral hypothalamus (LH, feeding center) projects directly to the superior salivatory nucleus (SSN) which is the primary parasympathetic center for the submandibular and sublingual salivary glands. Although abundant salivary secretion is observed during feeding in many animals, submandibular salivation is largely decreased in free-moving LH-lesioned rats. The hypothalamus including the LH contains neurons which produce many feeding-promoting peptides. Therefore, those peptides induce possibly the salivation by increasing SSN neuronal excitability. In this study, we mainly electrophysiologically investigated effects of some feeding-promoting peptides on SSN neurons. Whole-cell patch-clamp recordings were performed from neonatal rat SSN neurons retrogradely labeled with a fluorescent tracer. The membrane currents and potentials were analyzed in bath-application of the peptides. Among peptides tested, only orexin affected in many SSN neurons. When orexin was applied, SSN neurons induced depolarizations at the resting membrane potential (15/26), and inward currents at -70 mV in the presence of tetrodotoxin (18/24). The current responses were inhibited by antagonists for OXR1 and OXR2. The frequency of miniature excitatory postsynaptic currents scarcely increased (53/62). In immunohistochemical analysis, SSN neurons were positive to OXR1 and OXR2. These results suggest that orexin has the excitatory action on SSN neurons, which is mediated via postsynaptic orexin receptors. (COI: No)

1P-333

Withdrawn

1P-334

Edible sesquiterpene alcohols suppress cytotoxic chemotherapy side effects

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Chemotherapy with cytotoxic drug causes multiple side effects (i.e., Nausea, vomiting, and weight loss) that open interrupt drug treatment, thus, have been treated with multiple drugs. Despite that many patients still suffer during chemotherapy. To suppress those side effects, use of natural constituents are often recommended. Recently, we have been tested naturally occurring sesquiterpene alcohols (farnesol and nerolidol) effect on chemotherapy side effects. These constituents are widely used for perfumery and have been used for increase appetite for food. It also known to have antineoplastic, anti-mite efficacy, however, their effect on chemotherapy-induced side effects not known yet. In this experiment, these constituents effect on cisplatin-induced nausea and vomiting, food and water intake, weight loss was tested using the pica behavior (consumption of non-nutritive substances) of rats.

We found that farnesol and nerolidol treated rats consumed smaller amount of kaolin than saline treated animals. This results imply that farnesol and nerolidol have antiemetic efficacy. In addition, farnesol but not nerolidol treated animals consumed more food and lost less body weight than controls. In separate electrophysiology experiments, both of them inhibited 5-HT_3 receptor-mediated current in a non-competitive manner (This work was supported by the NRF-2017R1D1A1B03033436). (COI: No)

1P-335

Opposite effects of peripheral warming on autonomic nerve activities in the anesthetized rat

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We have investigated the effects of non-invasive and thermo-local cutaneous (NTLC) stimuli to the hind paws on the electrical activity of autonomic nervous system (ANS) to clarify the relationship between somatosensory system and ANS. For ANS activity, renal sympathetic nerve activity (RSNA), gastric vagus nerve activity (GVNA) and sympathetic nerve activity (SNA) on the epididymal white adipose tissue (EWAT) were picked up. In Sprague-Dawley rats anesthetized with intraperitoneal urethane (1.2 g/kg), suspending bipolar stainless steel electrodes were used to record each ANS activity. The nerve and electrodes were embedded in and stabilized with silicone rubber gel. For NTLC stimuli, hot pads lower than 42°C were attached onto the hind paws, and ANS activities were recorded and analyzed with a power lab. As a result, a magnitude of both RSNA and SNA on the EWAT during NTLC stimuli decreased sustainably comparing the reduction with the control level, and a small transient increase in GVNA was obtained by the same stimuli. We concluded that NTLC stimuli made ANS to cause almost opposite responses, that is, 'sustained decrease' in RSNA and SNA on the EWAT and 'transient increase' in GVNA. It was also interpreted that the present NTLC stimuli applied to drive the collaboration between somatosensory system and ANS might be effective similar to the whole body warming. (COI: No)

1P-336

Is sympathoexcitation by PVN-RVLM neurons augmented in heart failure?

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Excitation of paraventricular hypothalamic nucleus (PVN) neurons projecting to the rostral ventrolateral medulla (RVLM) (PVN-RVLM neurons) causes sympathoexcitation. The PVN-RVLM neurons reportedly become sensitized in heart failure. Thus, we tested the hypothesis that sympathoexcitation by the PVN-RVLM neurons is augmented in HF. In rats, myocardial infarction (MI) was chronically induced by left coronary artery ligation. More than three weeks after the ligation or sham surgery, a retrograde adeno-associated virus vector that encodes the light-activated excitatory opsin channelrhodopsin was microinjected into the RVLM bilaterally. More than three weeks later, intermittent bouts (0.5- to 1.5-s stimulation to non-stimulation) of photostimulation at 10, 20 or 40 Hz with a 5 ms pulse duration (473 nm wavelength, 9-10 mW) for 1 min were provided to the PVN bilaterally of the anesthetized rats. Photostimulation at each frequency increased renal sympathetic activity in sham [N = 10, 0 ± 0% of infarct size (IS)] and MI rats (N = 10, 52 ± 5% of IS). The renal sympathoexcitatory response to a bout of photostimulation in MI rats, as assessed by the area under the curve, did not differ ($P>0.05$) from those in sham rats, irrespective of stimulating frequency. These observations indicate that sympathoexcitation by the PVN-RVLM neurons is not augmented in heart failure. (COI: No)

1P-337

Role of Orexin neurons in the hypothalamus during social defeat stress in the rat

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It is known that neurons in the dorsomedial area (DMH) and the perifornical area (PeF) in the hypothalamus play an essential role on the autonomic cardiovascular response evoked by psychological stress. In addition, orexin (ORX) neurons are localized within the DMH and PeF, and may be involved in the cardiovascular response during the psychological stress. A social defeat stress (SDS) that mimics an interpersonal issue at workplace or school is a kind of psychological stress, but it is unclear how the ORX neurons in the hypothalamus participate in the cardiovascular response during the SDS. In the present study, we investigated the cardiovascular response and role of the orexin neurons during the temporal (one day) and repeated (2 weeks) SDSs in conscious Wistar rats. Blood pressure (BP), heart rate (HR) significantly increased during both SDSs. However, repeated SDS did not alter the baseline BP and HR. The number of c-Fos expressed neurons in the DMH and PeF increased after both SDSs, but the number of ORX neurons in the DMH and PeF did not change in both group compared to non-stress control animals. In the repeated SDS group, the ratio of c-Fos expressions in the ORX neurons of the DMH increased (~40%) compared to that of the temporal SDS group (~20%). These results suggest that neurons in the DMH play a crucial role in the cardiovascular response evoked by both SDSs and the repeated SDS for 2 weeks increased the excitability of the ORX neurons in the DMH. (COI: No)

1P-338

Effects of anaphylaxis on the gastric autonomic nerve activities in anesthetized rats

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Systemic anaphylaxis is life-threatening and involves various organs. We previously reported that gastric vasoconstriction at late phase preceded by transient vasodilation, increased gastric motility, and delayed gastric emptying were observed after antigen injection in the anesthetized rats sensitized with ovalbumin. However, the roles of the extrinsic autonomic nervous system in these antigen-induced gastric alterations were not known. Thus, we determined the changes in the efferent and afferent autonomic nerve activities in the stomach during anaphylactic hypotension. The efferent gastric sympathetic nerve activity (ef-GSNA), but not the efferent vagal nerve activity (ef-GVNA), increased only at early phase. This increased ef-GSNA may not account for anaphylactic gastric vasoconstriction since the increase in ef-GSNA was not coincident with the vasoconstriction. The increase in ef-GSNA and no change in ef-GVNA are inconsistent with anaphylaxis-associated increased gastric motility, whereas the increased ef-GSNA, which could induce pylorus sphincter contraction, is consistent with delayed gastric emptying. On the other hand, the afferent gastric vagal nerve activity (af-GVNA), but not the sympathetic afferent, increased during anaphylactic hypotension. In conclusion, ef-GSNA and af-GVNA increase during anaphylactic hypotension in anesthetized rats, suggesting that the extrinsic autonomic nervous system partly modulates the stomach function during systemic anaphylaxis. (COI: No)

1P-339

The efficacy of prosthetic retinal stimulation

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Suprachoroidal Transretinal Stimulation (STS) is the novel retinal prosthetic system for photoreceptor degenerating diseases. The stimulating electrode array of STS is implanted in sclera and do not directly contact retinal tissue to avoid physical damage of retina. Previously we investigated the response properties after STS by single-unit recording from cat lateral geniculate nucleus (LGN) relay neurons, and reported that single pulse stimulation elicits the burstic discharges which occur alternately on ON and OFF cells (PSJ meeting, 2017, 2018). Here we applied the continuous stimulation with various frequencies to investigate how stimulation frequency influences the number of spikes elicit by STS.

Single-unit activity of the cat LGN relay neuron was recorded, and the number of spikes per second was analyzed. The size of single electrode in the implanted array into sclera was 0.5 mm in diameter and 0.3 mm in height, which was the same as the device for clinical use. The parameter of single stimulation pulse was biphasic, 1000 uA amplitude, and 0.5 ms/phase duration. The stimulation frequency was changed from 1 Hz (1000 ms interval) to 100 Hz (10 ms).

The profile of the number of spikes per second elicited by continuous stimulation was neither single peak nor single trough against stimulation frequency. The maximum number of spike discharges was achieved by the stimulation not greater than 50 Hz, suggesting high frequency stimulation may less effective. (COI: Properly Declared)

1P-340

A possibility of intracortical neural prostheses with carbon-nanotube-based electrodes

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Neural prostheses and brain-computer interfaces have been facing various challenges for the next generation. One of those is the interface with neurons at relatively high spatial resolution. Previously, carbon nanotubes (CNTs) have been proposed to be useful as the surface material of electrodes for probing neuronal activities *in vivo,* and in turn, expected to enable further miniaturization of the electrodes. Besides, we have been suggesting a usability of CNTs also in stimulating electrodes for a neural prosthesis. In the present, we fabricated CNT-based electrodes that were around 10 micrometers in cross-sectional diameter. Those electrodes exhibited mechanical rigidity enough for penetration of the cerebral dura mater in the mouse *in vivo*, and acceptable electrode-electrolyte interface impedance for injecting micro-stimulus current without causing water electrolysis in the tissue. Moreover, we were able to visualize spatially confined neuronal excitations in response to the stimuli delivered from those electrodes, by using the voltage-sensitive dye imaging technique. These observations suggested a possibility of using our electrodes for multi-channel microstimulation in the cerebral cortex. (COI: No)

1P-341

Withdrawn

1P-342

In vivo otolith organs: clinical significance of its shape between normal and Meniere's disease

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Background and Purpose: The primary function of the otolith organs is the determination of body position and movements. Meniere's disease is characterized by recurrent spontaneous attacks of vertigo, fluctuating hearing loss, tinnitus, and aural fullness. The otolith organs are correlated with vertigo. We will investigate the shape of the otolith organs in normal subjects, and the clinical course of unilateral Meniere's disease and its unaffected ear. **Method:** In vivo 3D histology of the otolith organs from volume rendering was reconstructed by a conventional temporal HR-CT data. The imaging of the otolith organs was undergone by the volume rendering otolith algorithm.**Results:** In the clinical course of Meniere's disease, during the attack period, the utricular otolith was thinner, and the saccular otolith was larger than both pre and post status. **Discussion:** We provide 3D histological image of the otolith organs by which the internal state in vivo can be observed in detail using bioinformation associated with calcium carbonate. During suffering from Meniere's disease, the utricular otolith was decreased in volume. Otherwise, the saccular otolith was increased in volume. After effective treatments, the utricular otolith was increased and the saccular otolith was decreased in volume. These quantitative methods for assessment of vestibular otopathology have defined the changeable otolith in the utricle and saccule. (COI: No)

1P-343

A newly synthesized adenosine analogue COA-Cl increases dopamine secretion in mouse brain

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<Background> COA-Cl is a newly synthesized adenosine analogue with the molecular weight of 284. It is water soluble and very stable. We have found that COA-Cl activates tyrosine hydroxylase, a rate limiting enzyme for the biosynthesis of catecholamine in cultured neuronal cells, in association with protection against cellular damage induced by oxygen/glucose deprivation or by H2O2.

<Aim> In the present study, we aimed to explore whether or not COA-Cl increases the dopamine concentration in brain using living animals.

<Methods>

A microdialysis probe was set in dosal striatum of mouse brain. Ringer's solution containing COA-Cl was infused at 1μL/min. Dopamine concentration in the eluent was measured by HPLC. The location of the probe was confirmed by dissection after the experiment. To estimate the dose to tissue, the leakage of COA-Cl from the probe was also measured.

<Results and Conclusion>

COA-Cl increased dopamine concentration in a dose dependent manner. The increase was started 10-20min after the start of administration, and dopamine concentration reached 200% of base level at 60min when 100μM COA-Cl was infused. The dose was estimated about 20% of the total amount in the flow. Thus, we conclude that COA-Cl induces release of dopamine, a neuroprotective cathocolamine, in the mouse brain, a potential mechanism for its neuroprotective effects. (COI: Properly Declared)

1P-344

Neurotrophic Role of Glucagon-like Peptide-1 Promotes Neuronal Differentiation via PI3K-AKT Axis

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Purpose: Neurons are commonly developed from neuronal stem cells by stimulation of neurotrophic factors that act multiple roles on neurons such as neuronal development, growth, and protection. Glucagon-like peptide-1 (GLP-1) is an incretin that enhances glucose up-take to decrease blood sugar. Many studies suggested that stimulation of GLP-1 receptor leads to the similar function as neurotrophic factors. Hence, we hypothesized that GLP-1 has roles as neurotrophic factors on neuronal differentiation.

Method: Western blot was applied to measure the differential expression of NMDA, AMPA, dopamine, and acetylcholine receptors, vimentin, and synapsin 1. Immunofluorescent (IF) staining was used to check the morphology change and neuronal maturity by neuron-specific class III β-tubulin. Patch-clamp technique was used to detect action potential of differentiated neurons.

Result: Results of our study demonstrated that treatment of GLP-1 to neuroblastoma cells increased expression of NMDA, AMPA, and acetylcholine receptors as well as synapsin 1; but decreased vimentin expression. The IF images showed GLP-1 induced neurite process and neuronal morphology. The differentiated neurons can be induced action potential by patch-clamp. GLP-1 induced differentiation was suppressed by the PI3K inhibitor, but not the MEK inhibitor.

Conclusion: Our study suggests that GLP-1 is able to promote immature SH-SY5Y cells differentiating into physiologically mature neurons via PI3K-AKT signaling axis. (COI: No)

1P-345

Cholinergic induction of network oscillations in the slug olfactory neuron *in vitro*

Suguru Kobayashi (*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Japan*)

Synchronous oscillatory network is vital for cognitive functions of the brain of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC) and its frequency changes are suggested to encode the olfactory information and memory. We recently found oscillatory activity is generated spontaneously in dispersed cell culture of PC neurons. Application of acetylcholinesterase inhibitor or nicotine increased the number of spontaneous activities and furthermore, induced synchronous oscillatory activity. On the other hand, histamine and other biogenic amines often changed the number of spontaneous activities without generation of synchronous oscillation on PC neurons. These results suggest that acetylcholine can function as an excitatory modulator on the synchronous oscillatory activity of the PC neuron network via nicotinic acetylcholine receptors activation. (COI: No)

1P-346

Cycle duration-modulated information transfer of olfactory and vomeronasal sensory neurons in mice

Tomohiro Noguchi; Sadaharu Miyazono; Makoto Kashiwayanagi (*Department of Sensory Physiology, Asahikawa Medical University, Japan*)

The parallel processing of chemical ignals by the main olfactory system and the vomeronasal system has been known
to control animal behavior. The physiological significance of peripheral parallel pathways consisting of olfactory sensory neurons and vomeronasal sensory neurons is not well understood. Here, we show complementary characteristics of the information transfer of the olfactory sensory neurons and vomeronasal sensory neurons. A difference in excitability between the sensory neurons was revealed by patch-clamp experiments. The olfactory and vomeronasal sensory neurons showed phasic and tonic firing, respectively. Our estimation of the information carried by action potentials during one cycle of sinusoidal stimulation with variable durations revealed distinct characteristics of information transfer between the sensory neurons. Phasic firing of the olfactory sensory neurons was suitable to carry information about rapid changes in a shorter cycle (<200 ms). In contrast, tonic firing of the vomeronasal sensory neurons was able to convey information about smaller stimuli changing slowly with longer cycles (>500 ms). Thus, the parallel pathways of the two types of sensory neurons can convey information about a wide range of dynamic stimuli. A combination of complementary characteristics of olfactory information transfer may provide animals with solid olfaction. (COI: No)

1P-347

The Neuro-protective Role of Parkin-mediated Mitophagy in Ethambutol-induced Toxic Optic Neuropathy

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Ethambutol, one of the major components of combination pharmacotherapy regimen for multi-drug resistant tuberculosis, is the most common causative agent of toxic optic neuropathy (EON). Ethambutol is reported to have neuronal toxicity, especially for retinal ganglion cell, but the exact mechanism of retinal ganglion cell death is still obscure. We found that ethambutol induces depolarization of mitochondrial membrane potential ($\Delta\Psi m$) and caspase dependent apoptotic retinal neuronal death in retinal neuron. Ethambutol treatment also resulted in transcriptional up-regulation of PTEN-induced putative kinase 1 (PINK1)/PARK2, mitochondrial translocation of parkin, and phosphorylation of parkin at S65 in retinal neuronal cell. Ethambutol induced depolarization of $\Delta\Psi m$ and subsequent apoptosis of retinal neuron is exaggerated by si-RNA mediated PARK2 knock-down. Co-treatment with rapamycin, a representative autophagy inducer, ameliorates the ethambutol induced depolarization of $\Delta\Psi m$ and caspase dependent apoptosis in retinal neuron, in vitro. In the retinal tissue of EON mouse model, increment of mitophagy is observed and intravitreally delivered rapamycin significantly alleviated apoptosis of retinal ganglion cell. Collectively, parkin mediated mitophagy is a key neuroprotective mechanism in EON and rapamycin facilitates this process in retinal ganglion cell to protect it from ethambutol induced apoptosis.
(COI: No)

1P-348

Tregs Protect Dopaminergic Neurons against MPP^+ Neurotoxicity via CD47-SIRPA Interaction

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Purpose: Regulatory T cells (Tregs) have been associated with neuroprotection in animal models of Parkinson's disease. Herein, we show that Tregs directly protect dopaminergic neurons against MPP^+ neurotoxicity via an interaction between the two transmembrane proteins CD47 and SIRPA. **Methods:** Primary ventral mesencephalic (VM) cells or VM neurons were pretreated with Tregs before MPP^+ treatment. Live cell imaging system detected a dynamic contact of Tregs with VM neurons that were stained with CD47 and SIRPA, respectively. Dopaminergic neuronal loss was examined after silencing CD47 in Tregs or silencing SIRPA in VM neurons. **Results:** Tregs prevented MPP^+-induced dopaminergic neuronal loss and glial inflammatory responses. CD47-labeled Tregs dynamically contacted with SIRPA-labeled VM neurons. Silencing CD47 gene in Tregs impaired the ability of Tregs to protect dopaminergic neurons against MPP^+ toxicity. Similarly, SIRPA knockdown in VM neurons reduced the ability of Treg neuroprotection. Rac1/Akt signaling pathway in VM neurons was activated by CD47-SIRPA interaction between Tregs and the neurons. Inhibiting Rac1/Akt signaling in VM neurons compromised Treg neuroprotection. **Conclusion:** Tregs protect dopaminergic neurons against MPP^+ neurotoxicity by a cell-to-cell contact mechanism underlying CD47-SIRPA interaction and Rac1/Akt activation. (COI: No)

1P-349

Pathology-dependent mitochondria-cytoskeleton interaction in amyotrophic lateral sclerosis (ALS)

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease, where motor axons predominantly undergo degeneration and retract from their target muscle (denervation). It has been indicated that mitochondrial dysfunction, particularly defective mitochondrial dynamics, is relevant to the denervation process in ALS. However, the molecular mechanism underlying this process has not been fully elucidated. Here we show abnormal localization of Filamin A, an actin-binding protein, associating with mitochondria in motor axons of presymptomatic ALS model mice (SOD1-G93A tg). *In vitro* experiment suggested that Filamin A forms a tertiary complex with Dynamin-related protein (Drp) 1 and actin, playing a role as a machinery for mitochondrial fission. Consistently, the size and density of Filamin A – and Drp1 – positive foci within the motor axon was increased in SOD1-G93A tg mice compared to age-matched wild type mice, suggesting excessive mitochondrial fission. Furthermore, pharmacological inhibition of this complex formation delayed the denervation process in SOD1-G93A mice, suggesting that Filamin A – Drp1 complex accelerates the progression of ALS denervation. We will further discuss the underlying mechanism of the formation of Filamin A – Drp1 complex, and its pathological role in ALS.
(COI: No)

1P-350

Continuous laryngeal TRPV1 activation modulates swallowing initiation in anesthetized rats

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Purpose: Gastroesophageal reflux disease patients suffer from dysphagia. We hypothesized that TRPV1 activation by long-term gastric acid exposure causes dysphagia. This study aimed to investigate the effect of continuous laryngeal TRPV1 activation on swallowing initiation.
Methods: Experiments were carried out on urethane-anesthetized Sprague-Dawley male rats. To identify a swallow, EMG activity was recorded from the suprahyoid and thyrohyoid muscles. The number of swallows evoked by HCl (0.1 N), capsaicin (10^{-5} M) or airflow stimulation (40 ml/s) to the larynx was counted with or without pretreatment of TRPV1 blocker, SB366791 (10^{-2} M) application. Next, the number of airflow-evoked swallows was counted after 60-min either chemical stimulation. During capsaicin stimulation, the superior laryngeal nerve (SLN) was electrically stimulated over 10 sec (0.2 msec pulse duration, 30Hz) every 10 min. The threshold of the SLN-evoked swallow was recorded.
Results: TRPV1 blocker reduced the number of swallows evoked by HCl/capsaicin, but did not affect that evoked by airflow. The number of swallows during 60-min chemical stimulation decreased in a time-dependent manner. That of airflow-evoked swallows following 60-min chemical stimulation was decreased. The threshold of SLN-evoked swallow did not change during capsaicin stimulation.
Conclusion: The modulation of excitability in peripheral nervous system may be involved in swallowing reduction by continuous laryngeal TRPV1 activation. (COI: No)

1P-351

Prevention of Dry-Eye Pain by Diquafosol Sodium Administration

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Dry eye (DE) is known to cause ocular pain as well as unpleasantness in the eye. Recently, $P2Y_2$ agonist Diquas ® is used for the treatment of DE. Diquas accelerates moisture and mucin secretion from conjunctival epithelium and goblet cells. However, it's still unknown whether the tear secretion following Diquas treatment attenuates neuronal hyperactivity in brainstem neurons associated with DE, resulting in ocular pain.Tear volume, the number of eye blinks evoked by hypertonic saline application to the eye, and phosphorylation of extracellular signal-regulated kinase (pERK) and c-Fos expression in trigeminal subnucleus caudalis-upper cervical spinal cord (Vc-C1) were studied in exorbital grand-removed rats. Three percent Diquas (10 µl 6 times/day) was applied to the eye for 4 weeks in DE rats. Tear volume was significantly reduced, and the number of eye blinks elicited by the hypertonic saline application was substantially higher in DE rats compared with control rats. In DE rats, the reduction of tear volume and the number of eye blinks were recovered considerably after Diquas treatment. The number of pERK-IR cells and c-Fos-IR cells, and neuronal hyperactivity in Vc-C1 was also significantly suppressed following Diquas treatment.The present findings suggest that Diquas treatment causes the enhancement of tear secretion, and then the hyperactivity of Vc-C1 neurons in DE rats is attenuated, resulting in the prevention of DE pain. Acknowledgment: Santen Pharmaceutical Co. (COI: Properly Declared)

1P-352

Analysis of activated cortical area caused by food restriction in mice

Jihao Ma; Sakurako Yanase; Lisa Udagawa; Tomoyuki Kuwaki; Ikue Kusumoto-Yoshida (*Department of Physiology, University of Kagoshima, Japan*)

An appropriate regulation of food intake is essential for health. Rising rates of obesity, diabetes, and frailty pose significant threats to health, and thus understanding the brain regions that underlie aberrant food intake is critically important. It is well known that the hypothalamus regulates both food intake and energy homeostasis. However, interactions between the hypothalamus and other brain areas such as cortical regions require further elucidation.

In this study we adopted immunohistochemical c-fos signal mapping of both the hypothalamus and the insular cortex, which is known as the higher order sensory cortex, that integrate multiple modalities and play an important role in establishing homeostasis within the body. Neuronal activity that is related to food intake was induced by restricted feeding. It has been previously reported that mice fed a single daily meal at a regular time within the circadian range exhibit food anticipatory activity (Blaum ID et al., 2012).

Similar to the previous study, mice showed increased locomotion just before feeding time. Brain samples that were collected at feeding time showed increased c-fos signal positive neurons in the insular cortex and hypothalamic orexin neurons. These results suggest an important role of correlated activity for insular and orexin neurons in food anticipatory behavior. (COI: No)

1P-353

TLR2-dependent signaling relay of glial-neuronal circuits to regulate thermoregulation

Saki Murayama; Erkin Kurganov; Seiji Miyata (*Department of Applied Biology, Kyoto Institute of Technology, Japan*)

Toll-like receptor 2 (TLR2) recognizes components from Gram-positive bacteria viruses, fungus, and parasites. In the present study, we aimed to examine the localization and cellular phenotype of TLR2-expressing cells and TLR2-dependent signaling pathways regulating thermoregulation in mouse brain. TLR2 expression was prominent at both parenchyma microglia and perivascular macrophages in the circumventricular organs (CVOs), which lack typical blood-brain barrier and therefore blood-derived molecules pass blood vessels. A peripheral administration of TLR2 agonist Pam3CSK4 at a dose of 1 mg/kg significantly caused nuclear factor-kB (NF-κB) activation in microglia and macrophages. Moreover, the peripheral stimulation induced Fos expression in astrocytes and neurons in the CVOs and in neurons in hypothalamic thermoregulation-associated regions. A peripheral administration of Pam3CSK4 significantly induced a prominent and long-lasting fever and sickness behaviors such as a decrease in locomotor activity, food and water intake, and body weight. Preconditioning of TLR4 agonist lipopolysaccharide significantly elevated TLR2 expression in the CVOs and augmented fever response. These results indicate that TLR2 in microglia/macrophages of the CVOs directly recognize circulating Gram-positive bacteria component and transmits its information neighboring astrocytes and neurons to cause fever and sickness behaviors. (COI: No)

1P-354

A novel TRPM8 expressing "cold-neuron" in mouse hypothalamus and medulla

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Thermosensation is an important function in mammals to adapt to various conditions. Although thermosensation through periphery nerve endings has been well studied, brain temperature sensing is poorly understood. Recent studies have shown that warm-sensitive neurons are present in the preoptic area (POA) that express transient receptor potential melastatin 2 (TRPM2) as well as TRP vanilloid 1 (TRPV1) ion channels in the hypothalamus activated by increasing brain temperature. Here, we report the presence of neurons expressing cold-sensitive TRPM8 in somatodendrites of the medial preoptic nucleus (MPA), lateral septal nucleus (LS) and in axonal terminals of the median eminence (Me) in the mouse hypothalamus as well as in pons, medulla and spinal cord, but not in the cerebellum. TRPM8 is a cold and menthol receptor that is activated by chemical cooling agents and temperature dropping below <26 °C. Some TRPM8-expressing neurons possess relatively large somata and dendritic spines, but TRPM8 is not expressed with inhibitory neuronal marker proteins, parvalbumin, and somatostatin. Moreover, some axonal terminals of GnRH and VGlut2 neurons in the Me are colocalized with TRPM8. We also found TRPM8 expressing neuronal pathway from trigeminal ganglia (TG) to pons that forwarded to the medulla. Thus, our findings suggest that TRPM8 proteins are expressed at both excitatory and inhibitory neurons, and TRPM8-expressing neuron in the hypothalamus and medulla is a putative "cold neuron". (COI: No)

1P-355

Sensitivity of voltage-dependent Ca^{2+} channels in rat AVP neurons to an anthranilic acid derivative

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Ca^{2+} channels play an important role in secretion of arginine vasopressin (AVP) from the soma/dendrites and axon terminals in AVP neurons by triggering the $[Ca^{2+}]_i$ increment. An anthranilic acid derivative, flufenamic acid (FFA), was previously reported to inhibit L-type Ca^{2+} channels in rat arterial smooth muscle cells. Here, we examined the effects of FFA on the voltage-gated Ca^{2+} channels in AVP neurons isolated from the supraoptic nucleus of rat brain. When the extracellular isoosmotic bath solution was replaced with hyperosmotic solution, no significantly change was observed in the Ca^{2+} channel currents under voltage-clamp conditions. When FFA was applied, Ca^{2+} channel currents were significantly inhibited. The Ca^{2+} channel currents were not affected by an L-type Ca^{2+} channel blocker, nifedipine, and a P/Q-type Ca^{2+} channel blocker, ω-agatoxin IVA. On the other hand, an N-type Ca^{2+} channel blocker, ω-conotoxin GVIA (ω-CgTX), or a T-type Ca^{2+} channel blocker, NNC 55-0396 (NNC), suppressed the Ca^{2+} channel currents. When ω-CgTX and NNC were simultaneously administered, the Ca^{2+} channel currents were almost completely suppressed. FFA significantly inhibited not only the ω-CgTX-insensitive (T-type) component but also the NNC-insensitive (N-type) component of the Ca^{2+} channel currents. These results show that N- and T-type Ca^{2+} channels are functionally expressed in the soma/dendrites of AVP neurons, and both are sensitive to FFA. (COI: No)

1P-356

Behavioral and neural characteristics of recognition of the binary taste mixture in rats

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Introduction: Our recent study demonstrated that the rats have an ability to detect the component of binary taste mixture by using conditioned taste aversion (CTA) technic (Katagawa et al., 2016). In the present study, we conducted behavioral and neural experiments to investigate characteristics of recognition of components of binary taste mixture.

Methods: We used male Wistar/ST rats. In the behavioral study, we investigate the pattern of generalization to some concentration of component stimuli in the rats conditioned to binary taste mixture. The characteristics of behavioral pattern of CTA in the rats denervated their taste nerve, such as chorda tympani (CT) and glossopharyngeal (GL), were also investigated. In the electrophysiological study, whole nerve responses of CT to taste mixture and their components were measured. As binary taste mixture in above experiments, sucrose + HCl and sucrose + quinine hydrochloride were used.

Results: In the behavioral experiment, recognition of the component of binary taste mixture was obstructed by partner component. The rats without CT nerve could recognize component of taste mixture, but it was difficult for these rats to acquire CTA. Our electrophysiological experiment found that magnitude of response to mixture was smaller than that of sum of response to their components.

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1P-357

An imaging system for 3D detection of nano-vibrations in sensory epithelium of the inner ear

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Human audition can distinguish frequencies that are only 0.2% apart as well as perceive trillionfold differences in sound pressure level. These properties stem from sound-induced nanoscale vibrations in sensory epithelium of the inner ear. The epithelium is composed of three layers; sensory hair-cell, supporting-cell, and basilar-membrane layers. Although the spatially different distribution of the vibrations in each layer seems to be involved in the extraordinary hearing properties, this profile has not yet been precisely measured by conventional technologies. Therefore, we have developed an imaging system that can three-dimensionally detect the object's vibrations. The underlying technique is based on the optical coherence tomography, which is currently applied to medical diagnoses. First, a supercontinuum broadband light source is used to achieve high imaging performance. The spatial and depth resolutions are approximately 3.6 μm and 1.8 μm, respectively. Second, a vibrometry technique and an ultra-speed CMOS camera are incorporated into the system. This arrangement allows us to pursue nanoscale vibrations of up to 30 kHz in a wide area of a radius of 1 mm. Through an equipped microscope we scanned the traveling vibrations within the basilar-membrane layer in a live guinea pig. This system has a potential to determine physical networks across each layer of the epithelium and thereby it may contribute to finding a fundamental mechanism underlying auditory function. (COI: No)

1P-358

Withdrawn

1P-359

Massage-like stroking stimulation induces 50-kHz ultrasonic vocalizations

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Rats emit aversive 22-kHz ultrasonic vocalizations (USVs) in response to gentle touch with an unfamiliar human hand; however, tickling with a human hand mimicking conspecific rough and tumble play (heterospecific play) can induce appetitive 50-kHz USVs. We have shown that tickling stimulation in adolescent rat evokes 50-kHz USVs via dopamine in the nucleus accumbens (Hori et al., 2013), whicih is thought to play a pivotal role in reward process. Our previous studies have shown that massage-like stroking stimulation of the skin increases dopamine release in the nucleus accumbens in rats (Maruyama et al., 2012; Shimoju et al., 2017); however, it remains unknown whether the stroking stimulation elicits 50-kHz USVs. In the present study we investigated whether the stroking stimulation emits the 50-kHz USVs in rats. USVs were recorded using an UltraSoundGate Condenser Microphone. Acoustical analysis of the recordings was performed using Avisoft SASLab Pro. Stroking stimulation vigorously increased 50-kHz USVs during stimulus period and the calls showed various patterns such as flat (constant frequency), frequency-modulated and harmonic USVs. Present results suggest that massage-like stroking of the skin is an excellent handling method to make rats an appetitive state like tickling. (COI: No)

1P-360

Retinal circadian rhythm is entrained by the SCN via corticosterone secretion from the adrenal gland

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The retina transmits light signals to entrain the central circadian clock in the suprachiasmatic nucleus (SCN). Although the SCN generates entraining signals to the peripheral clocks, the mechanism to entrain the retinal circadian clock remains unclear since ambient light is also involved. Here we dissociated the two entraining factors by a jet lag experiment and examined which factor dominantly entrain the retinal clock. After an abrupt shift of the light/dark (LD; 12h light and 12h dark) cycle for 10 hours, the SCN of C57BL/6 mice took several days to restore the synchronization with the ambient LD cycle, during which the two entraining factors are supposed to be desynchronized. The circadian rhythm of the retina was examined by cFOS induction by light in the retinal ganglion cells (RGC). After the abrupt shift of the LD cycle, the phase delay of the cFOS induction by light took several days to restore the synchronization with the LD cycle. This suggests that the SCN is dominant to the ambient light in the entrainment of the retinal circadian rhythm. Further, adrenalectomy disrupted the cFOS induction rhythm by light and attenuated circadian rhythm of clock gene in the retina, which were restored by corticosterone (CORT) administration. Together with the finding that CORT receptor and clock protein PER1 were co-expressed in the RGC, the observations suggest that retinal circadian rhythm is entrained by the SCN via CORT secretion from the adrenal glands. (COI: No)

1P-361

Exercise capacity and intelligence in adults after betamethasone given to 4-day-old infant rats

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Purpose. The administration of betamethasone (BET) to pregnant women complicated with threatened premature delivery has been widely used in clinical practice to avoid respiratory distress syndrome in neonates. However, long-term side effects of BET administration on ability of the exercise capacity and intelligence have yet to be clarified; thus, repeated BET administrations have been contraindicated. We investigated the effects of BET administered during infancy on the exercise capacity and intelligence after growth using rat models. Method. We administered BET at 0.5 mg/body weight (kg) to 4-day-old rats (group B, n=6) and also established a control group (group S, n=5) by giving saline. We performed a suspension test by hooking rats' forelimbs on a horizontal bar at 40 cm high and measured the suspension time using 3-week-old rats for five consecutive days to test the motor capacity. We performed a step-down-type passive avoidance test (SDPAT) by measuring the staying-time on the insulted platform using 5-week-old rats after 2-day learning periods to avoid having to apply electric shocks. Results. The suspension time in group B was significantly ($p<0.05$) shorter than that in group S. There was no significant differences in staying-times in SDPAT between group B and group S. Conclusion. BET administrated in infancy might impair the motor capacity after growth. We have been performing more detailed investigations using mouse models. (COI: No)

1P-362

Characteristics of motor and memory functions in cerebral hypoperfusion model rat by microspheres

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[Introduction] The cerebrovascular embolism (CE) could induce multiple microinfarction, resulting in cognitive dysfunction like the vascular dementia (VD). However, the suitable VD animal model is not established. In this study, we investigated the optimal condition in microsphere (MS) induced CE model rats for memory impairment without motor dysfunction by measuring the cerebral blood flow (CBF), the motor and the memory function.

[Methods] CE were produced by MS (from 2,500 to 4,000 particles) injection into right internal carotid artery of rats with measurement of CBF in motor cortex and hippocampus. The pathological output including motor functions (physical deficit score and rotarod) and memory functions (Morris water maze) were observed.

[Results] CBF in both hippocampus and cortex decreased by MS injection, and the motor functions (score and rotarod) and the memory function (MWM) were aggravated dose-dependently. In this experiment, the injection of 3,000 particles of MS decreased in CBF to 74.7 ± 11.8 % and 73.9 ± 4.0 % vs control at hippocampus and motor cortex, respectively. These CBF decrease caused memory dysfunction but not motor dysfunction. Therefore, 3,000 particles of MS injected rat would be more appropriate as a VD model.

[Conclusion] MS injection decreased CBF and dose-dependently induced memory dysfunction like VD. (COI: No)

1P-363

H_2S Attenuates Maternal Cigarette Smoke Exposure-Induced Oxidative Stress in pFRG in Neonatal Rats

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We have reported that in neonatal rats, maternal cigarette smoke exposure (CSE) resulted in oxidative stress in parafacial respiratory group (pFRG), a brainstem region critical for mammalian central chemoreception. Hydrogen sulfide (H_2S), as a signaling molecule, plays an important anti-oxidative role in central nervous system. The aim of the present study was to elucidate whether nuclear factor erythroid 2-related factor 2 (Nrf2) serves a role in the anti-oxidative effect of H_2S in pFRG in neonatal rats. In a maternal CSE animal model, pregnant rats were randomly divided into four groups: control, CSE (pregnant rats received CSE during gestational days 1-20), CSE+NaHS (a donor of H_2S), and NaHS, and the corresponding neonatal rats (postnatal 2 days) were used. The experimental protocols were approved by the Animal Care and Use Committee of Sichuan University. CSE increased the generation of reactive oxygen species (ROS). NaHS pretreatment reduced the production of ROS, and increased the expression of superoxide dismutase, an important mammalian antioxidant enzyme that was inhibited by maternal CSE. However, expression of catalase and glutathione was not significantly different. Additionally, NaHS normalized the expression of Nrf2 and Keap1, which were up- and down-regulated, respectively, by CSE. These results indicate that maternal CSE leads to failure of Nrf2 to regulate some of its targets, and NaHS can reverse it and protect pFRG from oxidative stress in neonates. (COI: No)

1P-364

Maternal Cigarette Smoke Exposure Disturbs Excitatory/Inhibitory Balance in pFRG of Neonatal Rats

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We have previously reported that maternal cigarette smoke exposure (CSE) resulted in impairment of central chemoreception in neonatal rats in vivo and in vitro. It has been also reported that glutamatergic and γ-aminobutyric acid (GABA)-ergic innervation is involved in state dependent control of parafacial respiratory group (pFRG), a critical site for mammalian central chemoreception. The present study was carried out to test whether maternal CSE disturbs excitatory/inhibitory balance in pFRG of neonatal rats via techniques of liquid chromatography-tandem mass spectrometry, RT-PCR and immunohistochemistry, western blot and colourimetry. Pregnant rats were randomly divided into control and CSE (pregnant rats received CSE during gestational days 1-20) groups and the corresponding neonatal rats (postnatal 2-3 days) were used. The experimental protocols were approved by the Animal Care and Use Committee of Sichuan University. We found that maternal CSE significantly reduced glutamic acid and glutamine release, but did not affect GABA level, in pFRG. Meanwhile, the expression of vesicular glutamate transporter 2 was depressed, contrarily that of glutamate transporter 1 and GABA transporter 3 was elevated in the CSE group. In addition, the activity of glutaminase and glutamine synthetase was decreased. These results indicate that maternal CSE causes an excitatory/inhibitory imbalance in pFRG, which might contribute to suppression of central chemoreception in neonates. (COI: No)

1P-365

Brown adipose tissue is involved in anti-obesity effects of royal jelly in high fat diet-fed mice

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Purpose: It has been reported that royal jelly (RJ) supplementation improves insulin sensitivity; however, its impacts on energy expenditure and adiposity remain elusive. We investigated anti-obesity effects of RJ supplementation and their relation to physical activity levels and thermogenic capacities of brown (BAT) and white adipose tissue (WAT).

Methods: C57BL/6J mice were fed under four different experimental conditions for 17 weeks: normal diet (ND), high fat diet (HFD), HFD with 5% RJ, and HFD with 5% honey bee larva powder (BL). Spontaneous locomotor activity, hepatic triglyceride (TG) content, and blood parameters were examined. Gene and protein expressions of thermogenic uncoupling protein 1 (UCP1) and mitochondrial cytochrome c oxidase subunit IV (COX-IV) in BAT and WAT were investigated.

Results: Dietary RJ, but not BL, suppressed HFD-induced accumulations of WAT and hepatic TG without modifying food intake. Consistently, RJ improved hyperglycemia and the homeostasis model assessment-insulin resistance (HOMA-IR). Although dietary RJ and BL unchanged locomotor activity, gene and protein expressions of UCP1 and COX-IV in BAT were increased in the RJ group compared to the other experimental groups. Neither the RJ nor BL treatment induced browning of WAT.

Conclusion: Our results indicate that dietary RJ ameliorates diet-induced obesity, hyperglycemia, and hepatic steatosis by promoting metabolic thermogenesis in BAT in mice. (COI: No)

1P-366

Withdrawn

1P-367

Ischemic postconditioning induced by opening of mK^+_{ATP} channels and NMDAR silencing by mPTP opening

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[Background] Cerebral ischemic postconditioning (IPoC) has been shown to reduce infarct volume in cerebral ischemia/reperfusion (I/R) injury and has association with a mitochondrial ATP-dependent potassium (mK^+_{ATP}) channel. Recently, some reports have demonstrated that *N*-methyl-D-aspartate receptor (NMDAR) silencing induced by mild opening of the mitochondrial permeability transition pore (mPTP) was a crucial determinant of neuroprotection. In the present study, we examine the precise mechanisms of IPoC using electrophysiological approach.

[Methods] C57BL/6J mice hippocampal slices were used in all experiments. We simulated severe neuronal ischemia by exposing slices to nitrogen-contained solution. We measured the NMDAR currents and intracellular Ca^{2+} concentration, mitochondrial membrane potential with patch-clump technique and the fluorescent probes in mice hippocampal neurons.

[Results] NMDARs and anoxia mediated increase in Ca^{2+} were silenced during IPoC ($P<0.05$) and mK^+_{ATP} channel opener diazoxide prevented the anoxia-mediated increase in Ca^{2+} and reduction in NMDAR currents ($P<0.05$). The mPTP blocker cyclosporine A prevented the IPoC effect that NMDAR currents reduced ($P<0.05$). Furthermore, Φ_m depolarization was induced by the activation of mK^+_{ATP} channels during IPoC.

[Conclusion] The present study indicates that mitochondria plays a pivotal role for neuroprotection of IPoC induced by opening of mK^+_{ATP} channels through NMDAR silencing by mild mPTP opening. (COI: No)

1P-368

Withdrawn

1P-369

Low frequency stimulation targeting the subiculum reverses drug resistance in temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is easily drug resistant and not well control by current treatments. Thus, mechanisms of drug resistance need to be evaluated and further develop better methods to cure those with drug resistant epilepsy. In this study, using *in vivo* neuronal activity recording in a classic multi-drug resistant TLE model, we found that subicular pyramidal neuron activity was specifically not inhibited by the anti-epileptic drug phenytoin in drug resistant rats. Long-term low frequency stimulation (LFS) at the subiculum, which is clinically feasible, inhibited the activity of subicular pyramidal neurons and reversed drug resistance. Meanwhile, LFS significantly alleviated the severity of seizures, as indicated by lowering seizure stage, shortening afterdischarge duration and generalized seizure duration. Further, electrophysiology data showed the dysfunction of sodium channels in subicular pyramidal neurons is involved in drug resistance. These results suggest that subicular pyramidal neurons may be a key "switch" mediating drug resistance epilepsy and represent a new potential target for more precise treatment of drug resistant TLE. (COI: Properly Declared)

1P-370

Utilizing the TRPV1 and TRPM8 channels to facilitate the swallowing

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The larynx and associated laryngopharyngeal regions are important for swallowing. Transient receptor potential (TRP) channels have been detected in these regions, however, their role in evoking swallowing reflex has not been fully elucidated. Our purpose was to understand whether we can utilize the TRP channels to improve swallowing. Immunohistochemistry was conducted to detect TRPV1 and TRPM8 in the cell bodies of afferents that supply the regions. The superior laryngeal nerve (SLN, a nerve that supply the regions) activity was recorded following topical application of different solutions including TRPV1 and TRPM8 agonists and antagonists. The number of evoked swallowing reflexes following topical application of the solutions was counted. TRPV1 and TRPM8 were detected majorly in the unmyelinated afferents. The SLN activity was modulated by the topically applied TRP channel agonists. The agonist evoked response was attenuated by prior topical application of the respective antagonist. In addition, topical application of the agonists evoked a number of swallowing reflexes that was significantly more than the reflexes evoked by distilled water. Furthermore, prior topical application of the antagonist significantly reduced the number of agonist evoked reflexes. The findings suggest that activation of TRP channels facilitates the evoking of swallowing reflex. Utilization of TRP channels can be a therapeutic strategy in management of dysphagia and to prevent pulmonary aspiration. (COI: No)

1P-371

Mating with SFPs deficient males cause the suppression of NaCl intake in females in *Drosophila*

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In *Drosophila* females, mating induces behavioral switch that is both dramatic and varied, including a reduction in receptivity, an increase in egg laying, alterations in sleep cycles, and changes in dietary preference. Female fruit flies intake yeast and sodium chloride (NaCl) more after mating. These post mating responses are mediated by the male-derived seminal fluid proteins (*SFPs*) , especially sex-peptide (*SP*), and the receptor (Sex peptide receptor , *SPR*) in the female nervous system.

In this study, we used *SFPs* and *SPR* deficient flies to investigate the effect of mating on the intake of NaCl, the amino acid mixture and sucrose. In the wild type females, intake of the amino acid mixture increased after mating and intake of sucrose are reduced slightly but significantly; these post-mating responses were not observed in the *SPR* deficient females. NaCl intake increased in the wild type females after mating, but decreased in the *SPR* deficient females and wild type females mated with *SP* deficient males. In addition, in the previously reported post-mating circuitry, inactivation of *SP* sensing neurons (SPSNs) did not cause any significant change in NaCl intake after mating. However, the excessive NaCl intake caused by inactivation of second-order neurons which are called SAG neurons and mediate *SP* sensing, was suppressed by mating. These results suggest the existence of a *SP*-*SPR* independent post mating circuitry which specifically suppresses NaCl intake after mating. (COI: No)

1P-372

Mood stabilizing drugs activate adult neural stem cell-neurogenesis system

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Neural stem cells (NSCs), which produce all neurons and glial cells in the developing brain and provide new neurons in the adult brain and provide new neurons for the olfactory bulb and the hippocampus. Neurons play significant roles in the olfaction and some types of memory. NSCs attract much attention as therapeutic method for impaired central nervous system. However, efficient and clinically feasible strategy to activate endogenous NSCs has never established. We have previously demonstrated that mood stabilizing drugs, which are used for the patients with bipolar disorder, enhance the self-renewal of mouse NSCs in vitro at therapeutically relevant concentrations in the cerebrospinal fluid. The pharmacological effects of classical mood stabilizers are mediated by the activation of Notch signaling in the NSC.

In this study, we examined the effect of lamotrigine(LTG), a novel type of mood stabilizer, on self-renewal of NSCs and neurogenesis in the subependymal zone, the dentate gyrus and other regions such as the cortex by chronic administration of LTG. Our data suggest that LTG promotes self-renewal of NSCs, which are similar to classical mood stabilizers, such as valproate and carbamazepine. We are also assessing whether LTG-induced neurogenesis influences to behavior. (COI: Properly Declared)

1P-373

Chebulinic acid negated the development of streptozotocin induced experimental dementia in rats

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Chebulinic acid (CA) the main active constituent of Terminella chebulla belonging to the Combretaceae family has various neuroprotective effects and improves cognitive function in animal models of neurodegenerative disorders. In the current study, the protective effects of CA in an intracerebroventricular (ICV)- Streptozotocin (STZ)-induced sporadic Alzheimer's disease (SAD) was determined. STZ was infused bilaterally at the dose of 3 mg/kg/icv on day 1st and 3rdafter surgery. CA was tested at doses of 25, 50 and 100 mg/kg/po from 7th day onwards after ICV-STZ. Spatial and non-spatial memory was evaluated using Morris water maze (MWM) and object recognition task (ORT) in rats. On day 22 rats were sacrificed and hipoocampal brain regions were used to identify biochemical, neurochemical, neuroinflammatory and histopathologicsal alterations. ICV-STZ was found to significantly shorten the latency time on the MWM and ORT which was associated with increase in oxidative stress (lipid peroxidation and nitrite), compromised antioxidant defense (reduced GSH), neurotransmitter alteration (AChE, DA, Nadr, 5-HT, GABA and glutamate), elevation in neuroinflammatory cytokine (IL-1 β, IL-6 and TNF- α) levels and degenerate hippocampus architecture. The study demonstrates that CA treatment significantly prevented all ICV-STZ-induced detrimental changes induced by STZ. Thus, CA may have a therapeutic value for the treatment of SAD. (COI: No)

1P-374

Chronic EEG recording from rodents using ceramic-guided wire electrodes

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Rodents are a useful animal model to study oscillations of the brain activity because of its toughness against invasive manipulations such as electrode implantation and easiness for long-term chronic recording. We used two different types of electrodes (screw and ceramic-guided twisted-pair wire) chronically implanted into the rat brains and compared their characteristics as probes for neural activity. As expected, the epidural surface recording using the conventional screw electrodes were suitable for detecting the low-frequency, global oscillations. We could stably record the infra-slow range (about 0.1Hz) oscillation with the screw electrodes. In contrast, wire electrodes were better for detecting action potentials and local field potentials at higher frequencies. We recorded the EEG from the epileptic rats (GAD65 deficient mutant rats) and found that wire electrodes are suitable for detecting paroxysmal seizure waves, which were often missed with the screw electrodes. We further manipulated the brain oscillations by administering ketamine and examined how the seizure waves interact with the ketamine-induced slow oscillation of EEG. Recordings with wire electrodes clearly demonstrated that spike-and-wave complex tended to occur during the cortical up-state, rather than the down-state. Our results indicate that twisted-pair wire electrodes, although more invasive, are suitable for a detailed epilepsy wave form analysis than the conventional screw electrodes. (COI: No)

1P-375

Retinal ON pathways contribute to temporal characteristics of visual motion processing in mice

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There are two pathways of visual processing in the retina: ON and OFF pathways that originate from ON and OFF bipolar cells. ON bipolar cells use metabotoropic glutamate receptors to receive the signals from photoreceptors with the effector protein TRPM1 to influence membrane potential, whereas OFF bipolar cells use ionotoropic glutamate receptors that immediately change the membrane potential. To understand differences in temporal characteristics of visual processing between these pathways, we examined optokinetic responses (OKRs) elicited by two-frame animations presented with an inter-stimulus interval (ISI) in mutant mice with dysfunctional ON-bipolar cells (TRPM1$^{-/-}$) and wild-type mice. In general, two-frame animations elicited OKRs in the veridical direction without an ISI. As the ISI became longer, the OKRs reduced and eventually reversed their directions. The OKRs of TRPM1$^{-/-}$ mice showed more progressive reduction and the directional reversal at shorter ISIs (53.3 ms) than the wild type mice (213.4 ms). We also simulated OKRs with dependence on ISIs using a model of visual motion processing (the Reichardt motion detector). Further analyses using the model revealed that temporal frequency characteristics of visual processing were different between TRPM1$^{-/-}$ and wild-type mice with higher optimal temporal frequency for the TRPM1$^{-/-}$ mice. The results suggest that the ON pathway may improve sensitivity to slower changes in visual signals.
(COI: No)

1P-376

Distribution of Smad mRNA and proteins in the rat brain

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Smad proteins are known to transduce the action of TGF-β superfamily proteins including TGF-βs, activins, and bone morphogenetic proteins (BMPs). In this study, we examined the expression of Smad1, -2, -3, -4, -5, and -8 mRNA in the rat brain by means of in situ hybridization (ISH). In addition, we examined the nuclear accumulation or phosphorylation of Smad1, -2, -3, -5, and -8 proteins after intracerebroventricular injection of TGF-β1, activin A, or BMP6 with immunohistochemistry to investigate whether TGF-β, activin, and/or BMP activate Smads in the rat brain. Nine-week-old male Sprague-Dawley rats were used. ISH signals for Smad1, 2, 3 and 4 mRNA were widely detected in the brain. Especially, ISH signals for Smad2, 3 and 4 mRNA were abundantly detected in forebrain regions including the olfactory bulb, olfactory tubercle, piriform cortex, basal ganglia, cingulate cortex, hippocampus, and cerebellar cortex. ISH signals for Smad5 and Smad8 mRNA were restricted to a small number of brain regions, the signal intensity of which was weak. Intracerebroventricular injection of activin A induced nuclear accumulation of Smad2 and Smad3 immunoreactivity in neurons. Activin A also induced phosphorylation of Smad3 in microglia. On the other hand, BMP6 induced phosphorylation of Smad1/5/8 in astrocytes. These results suggest that neurons and microglia utilize activin-Smad2/3 signaling and astrocytes utilize BMP-Smad1/5/8 signaling for brain homeostasis. (COI: No)

1P-377

Event related potentials in the first-person shooter game with virtual reality environment

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In the neuroergnomics research (applying neurotechnology to ergonomics), measuring event-related potentials (ERPs) is quite important but still not easy in the real world due to difficulties in the synchronization of the events and electroencephalogram (EEG) recordings. This difficulty can be solved in the Virtual Reality (VR) environments, although many of the previous VR studies used EEG frequency domain analysis.

In this study, we measured ERPs from 20 healthy adults using a 64-channel wireless wearable EEG system with dry electrodes to record participants playing a first person shooting (FPS) game with a VR head mount display (HMD). The purpose of the game was to search and destroy terrorists in an enclosed area. The event markers used were 1) player pulling a gun trigger, 2) out of sight gunshots, and 3) being hit by the enemy.

Informed consent was obtained from all participants, and the methods applied in the study adhered to the tenets of the Declaration of Helsinki for the use of human subjects in biomedical research. All study protocols were approved by the institutional ethics committee of National Defense Medical College.

ERP components were elicited when 1) participants missed the enemy (50-100 ms after participants response), 2) participants were hit (200-250 ms after visual and sound feedback), and 3) out of sight gunshot sounds were presented (180-200 ms after sound onset). The relationships between those components and behavioral measures were also examined. (COI: No)

1P-378

Changes in reproductive hormones-related genes in hippocampus of cognitive impaired male rats

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Androgen deficiency, an indicator of reproductive senescence, can conduce towards many diseases including cognitive impairment. Meanwhile, hippocampus, a brain region functioning for cognition, can also express genes associated with reproductive hormone receptors and locally synthesize reproductive hormones as seen in the hypothalamic-pituitary-testicular (HPT) axis. From our previous study, the androgen deficient status (or significantly lowered serum testosterone levels) could be detected in male rats when they reached the middle-age (12 months old), but the phenomenon was initiated at 8 months old at the hypothalamus and pituitary levels. Thus, it is interesting to know how the reproductive hormone-related genes in hippocampus expressed in cognitive impaired male rats. Male rats at the ages of 4, 6, 8, 10 and 12 months old were subjected for this study. Rats were tested for cognitive performance using Morris water maze test. After the test, hippocampus was collected, and mRNA expression levels of reproductive hormones-related genes were determined by qrt-RTPCR techniques. Cognitive impairment was detected at 8 months old. In hippocampus, *Kiss1*, *Gnrhr*, *Lhβ*, *Ar*, *Esr1* and *Esr*2 mRNA levels were significantly elevated at 8 months old. This denotes that the hippocampus responses to changes of HPT axis, when males enter reproductive senescence, by up-regulating sex steroid receptors and reproductive hormone syntheses encoding genes in an attempt to retain the cognitive function. (COI: No)

1P-379

Agomelatine protects against on permanent cerebral ischemia model through Nrf2-HO-1 pathway

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Stroke is the major cause of death and permanent disability worldwide. It has been reported that 85% of patient are ischemic stroke.Nowadays,the standard treatment is recanalization.However, only 5% of patients can access this treatment.Therefore,the new strategies for permanent ischemic stroke need to be investigated.Agomelatine is one of melatonergic agonist that acts on MT1/2 receptors and antagonist to 5-HT2c receptor.It has been pleiotropic effect such as anti-oxidant, anti-inflammation. In this study, we focused on the effect of agomelatine on permanent cerebral ischemia rat model.All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the Faculty of Medicine, Chiang Mai University, Thailand (Permitted number: 12/2559).Male Wistar rats were randomly divided into four groups (n=6/group). Sham operating group, permanent ischemic model group, permanent ischemic model plus agomelatine (40 mg/kg, i.p) group and permanent ischemic model plus melatonin (10 mg/kg, i.p) group. After 24 h of ischemic onset, we investigated neurological deficit and infarct volume by using neurological deficit scores, TTC and TEM.Moreover, we determined Nrf2-HO-1 protein expression by western blotting.The results showed that agomelatine and melatonin decrease neuronal injury and also promote Nrf2-HO-1 signaling pathway. These findings suggest that agomelatine and melatonin exerts beneficial effect on permanent cerebral ischemia model. (COI: No)

1P-380

Withdrawn

1P-381

Effects of quercetin on neuronal activity in the hypothalamic food intake regulating areas

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A flavonoid quercetin possesses anti-obesity activity but there is no evidence to suggest its central effects, especially for the hypothalamic brain regions that play an importance role in regulation of energy balance. Hence, this study investigated the effects of quercetin on neuronal activity in the hypothalamic food intake regulating areas including the arcuate nucleus (ARC), the ventromedial hypothalamus (VMH), and the dorsomedial of hypothalamus (DMH) in male Wistar rats. Rats received either vehicle (1 ml/kg, p.o.) or quercetin (100, 200 and 400 mg/ml/kg, p.o.) for 30, 60, 90 and 120 min. Free-floating brain sections (30 μm) of the ARC, VMH and DMH were subjected to immunohistochemical staining for Fos, a neuronal activation marker.The highest number of Fos+ neurons/area was found in the ARC in the rats treated with 100 mg/ml/kg of quercetin for 120 min. Significantly more number of Fos+ neurons/area in the ARC and significantly less number of Fos+ neurons/area in the VHM in quercetin treated group than vehicle-treated group were demonstrated. Number of Fos+ neurons/area in the DMH were not different between quercetin and vehicle treatments. The results suggested that quercetin may involve in the regulation of food intake and energy homeostasis by activate neurons in the ARC of the hypothalamus and inactivate neurons in the VMH of the hypothalamus. Further studies are required to clarify which neuronal types in the ARC are activated by quercetin. (COI: No)

1P-382

Dihydrocapsaicin improves functional recovery after cerebral ischemia and reperfusion in rat model

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This study investigated the effects of dihydrocapsaicin (DHC) on cerebral damage in cerebral ischemia and reperfusion (I/R) rats. Middle cerebral artery occlusion was induced in I/R rats for 2 h, followed by reperfusion. The animals were divided into three groups: sham, I/R + vehicle, and I/R + DHC (10 mg/kg BW). All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the Faculty of Medicine, Chiang Mai University, Thailand. After 14 d of reperfusion, DHC-treated I/R rats significantly decreased neurological deficit scores, infarct volume, and brain morphology changes. Moreover, DHC also induced angiogenesis via increased the expression of hypoxia inducible factor 1α (HIF-1α), vascular endothelial growth factor (VEGF), and matrix metalloprotease 9 (MMP-9) and also increased the expression of angiogenic inhibitors like angiopoietin 1 (Ang-1) and its receptor tyrosine kinase (Tie-2). DHC-mediated angiogenesis was confirmed by the significantly increased in the positive BrdU-labeling co-localized with the von Willebrand factor (endothelial cell marker). Furthermore, it was demonstrated by rotarod and pole tests that DHC promoted functional recovery. Therefore, this study offers to aid future development for protection against cerebral I/R injury and also improves function recovery after an ischemic stroke mediated by angiogenesis.

There is no actual or potential conflict of interest in relation to this presentation. (COI: Properly Declared)

1P-383

The Effect of difference of cognitive control levels in SRK model on EEG frontal theta band

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Many models which define human cognitive behavior have been proposed so far. In this study, we employed the Skill-Rule-Knowledge (SRK) model (Rasmussen, 1983) in defining for cognitive control levels for human performance. In addition, the effectiveness of this model confirmed, however, studies on EEG corresponding to the levels of cognitive control have not enough done. The purpose of this study is to explore the effect of cognitive control levels on theta band of EEG activity in the frontal region.

Eighteen healthy right-handed men (19-23 years of age (20.8±1.76 years)) participated in the experiment. All experiments performed in accordance with the Declaration of Helsinki. As an experimental task, a mental arithmetic task based on the SRK model in Rasmussen (1986) was created. This task consists of Skill task (ST), Rule task (RT), Knowledge task (KT). The EEGs recorded on the scalp by 128 channels. The electrodes to be analyzed were Fpz, Fz, Cz on the ten-twenty electrode system.

As a result, activity of the frontal midline theta was observed at 5.4-7 Hz. In the activity of frontal theta (5.4-7 Hz), KT appeared more than ST and RT. Furthermore, in the result of Fpz, there was a difference between all the tasks.

The results indicated that the higher conscious control of cognitive behavior requires, the more activity of the frontal midline theta band increase.

The activity in the theta band at the frontal region may be an indicator of the cognitive control of the SRK model. (COI: No)

1P-384

Parvalbumin positive neurons in the basolateral amygdala and anxiety-like behavior in OLETF rats

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Otsuka Long-Evans Tokushima Fatty (OLETF) rats lacking the CCK-A receptor due to spontaneous genetic mutation were reported as an animal model of type 2 diabetes and also to exhibit the anxiety-like behavior. In the basolateral amygdala (BLA), basket cells mainly express cholecystokinin (CCK) or parvalbumin (PV) in the perikaryon, however, it remains unclear whether PV positive neurons in OLETF rats also change or not. Therefore, we aimed to examine the number of PV positive neurons in the BLA of OLETF rats compared to control rats.

Male OLETF and control male Long-Evans Tokushima Otsuka (LETO) rats (both 20-week-old) were used. The open field test was performed for assessment of anxiety-like behavior. The number of PV positive neurons in the BLA was also evaluated by the method of immunohistochemistry.

In the open field test, total locomotion was not changed, whereas the distance and time in the center zone were significantly smaller in OLETF rats than LETO rats. OLETF rats showed more stretched attend postures and less rearing compared to LETO rats. No significant differences were shown between OLETF and LETO rats in the number of PV positive neurons in the BLA.

These data suggested that behavioral abnormality in details of OLETF rats might not be due to PV positive neuronal system, and adaptive changes of PV positive neurons might not cause in the BLA. (COI: No)

1P-385

Role of the medulla in the regulation of slow wave sleep

Yoshimasa Koyama; Kazuki Kobayashi; Hayato Iwata; Tatsuya Suzuki; Kaname Mochizuki; Yoshifumi Arai (*Department of Science and Technology, Fukushima University, Japan*)

It is well known that sleep-wake cycle is regulated by waking centers in the posterior hypothalamus and brainstem and sleep center in the preoptic area (POA). Some neurons in the POA start to fire prior to the start of slow wave sleep (SWS) and continue firing during SWS, suggesting that the POA initiates and maintains SWS. It is also widely accepted that the brainstem including pons and medulla has a role in regulation of wakefulness and REM sleep, however little attention has been paid on the involvement of medulla in SWS regulation, except the reports that neurons in and around the nucleus of solitary tract (NST) are sleep active and that GABAergic neurons around the facial nerve have some roles in SWS regulation.In the present study, to examine the role of medulla in regulation of SWS, we systematically applied electrical micro stimulation to wide areas in the medulla through carbon fiber electrode and found that synchronized EEG similar to that observed during SWS was evoked from several areas including those around the NST, but not around the facial nerve. Next, using head restrained, un-anesthetized rats, we recorded single neuronal activity under sleep-wake cycles from the medulla, mainly in and around the NST and the facial nerve. Most of the neurons recorded were most active during REM sleep, less active during SWS and least active during waking or highly active during both REM sleep and waking. Mechanisms of SWS regulation in the medulla would be discussed. (COI: No)

1P-386

The Protective Effect of Neferine on Permanent Ischemic Brain Injury in Rats

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Cerebral ischemia or low blood supply to brain is the cerebrovascular disease as a result of occlusion or narrowing of brain artery, which is the most common cause of disability and death. Generally, reduced of blood flow affects to brain by evoke excitotoxicity, calcium (Ca^{2+}) accumulation and inflammation, lead to neuronal cell death. Nowadays, several natural compounds are ascertained for using as alternative treatments for cerebral ischemia. Neferine is one of the popular natural compounds, isolated from embryo seed of *Nelumbo nucifera* or lotus. The pharmacological activities of neferine are including anti-oxidant, anti-inflammatory effects, together with neuroprotection. This study investigated the neuroprotective effect of neferine on permanent cerebral ischemia in rats. The models were induced by middle cerebral artery occlusion (MCAO) for 24 h. Male Wistar rats were used and approved by the Animal Ethics Committee and conducted in compliance with under the Chiang Mai University's guidelines for care and use of laboratory animals. Rats were divided into 3 groups: control group; vehicle group; and 50 mg/kg BW neferine treated MCAO group. After 24 h of MCAO, we found that neferine significantly reduced the area of the infarction, morphology changes in the neuronal cells including apoptotic cell death. Moreover, neferine also preserved the healthy neurons in the penumbra area. In conclusion, neferine can improve the neuronal cell death in cerebral ischemia injury. (COI: No)

1P-387

Effects of NSAIDs on cerebral glucose metabolism measured by [^{18}F]FDG uptake in rat brain slices

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Objectives: Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) that inhibits cyclooxygenase-2 (COX-2) more selectively than COX-1 and thus is used as a drug with less gastric side effects. Several studies have indicated that COX-2 selective NSAIDs including celecoxib increase intracellular Ca^{2+}concentration and cause apoptosis in various tumor cells. We have examined whether celecoxib and other NSAIDs have any effect on cerebral glucose metabolism by measuring [^{18}F]FDG uptake in rat brain slices.

Methods: Sagittal brain slices (300-μm thickness) were prepared from Wistar rats (6–8 weeks old). The slices were incubated with 100 kBq/mL [^{18}F]FDG in oxygenated Krebs-Ringer solution at 36°C, and serial two-dimensional time-resolved images of [^{18}F]FDG uptake were obtained by replacing imaging plates every 15 min. The frontal cortical region was chosen as a region of interest.

Results: Celecoxib and another COX-2 selective nimesulide increased [^{18}F]FDGuptake rate gradually with time over 2 h. Addition of membrane-permeable Ca^{2+}chelator BAPTA-AM reducedthe increasing effect of theses two drugs. Among other NSAIDs tested at concentration of 300 μM, diclofenac, indomethacin, mefenamic acid, and piroxicam increased [^{18}F]FDG uptake rate whereas aspirin, sodium salicylate, ibuprofen, and ketoprofen had no effect.

Conclusion: These results show that various NSAIDs have effects to increase cerebral glucose metabolism probably by elevating intracellular Ca^{2+}concentration. (COI: No)

1P-388

MLCK isoforms regulate intestinal epithelial hyperpermeability under inflammatory stress

Yu Chen Pai; Tsung-Chun Lee; Chia-Hui Yu (*Graduate Institute of Physiology, National Taiwan University College of Medicine, Taiwan*)

Patients with IBD showed epithelial barrier damage and increased proinflammatory cytokines, including IFNg and TL1A. IFNg induced tight junctional (TJ) defects and bacterial endocytosis both mediated by MLCK. To investigate the roles of MLCK variants in the differential regulation of epithelial hyperpermeability under proinflammatory stress. Mouse models of colitis by DSS and IO were assessed at early and late time points. Human Caco-2BBe cells were treated with IFNg or TL1A for measurement of barrier function. *In vivo*, bacterial endocytosis and brush border fanning correlated with increase of mucosal TL1A expression in DSS-1d and IO-6h mice and can be abolished by neutralizing anti-TL1A or MLCK inhibitors. TJ damage were observed at DSS-4d and IO-24h, also in a MLCK-dependent manner. *In vitro* studies showed that TL1A and low dose IFNg increased *MLCK2* transcripts, terminal web MLC phosphorylation and bacterial endocytosis without TJ defects; high dose IFNg induced TJ disruption instead. Bacterial endocytosis was inhibited by MLCK inhibitors and si*MLCK*, but not by si*MLCK1*. In contrast, high dose IFNg-induced TJ disruption were inhibited by si*MLCK*, si*MLCK1*. Lastly, Caco-2 cells with MLCK knockout displayed increased bacterial endocytosis after overexpression of MLCK2 but not MLCK1. Low dose IFNg and TL1A induced bacterial endocytosis via MLCK2-activated terminal web MLC phosphorylation, whereas high dose IFNg caused TJ disruption in a MLCK1-dependent manner.

(COI: No)

1P-389

TRPV6 mutations cause neonatal transient hyperparathyroidism

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Neonatal transient hyperparathyroidism (NTHP) is a skeletal disorder with a decreased bone mineral density, thin limb and fractures. Although placental etiology is suggested, its molecular mechanisms are unclear. Here we report that mutations in TRPV6, a Ca^{2+}-selective epithelial ion channel, cause NTHP. Patch-clamp recordings, intracellular Ca^{2+}-imaging, and Western blotting of biotinylated proteins revealed that there were several types of mutations: 1) abnormal plasma membrane trafficking, 2) impaired protein stability, 3) incorrect regulation by intracellular Ca^{2+} concentration. There results suggest that TRPV6 is involved in the maternal-fetal Ca^{2+} transport in the placenta for sustaining fetal bone mineralization. TRPV6 mutations cause NTHP affecting the Ca^{2+} transport through distinct mechanisms. (COI: No)

1P-390

Involvement of EP receptors in the regulation of Short circuit current by prostaglandins in A6 cells

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Water secretion across epithelial tissues driven by their epithelial Cl^- secretion contributes to regulation of body fluid content as well as prevention of our body from bacterial/viral infection. Short circuit current (Isc) measurement is a good technique to clarify the ion transport through epithelial cells. A6 cells derived from Xenopus laevis are good model to study ion transport in principal cells of renal cortical collecting duct. Prostaglandin E2 (PGE2) plays an important role in the regulation of salt transport in the mammalian distal nephron especially in the cortical collecting tubule. In A6 cells, Isc was increased by exposure to PGE2. The effect was partially abolished by either apical amiloride, an epithelial Na+ channel blocker, or NPPB, a Cl− channel blocker. Several articles suggested that protein kinase C (PKC)- and protein kinase A (PKA)-mediated intracellular signaling pathways might be involved in the PGE2-mediated ion transport in A6 cells. There are 4 subtypes of PGE2 receptors (EP receptors); EP1, EP2, EP3 and EP4. However, there has been no report which EP receptors are involved in the PGE2-mediated ion transport in A6 cells. The present study shows that PGE2 stimulated ion transport is mainly induced by chloride, and Butaprost, the EP2 agonist can mimic the effect of PGE2 on the ion transport, suggests that EP2 is involved in PGE2-induced Cl- secretion in A6 cell.

(COI: No)

1P-391

Oligomerization of Na^+/H^+ exchanger isoform 3 (NHE3) and its role in the transport mechanism

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Na^+/H^+ exchangers (NHEs) are electroneutral transporters that mediate a one for one exchange of extracellular sodium and intracellular protons. NHE isoform 3 (NHE3) is expressed predominantly in the apical membrane of intestinal and renal epithelia, where it plays a pivotal role in salt and fluid absorption and acid-base homeostasis. NHE3 localizes to both the plasma membrane and endosomal compartments in the cells. Acute up-regulation of NHE3 transport activity is mediated by altering the total number of exchangers in the plasma membrane as well as their individual activity. We have previously shown the existence of a slow activation mode of individual NHE3 activity, which induced intracellular acidification and required ~ 5 min to attain completion. This slow response is may result from conformational changes of exchangers such as oligomerization of exchangers and/or ancillary proteins that regulate its activity. However, definitive demonstration of the existence of NHE3 oligomerization at the surface of living cells is still lacking. To investigate whether oligomeric structure is required for NHE3 function, extracellular epitope-tagged NHE3 was stably transfected into NHE-deficient cells. In addition, to test whether individual subunits of NHE3 are the minimum functional unit for Na^+/H^+ exchange, we co-expressed NHE3 with an extracellular epitope-tagged transport-deficient mutant of NHE3. (COI: No)

1P-392

Computer simulation of intracellular HCO_3^-/CO_2 buffering in pancreatic duct cell

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[Aim] Previously we constructed a mathematical model of pancreatic duct cell using MATLAB/Simulink (Yamaguchi *et al*. 2017). In the model, intracellular HCO_3^-/CO_2 buffering reaction ($CO_2 + H_2O \rightleftarrows H^+ + HCO_3^-$) was set quick enough to settle intracellular pH (pH_i) and reproduce HCO_3^--rich fluid secretion. To simulate transient changes of pH_c by NH_4^+ pulse, etc. in guinea-pig pancreatic duct cell, we have tried to construct a more precise HCO_3^-/CO_2 buffering system.

[Methods] In 2 buffering phases: $CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$, the equilibrium constants (pK_a) of reactions 1 and 2 were set as 2.3 and 3.8, respectively (thus the net pK_a of total buffering is 6.1) (Eigen 1964). The forward and backward reaction coefficients in the absence of carbonic anhydrase (CA) were set to the reported values (Welch 1969).

[Results] To determine the coefficients of reaction 1 (activity of CA), the bathing was changed from HCO_3^-/CO_2-buffered solution to HCO_3^-/CO_2-free, HEPES buffered solution. While pH_c was not stable in the absence of CA, pH_c showed biphasic changes (transient alkalinization followed by slower acidification) when the reaction coefficients were increased by 100 times. Then, pulse application of NH_4^+ (20 mM) to the bath caused 4-phasic complex pH_c changes similar to experimental data.

[Conclusion] Construction of a precise HCO_3^-/CO_2 buffering system reproduced transient pH_c changes by NH_4^+ pulse in pancreatic duct cell. (COI: Properly Declared)

1P-393

Secretory reflex pathway of Xenin-25 in the rat ileum

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Xenin-25 is secreted by enteroendocrine cells in the small intestine. The neuronal circuits mediating the Xenin-25-induced anion secretion were characterized using Ussing chambered mucosa-submucosa preparations from the rat ileum. All studies were performed with approval of the Committee for Animal Research of Kyoto Prefectural University of Medicine (M27-482). Serosal application of Xenin-25 increased the short-circuit current (*Isc*) in a concentration-dependent manner. The responses were abolished by the combination of Cl^--free and HCO_3^--free solutions. The responses were almost completely blocked by TTX (10^{-6} M), but not by atropine (10^{-5} M) or hexamethonium (10^{-4} M). The selective antagonists for NTSR1, NK1, VPAC1, VPAC2 and capsaicin inhibited the responses to Xenin-25. Immunohistochemical staining showed the colocalization of NTSR1 and NK1 with SP- and calbindin-immunoreactive neurons in submucosal plexus, respectively. Xenin-25-induced Cl^-/HCO_3^- secretion is involved in NTSR1 activation on intrinsic and extrinsic afferent neurons, followed by the release of SP and subsequent activation of NK1 expressed on non-cholinergic VIP secretomotor neurons. Finally, the secreted VIP may activate VPAC1 on epithelial cells to induce Cl^-/HCO_3^- secretion. Activation of VIP-positive secretomotor neurons by IPANs and extrinsic afferent neurons by postprandially released Xenin-25 may account for most of the neurogenic secretory response induced by Xenin-25. (COI No)

(COI: Properly Declared)

1P-394

Secretory reflex pathway of SCFA in the rat distal colon

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Short-chain fatty acids (SCFAs) are end products of bacterial fermentation in the colon. Colonic chloride secretion is induced by SCFAs via the enteric nervous reflex. Previous study showed that luminal propionate transiently stimulated the colonic chloride secretory response via sensory and cholinergic systems of the mucosa in rat distal colon [J. Physiol., 403, 559-575, 1988]. Furthermore, same group reported that the non-neuronal release of acetylcholine (ACh) from colonocytes coupled with propionate stimulation plays a key role in chloride secretion [J. Physiol., 589(Pt 4):953-962, 2011]. However, previous studies did not identify the afferent neural pathway in the enteric nervous system that induced ion transport stimulated by SCFAs. In the present study, we have examined secretory reflex pathway of SCFAs on rat distal colon using Ussing chamber method. In the present experiment, tachykinin receptor antagonists were used to identify afferent neuronal pathway since intrinsic primary afferent neurons contain tachykinins. The addition of propionate to the luminal bathing solution concentration-dependently induced transient K^+ and Cl^- and/or bicarbonate secretion. The propionate-induced transient K^+ and Cl^- and/or bicarbonate secretion was modulated by neurokinin receptor 1 (NK1) antagonist, CP96346 and NK3 antagonist, osanetant, respectively. These findings indicate that epithelial ion transport is modulated by tachykinin receptors in rat distal colon. (COI: No)

1P-395

Epithelial ion secretion of human bronchial ciliary epithelium

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Airway epithelia play key roles in maintaining the volume and composition of airway surface liquid by regulating transepithelial ion transport. However, little information is available on ion transport in upper airway epithelia. We studied transepithelial ion transport of airway epithelia by measuring the short circuit current (Isc) and surface pH using surface enhanced Raman spectroscopy (SERS) in human bronchial ciliary cellscultured under air-liquid interface conditions. In human bronchial ciliary epithelia, the amount of anion secretion was equal to that of Na^+absorption. To focus on the anion transport, experiments were carried out under a condition ofblocking Na^+absorption by benzamil. Under a Cl^-/HCO_3^--containing condition, the basolateral addition of DIDS, a blocker of AE, NBC and Cl^-channels, decreased Isc. Under a Cl^--free HCO_3^--containing condition, Isc was smaller than that under a Cl^-/HCO_3^--containing condition. Finally, under a HCO_3^--free Cl^--containing condition, Isc was smaller than that under a Cl^-/HCO_3^--containing condition and similar to that under a Cl^--free HCO_3^--containing condition. Apical surface pH showed a decreasing trend after addition of DIDS, NPPB and a Cl^--free HCO_3^--containing solution.These results suggest that human bronchial ciliary cellssecrete not only Cl^-but also HCO_3^- via cooperationbetween Cl^-channel and anion exchanger, contributing to control of the volume and composition of airway surface liquid. (COI: No)

1P-396

Zinc finger protein 521 involved in small intestinal function and stem cell differentiation

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Zinc finger protein 521 (ZFP521) regulates differentiation of stem cells. It is known that ZFP521 suppresses differentiation of mesenchymal stem cells into adipocytes. Contrary to this report, the body size of *Zfp 521*-deficient (*Zfp521*$^{-/-}$) mice were obviously smaller than that of their wild type (*Zfp521*$^{+/+}$) littermates. Therefore, we investigated adipose tissues and nutrient absorption of *Zfp521*$^{-/-}$ mice to evaluate the reasons for the poor growth of *Zfp521*$^{-/-}$ mice. Initially, we examined the differences in adipose tissues, blood glucose and the amount of food intake between *Zfp521*$^{-/-}$ mice and *Zfp521*$^{+/+}$ mice. In *Zfp521*$^{-/-}$ mice, the size of white adipocytes in any tissues were small and the levels of blood glucose and leptin were low. Additionally, the amount of food intake and the amount of glucose in their feces increased extremely as compared with those in *Zfp521*$^{+/+}$ mice. Then, we performed glucose tolerance tests to examine the blood glucose level. There was no difference in the levels of blood glucose between *Zfp521*$^{-/-}$ mice and *Zfp521*$^{+/+}$ mice after intraperitoneal administration, but the levels of blood glucose in *Zfp521*$^{-/-}$ mice were lower than those in *Zfp521*$^{+/+}$ mice after oral administration. These results suggested *Zfp521*$^{-/-}$ mice have malfunction in the small intestine, which may be a cause of poor growth of adipose tissue. We are currently investigating whether there is an abnormal differentiation of intestinal epithelial stem cells in *Zfp521*$^{-/-}$ mice. (COI: No)

1P-397

Role of cysteine protease inhibitors in malignancy of oral squamous cell carcinoma

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Purpose: Oral squamous cell carcinoma (OSCC) is the most common oral malignancy with poor prognosis. 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] is considered as an anticancer agent. Addition of 1α,25$(OH)_2D_3$ reduced cell proliferation and increased apoptosis of some cell lines of OSCC, but the mechanism is not clear. We focused on cysteine proteases inhibitors, which have been reported to regulate progression and suppression of cancers.

Methods: Two established cell lines, HSC-3 and SAT, which are derived from human oral squamous cell carcinoma, were used. HSC-3 has been reported to have high metastatic potential, while SAT shows non-metastatic characteristics. The cells were cultured in the DMEM with 10% charcoal-treated FBS in the absence and presence of 1α,25$(OH)_2D_3$. After treatment with 1α,25$(OH)_2D_3$, cells were harvested at 6h-3days. Total RNA was purified and the expression levels of cystatin C (CST3) and cystatin D (CST5) were determined by real time RT-PCR.

Results: The expression level of CST5 was increased by addition of 1α,25$(OH)_2D_3$ in HSC-3, while that of CST3 was not significantly changed. In SAT, the expression levels of both CST3 and CST5 were not affected by addition of 1α,25$(OH)_2D_3$.

Conclusion: The expression of cystatin D was induced by active vitamin D_3, which may be negatively correlated to the malignancy of oral cancer.

(COI: No)

1P-398

Renal impairment disturbs the intestinal microbiota and alters intestinal motility

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Background

It has been reported that segmental and total colonic transit times were longer in patient on hemodialysis or peritoneal dialysis than in the general population. However, the mechanism of intestinal motility dysregulation in patients with kidney disease remains unclear. In this study, we investigated the mechanisms of intestinal motility dysregulation caused by renal impairment.

Methods

We examined the responses to electric field stimulation (EFS) in the longitudinal muscle obtained from the distal colon in 5/6 Nephrectomy mice (5/6 Nx) and sham operated mice (sham). Next, we measured expression levels of inflammatory cytokines in distal colon and we investigated populations of bacteria in stool. In addition, we investigated the responses in the distal colon with antibiotic treatment. At last, we investigated whether uremic toxin is involved in inflammation.

Results

In the distal colon, magnitudes of EFS induced-relaxation were smaller in 5/6 Nx. The expression of IL-6, TNFa, and iNOS were increased in 5/6 Nx. *Lactobacillus spices* and *Fusobacterium spices* were decreased in stool of 5/6 Nx. The magnitudes of EFS-induced relaxation in distal colon obtained from 5/6 Nx with antibiotic treatment were similar to those observed in sham with antibiotic treatment. Indole sulfate and trans-aconite acid enhanced LPS-induced TNFa production. Thus, microbiota and uremic toxin contribute to intestinal motility dysregulation induced by renal impairment. (COI: No)

1P-399

Down-regulation of PDGFRα+ cells caused colonic dysmotility in DSS-induced colitis mice

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Background

Inflammatory bowel disease (IBD), which consists of ulcerative colitis (UC) and Crohn s disease. In this study, the contributions of PDGFRα$^+$ cells and SK3 channels were investigated in colonic transit dysfunction of DSS-treated colitis mice.

Methods: Colonic migrating motor complexes (CMMCs), smooth muscle spontaneous contractile experiments, intracellular recordings, western blotting analysis and quantitative PCR were applied in the present study.

Results: In CMMC experiments, the colonic transmission was disordered with the contractile amplitudes and frequencies inconsistent and was not sensitive to the SK3 antagonist and agonist (apamin and CyPPA) in DSS-colitis compared with control mice. Similarly, in contractile experiments, the colonic smooth muscles were also insensitive to CyPPA and apamin. Intracellular recordings showed that the reactions of membrane potentials to apamin and CyPPA had no more significant pharmacodynamic effect in DSS-colitis mice.

Conclusions: These results demonstrate that colonic transmission disorder in the DSS-colitis mice is the contributions of down-regulated purine/PDGFRα$^+$ /SK3 signaling pathway.

KEYWORDS

Colonic transit disorder, colitis, PDGFRα$^+$ cells, SK3 channel, Purine, SIP syncytium

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(COI: No)

1P-400

Neurogenic relaxation of Xenin on spontaneous circular muscle contractions in rat distal colon

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Xenin1-25(Xen) is a 25 amino acid neurotensin-related peptide produced by enteroendocrine cells and it increases the insulin release at physiological glucose concentrations. Xen also delays gastric emptying, reduces food intake, induces gall bladder contractions, suggesting that some effects of Xen could be mediated by enteric neurons. However, the effects of Xen in the distal colonic circular muscle have not been previously investigated. In this study, we aimed to investigate the neuronal pathways underlying Xen-induced relaxation on spontaneous circular muscle activity. The muscle activity in the strip preparations of the rat distal colon was recorded in isometric condition by the Mugnus chamber system. Na^+ channel blockers (TTX & A803467) significantly reduced the Xen-induced relaxation time. Xen-induced relaxation time was not altered by atropine. Xen-induced relaxation time was not changed by the Ca^{2+}- dependent K^+ channels blocker (Apamin). Furthermore, the relaxation time was not reduced by the Guanylate cyclase inhibitor (ODQ) and the P2Y1 receptor antagonist (MRS2500). In contrast, the relaxation time was significantly reduced by Neurotensin receptor (NTSR1) antagonist and by VIP receptor (VPAC2) antagonist. These results suggest that Xen induces the relaxation of spontaneous circular muscle contraction by activating VIP positive non-cholinergic motor neurons and NTSR1 in rat distal colon. (COI. No)

(COI: Properly Declared)

1P-401

CRF regulates colonic motility through CRF-PDGFRα$^+$/ICC pathway

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CRF released during stress has been reported to be involved in the abnormal colonic motility during IBS, but the exact mechanism is still unclear. SIP syncytium includes ICC, PDGFRα$^+$ cells and smooth muscle cells (SMCs) electrically coupled possibly via gap junctions in the smooth muscle layer, which is the final effector of smooth muscle contraction. Our aim in the present study is to explore the role of CRF/ PDGFRα$^+$ cells or ICC pathway in heterotypic stress-induced colonic transit disorder mice. We found that the number of fecal pellets during the stress was much more in the stress-induced mice than in the control ones, but after 24 h, the fecal pellets seemed similar between the control mice and the stress-induced mice. Colonic transit in the control mice was faster than in the stress-induced mice in CMMC experiment. Urocortin induced increase of CMMC which could not be inhibited by tetrodotoxin. The expression of PDGFRα was higher in the colon of stress-induced mice than in the control ones, but the expression of ICC was lower in the colon of stress-induced mice than in the control ones. These results indicate that CRF can regulate the colonic transit through CRF-PDGFRα$^+$/ICC pathway, which may be the reason underlying the heterotypic stress-induced colonic transit disorder.

Keywords: colonic transit; CRF; SIP syncytium

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1P-402

Regulation of gastric motility by histamine via interstitial cells of Cajal in the Syrian hamster

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Gastric motility is controlled by the autonomic and enteric nervous systems and by interstitial cells of Cajal (ICCs). Although histamine is released from enterochromaffin-like cells in the gastric mucosa, its regulatory roles in gastric motility are still controversial. Therefore, we investigated the functional roles of histamine in gastric motility. Adult male Syrian hamsters (*Mesocricetus auratus*) were anesthetized with isoflurane and exsanguinated via the axillary arteries. The stomach preparations were dissected out and were mounted in a Magnus tube, and mechanical responses were recorded using a force transducer. Exogenous application of histamine evoked regular, periodic contractions in the stomach smooth muscle. An H_1 receptor agonist reproduced the contractile responses and an H_1 receptor antagonist blocked histamine-evoked contractions. Atropine and tetrodotoxin did not affect the histamine-evoked contractions. Pretreatment with drugs that inhibit the activity of ICCs abolished the effects of histamine. In conclusion, the findings suggest that histamine regulates gastric motility by acting on ICCs via H_1 receptors in the hamster. The remarkable ability of histamine to induce rhythmic contractions would be useful for treatment of gastric dysmotility. (COI: No)

1P-403

Changes of colonic transit in feeding state after abdominal open surgery in conscious rat

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The aims of this study were to investigate changes of colonic transit(CT) after abdominal surgery in conscious rats over a time series and to demonstrate the influence of feeding on CT following surgery. The operation had been demonstrated under the isoflurane for set the cannula into the cecum to connect the proximal colon. Other side of the cannula was through under the back skin to inject makers for the measure of CT. This operation is considered to represent surgical stress(SS) in this study. The rats were divided into four groups as follows: measurement at five days after surgery without/with feeding defined as the recovery group(REC and REC+F group): measurement on the day after surgery without/with feeding(SS and SS+F group). At five days or the day after the surgery, 20 metal radiopaque markers were administered to the proximal colon and visible throughout the gastrointestinal tract via soft X-ray immediately after the administration of markers up to 240 min. CT was calculated by the geometric center(GC) on the images of those. There were no differences in GC among REC and REC+F group, which means that CT was not affected by feeding in the normal state. Although GC of SS group was delayed compared to REC group, GC of REC+F group was accelerated compared to REC+F. The results show that CT following surgery was delayed and the delay in CT was accelerated by feeding. It suggests that colonic disfunction after surgery is affected by contents of the intestine. (COI: No)

1P-404

The mechanism of sexually dimorphic responses of colorectal motility by noxious stimulation in rats

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We have previously demonstrated that activation of the descending pain inhibitory pathway enhances colorectal motility in male rats. In contrast, in female rats, noxious stimulation did not enhance the motility. In this study, we aimed to clarify the mechanism underlying the sexually dimorphic responses. Colorectal motility was measured in vivo in anesthetized rats. Administration of a noxious stimulant capsaicin into the colorectal lumen enhanced colorectal motility in male but not in female. However, when a GABAA receptor inhibitor was intrathecally administered to the L6-S1 level of the spinal cord, colorectal motility was facilitated in response to the intracolonic capsaicin even in females. The capsaicin-induced responses in males were inhibited by spinal injection of both serotoninergic and dopaminergic blockers, but in females, only the serotonergic blockers effectively inhibited the response. Our findings demonstrate that the intracolonic noxious stimulation activates GABAergic and serotoninergic descending neurons in females, but serotoninergic and dopaminergic neurons are dominantly activated in males. Although both GABA and serotonin/dopamine would suppress pain transmission in the spinal cord, they exert opposite effects on the pelvic nerves, which promote enhancement of colorectal motility. Thus, the difference in the descending neurons operating after noxious stimulation would be responsible for the sexually dimorphic responses of colorectal motility. (COI: No)

1P-405

Recovery of tight junctional localization and Mg^{2+} transport of claudin-16 mutant by primaquine

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Claudin-16 (CLDN16) is expressed in the thick ascending limb (TAL) of Henle's loop and regulates the paracellular reabsorption of magnesium (Mg^{2+}). Genetic disorders of CLDN16 cause mislocalization of CLDN16, resulting in hypomagnesemia. Here, we searched for a novel drug to restore tight junctional localization of a CLDN16 mutant. A D97S mutant, which has a mutation in the first extracellular loop (ECL) of CLDN16, was mainly colocalized with endosome marker. The protein stability of the D97S mutant was lower than that of the wild-type (WT). The expression level of the D97S mutant was increased by lactacystin, a proteasomal inhibitor. Endocytosis inhibitors increased the tight junctional localization of the D97S mutant. We found that primaquine, an antimalarial agent, increased the protein stability and cell surface localization of the D97S mutant. Paracellular Mg^{2+} transport was increased by primaquine in D97S mutant-expressing cells. The expression of chaperon proteins, proteasome activity, and lactate dehydrogenase release were decreased by primaquine, and the proportion of viable cells increased. In contrast, these effects were not observed in cells expressing WT CLDN16. These results suggested that primaquine increases the tight junctional localization of the D97S mutant, resulting in a reduction in ER stress and cytotoxicity. Primaquine may become an effective treatment drug for selected patients with mutant CLDN16. (COI: No)

1P-406

Endocytosis of NKCC2 is impaired in renal tubule in moesin knockout mice

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Moesin is a member of the ezrin-radixin-moesin (ERM) family protein, which is a crosslinker between membrane proteins and actin cytoskeleton. Recently, moesin was reported to interact with $Na^+/K^+/2Cl^-$ cotransporter type 2 (NKCC2). NKCC2 plays an essential role in regulating body salt levels *via* the reabsorption in the thick ascending limb of Henle (TAL). However, the physiological roles of moesin in the kidney remain unclear. Here, we examined the physiological roles of moesin in the regulation of renal function *in vivo* by using male moesin-null (*Msn*$^{-/y}$) mice.

To evaluate the cell surface expressions and endocytosis of NKCC2, we analyzed these changes using protein biotinylation in tubular suspension including TAL. We investigated the distribution of NKCC2 in lipid raft. To examine the renal physiological roles, we performed biochemical analysis of plasma and urine.

In these results, we found that apical surface expression of NKCC2 was significantly increased in *Msn*$^{-/y}$ TAL. Internalized NKCC2 was significantly reduced in the *Msn*$^{-/y}$ TAL. Lipid raft expression of NKCC2 was significantly decreased in *Msn*$^{-/y}$ mice. Significant increase of plasma Cl^- concentration was observed in *Msn*$^{-/y}$ mice. Urinary absolute excretions of Na^+ and Cl^- in *Msn*$^{-/y}$ mice were lower than those of WT mice. These results suggest that moesin regulates the apical surface expression level of NKCC2 by targeting NKCC2 to lipid raft and plays important roles in the renal electrolyte handling. (COI: No)

1P-407

Quantitative analysis of epithelial transport in proximal tubule with mathematical model

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Proximal tubule (PT) is known to reabsorb 60 – 70% of Na^+, K^+, Cl^- and water and most of necessary solutes such as HCO_3^-, glucose etc. It is generally accepted that Na^+ transport is coupled with that of partner solutes such as HCO_3^-, glucose and others from its lumen to epithelial cytoplasm, and that Na^+ is actively transported to interstitium by Na^+, K^+-ATPase in basolateral membrane. But this idea is questionable, because the total amount of partner solutes in the lumen is too small to explain the large amount of proximal Na^+ reabsorption. In addition, K^+ is reabsorbed in the PT, despite the driving force of K^+ transport generated by the Na^+, K^+-ATPase directs from the interstitium to the lumen. Wareing et al (J. Physiol. 488: 153-162, 1995) proposed that K^+ was reabsorbed by solvent drag, because K^+ reflection coefficient measured in rat PT was small. If the idea is true, K^+ cannot be transcellularly reabsorbed by the solvent drag via aquaporins highly selective to water. K^+ should be dragged by paracellular osmotic water reabsorption via "water pore" in tight junction. But it is unclear whether 60 – 70% of the filtered K^+ can be reabsorbed by this mechanism. Furthermore, no experimental techniques are available to separate transcellular and paracellular transports. To explore the reabsorption mechanisms of Na^+ and K^+, we aimed to quantitatively analyze their transports by constructing a mathematical model of PT and to propose a possible transport mechanism. (COI: No)

1P-408

Low-Pi diet-induced metabolic acidosis with alkalinuria was reversed in the Pendrin KO mice

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Although mice fed low-Pi diet (1 wk) showed hypercalcemia and metabolic acidosis with alkalinuria (Yasuoka et al, ASN2015, abstract), the pathophysiological roles of Pendrin and Ca-sensing receptor (CaSR), in the luminal and basolateral membranes of the kidney cortical collecting duct type B intercalated cells (IC-B), respectively, (Yasuoka et al, 2015), are unknown. Methods: Pendrin KO and WT mice (10 weeks, male) were fed either normal diet (1% Pi + 1%Ca) or low-Pi (LP) diet (0.02% Pi + 1% Ca). On day 7, blood and 24-hr urine samples were collected and analyzed. Results: Plasma Ca concentrations were similarly and significantly (**P < 0.005) increased in the both mice with LP diet [KO, 9.0** mg/dl; WT, 9.8** mg/dl], compared with those in normal diet [KO, 7.3 mg/dl; WT, 7.7 mg/dl]. Plasma pH significantly (*P < 0.05) decreased from 7.37 (control) to 7.32* (LP) in WT mice but not in KO mice (from 7.36 (normal) to 7.38 (LP)). Surprisingly, but as expected, decreased urine pH in KO mice with normal diet significantly and further decreased from 5.2 (normal) to 5.0** (LP), whereas it increased from 6.2 (normal) to 7.3** (LP) in WT mice during the same conditions. Conclusion: Paradoxical metabolic acidosis with alkalinuria during LP diet-induced hypercalcemia may be due to upregulation of luminal Pendrin through stimulation of the basolateral CaSR in IC-B. (COI: No)

1P-409

Atorvastatin ameliorates renal injury in high-fat diet-induced obese rats

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Purpose

The common cause of obesity is the consumption of high-fat diet leading to renal lipotoxicity and subsequent renal dysfunction through several pathways including oxidative stress and lipid accumulation. Atorvastatin is a popular lipid lowering drug in clinical treatment. This study aimed to investigate the effects of atorvastatin on metabolic profiles and kidney function in high-fat diet induced-obese rats.

Methods

The rats were divided into normal diet and high-fat diet (HF). After obesity being induced by high-fat diet for 16 weeks, the HF group was randomly divided into HF and high-fat diet treated with atorvastatin (HFA) by oral gavage. After 20 weeks, the rats were sacrificed, blood and kidney tissue samples were collected to investigate the effects of atorvastatin on kidney function, lipid accumulation and oxidative stress.

Results

Plasma cholesterol, insulin, glucose, and HOMA index as well as the elevation of renal cholesterol and triglyceride content were markedly increased in HF group. HF also showed to impair renal function including renal organic anion transporter 3 along with the increases in renal oxidative stress and apoptosis. After treated with atorvastatin, lipid accumulation, oxidative stress and kidney function were improved.

Conclusion

These data indicated that atorvastatin can restore kidney dysfunction in obese rats. These alterations may be due to the effects of atorvastatin on metabolic parameters, insulin resistance, renal lipid accumulation. (COI: No)

1P-410

Protective role of COUP-TFII against cisplatin-induced acute kidney injury

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[Purpose] The chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) plays essential roles in organogenesis of embryos. Recently COUP-TFII is also implicated in several diseases in adults. Here we focus on the role of COUP-TFII in cisplatin-induced acute kidney injury (AKI). [Methods] All animal experiments follow the National Institutes of Health guide for the care and use of Laboratory animals. Male tamoxifen-inducible COUP-TFII-knockout mice or control mice were treated with cisplatin at 12 weeks old. The kidney samples were subject to morphological studies, TUNEL assay, immunohistochemistry and RT-qPCR. Serum levels of creatinine and TNF-a were measured. [Results] Administration of cisplatin induced a more severe AKI in adult COUP-TFII-knockout mice. An increase in dead cells in both the proximal tubules and thick ascending limb of Henle's loop (TAL) was observed in knockout kidneys. The expression levels of COUP-TFII decreased in the TAL by cisplatin administration. There was no difference in the expression levels of transporter mRNAs responsible for cellular cisplatin uptake between control and knockout kidneys. COUP-TFII-knockout mice exhibited an elevation in serum TNF-a levels 24 hours after cisplatin exposure, suggesting the involvement of the TNF-a pathway. [Conclusions] COUP-TFII protects kidneys against cisplatin-induced AKI and may be a potential therapeutic target for AKI. (COI: No)

1P-411

Possible Role of Garlic Oil in Ameliorating Renal Injury after Liver Ischemia/Reperfusion in Rats

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Acute liver failure induces kidney injury through inflammation and oxidative stress. This study investigated the effects of garlic oil supplementation on renal functions after liver ischemia/reperfusion (I/R). Forty adult female Wistar rats were randomly divided into control, garlic oil-supplemented, liver I/R, and garlic oil-supplemented I/R groups. Liver ischemia was performed in anesthetized rats for 45 min then reperfusion was for 24 hours in metabolic cages. Garlic oil was administered 2 weeks prior to I/R. Plasma samples were used for determination of liver enzymes and creatinine levels. 24 hour urinary samples were assayed for albumin, volume and creatinine concentration to calculate glomerular filtration rate (GFR). Right kidney specimens were used for determination of hemoxegenase1 (HO1). Compared to control group, liver I/R group exhibited significantly elevated liver enzymes, plasma creatinine, urinary albumin, mitochondrial NAD+ in liver and kidney tissues and lowered GFR. The treated I/R group had significantly lowered plasma levels of liver enzymes, urinary albumin and mitochondrial NAD+ in liver and kidney tissues compared to liver I/R group in addition to upregulated HO1 gene expression in renal tissues. Liver I/R caused renal impairment through oxidative stress, while garlic oil supplementation partially improved such impairment by limiting oxidative stress and enhancing gene expression of HO1 in renal tissues. (COI: No)

1P-412

A novel NEU mutagenesis model rat of chronic kidney disease

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Purpose: The aim of this study is characterization of a new model rat of chronic kidney disease. The rat was generated by chemical mutagen ethylnitrosourea (ENU), which induces random point mutation on the genome. To eliminate mutations that might have been generated by ENU in other chromosomal regions of the locus, more than five backcross generations were performed against the F344/NSlc inbred background. A single gene was confirmed by linkage analysis and direct sequencing of some candidate genes using a next generation sequencer.Methods: Biochemical examinations including serum albumin, urinary albumin, BUN, total cholesterol, glucose, and hemoglobin were conducted at 3, 5, 7, 9, and 11 months of age in wild type, heterozygous and homozygous mutant rats. Pathological examinations of multiple organs were conducted.Results: The most prominent phenotype of this model rat was naturally progressive urinary albumin. Cardiovascular disease, chronic obstructive pulmonary disease, lipid metabolism abnormality, anemia, and osteoporosis were also exhibited in the course. Conclusions: This model rat is useful in the disease state analysis of multiple organ diseases because previously mentioned multiple disease occurs at the same time. Examples would be cardio-renal, cardio-pulmonary, and renal-brain relations. (COI: No)

1P-413

Pathogenic role of ERK1/2-mTORC1 axis in adriamycin-induced glomerulosclerosis

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TGF-β plays crucial roles in the development of focal segmental glomerulosclerosis (FSGS), however, key molecular pathway remains unknown. Here we described the pathogenic mechanism that links mTORC1 activation by TGF-β in murine model of FSGS induced by adriamycin (ADR) injection. ADR clearly augmented p-Smad3 and p-S6RP expression after day 1 and these changes persisted till day 14. ADR-induced mTOR activation was completely prevented by SB431542, a pharmacological inhibitor of TGF-β receptor-I activation. Treatment of SB431542 or rapamycin significantly suppressed glomerular fibrosis, Col4α3 and PAI-1 expression and restored nephrin, synaptopodin, WT-1, podocin and podocalyxin caused by ADR. FDA-approved MEK inhibitor trametinib blunted TGF-β1-induced mTORC1 activation in podocytes. Trametinib, even at lower doses, effectively ameliorated ADR-mediated TGF-β, PAI-1, fibronectin, α-SMA upregulation and prevented proteinuria with elevated serum albumin level. Notably, rapamycin suppressed upstream Smad3 and ERK1/2 phosphorylation *ex vivo* and *in vivo* studies. ERK inhibition also down-regulated p-Smad3, indicating pathologic paracrine signalings by forming a positive feedback loop. Taken together, accentuated TGF-β-ERK1/2-mTORC1 pathway is suggested as an important therapeutic target for glomerulosclerosis. Blocking this vicious loop by trametinib might offer a new strategy in ameliorating albuminuria and FSGS progression. (COI: No)

1P-414

Role of TRPV3-ANO1 interaction in keratinocyte wound healing

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TRPV3 is a member of highly calcium-permeable nonselective cation channel. This channel is strongly expressed in skin keratinocytes, and involved in warmth sensation, itch, wound healing, and several cytokine secretions. Previous studies have shown that anoctamin1 (ANO1), a calcium-activated chloride channel, is activated by calcium influx through TRPV1, TRPV4 or TRPA1. These TRPs-ANO1 interactions are important for TRPs-mediated physiological functions. ANO1 is also expressed in epithelial cells. Therefore, ANO1 could have physiological significance with TRPV3 in keratinocytes. The aim of this study is elucidation of interaction and physiological function of TRPV3-ANO1 interaction in keratinocytes. We investigated TRPV3-ANO1 interaction in HEK293T cells, and observed ANO1-mediated currents upon TRPV3 activation. Furthermore, we studied their functional interaction in normal human epidermal keratinocytes (NHEK). We observed chloride currents upon TRPV3 activation in NHEK. Moreover, these chloride currents depended on extracellular calcium. This result suggests that ANO1 interacts with TRPV3 in keratinocytes. Then we investigated effects of an ANO1 blocker with an *in vitro* wound-healing assay using NHEK. An ANO1 blocker inhibited cell migration. Low chloride medium also inhibited the wound-healing process. These results indicate that chloride influx through ANO1 activity enhances the wound healing in keratinocytes. (COI: No)

1P-415

Functional analyses for a Ca^{2+} binding site of TRPM4 and TRPM5 channels

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Transient receptor potential cation channel subfamily M member 4 (TRPM4) and member 5 (TRPM5) are Ca^{2+}-activated nonselective cation channels. Recently, electron cryo-microscopy structures of TRPM4 were reported from several laboratories. A structure of TRPM4 contains Ca^{2+} within an intracellular cavity, which is thought to be a Ca^{2+}-binding site. However, the structure is in a closed state and it has not been evaluated whether the amino acid residues which were proposed to form the Ca^{2+}-binding site are essential for the Ca^{2+}-sensitivity of TRPM4 by functional analyses. Therefore, we examined the effects of mutations of the amino acid residues at the proposed Ca^{2+}-binding site of TRPM4 and also TRPM5 using mutagenesis, inside-out patch clamp technique, and whole-cell patch clamp technique. As a result, the mutations of the proposed amino acid residues of TRPM4 severely reduced Ca^{2+}-sensitivity. The mutations of the same amino acid residues of TRPM5 also reduced their Ca^{2+}-sensitivity. From these results, the proposed Ca^{2+}-binding site of TRPM4 has been proved to be the actual one by functional analyses, and in the same manner the corresponding amino acid residues of TRPM5 also has been revealed to form a functionally necessary Ca^{2+}-binding site. (COI: No)

1P-416

Enhanced activity by NKCC1 and SLC26A6 in cardioplegic arrest of db/db heart

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Diabetic heart dysfunctions during cardiac surgeries have revealed several clinical problems associated with ion imbalance. In this study, we modified the Langendorff-free cardioplegia and identified the involved ion transporters in db/db heart. Enhanced expression and mis-localized Na^+-K^+-Cl^- cotransporter NKCC1 was observed in the db/db heart. Enhanced NKCC1 activity was observed in the left ventricle of db/db during cardioplegia-induced arrest. Intracellular pH changes by chloride/bicarbonate exchange activities in the left ventricle were enhanced in db/db. The Cl^- transporting activity in left ventricle strips of db/db was increased as compared with that in wild type. The expression of SLC26A6 was evaluated its role in enhanced Cl^- movement. Expression of SLC26A6, as well as carbonic anhydrase IV, was increased. As such, the enhanced Cl^- transporting activity by NKCC1 and SLC26A6 in db/db during cardioplegia-induced arrest provides greater insight into pH dysregulation and ion imbalance-mediated diabetic heart dysfunction.

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1P-417

Involvement of thermosensitive TRP channels in temperature-dependent microglia movement

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Microglia maintain the homeostasis of the central nervous system and migrate *via* chemotaxis in their activated state. Interestingly, hypothermia was shown to reduce the microglial migration induced by ischemia, suggesting a modulation of microglia movement by temperature. Although several ion channels and transporters are known to support the microglia movement, the molecular mechanism behind the temperature-dependent movement of microglia has not yet been elucidated. Some members of the transient receptor potential (TRP) channels superfamily which exhibit thermosensitivity, constitute strong candidates for the mediation of this phenomenon. Here, we report a clear regulation of mouse microglia movement by temperature. Time-lapse experiments showed a decreased microglia movement by exposure to mild cooling, while mild heating had the opposite effect. Among the thermosensitive TRP channels expressed in mouse microglia, TRP Melastatine 4 (TRPM4) and TRP Vanilloid 4 (TRPV4) were of particular interest, and shown to be functional in microglia. By using 9-phenanthrol, an inhibitor targeting both TRPM4 and TRPV4 channels, and TRPV4 KO microglia, we observed a significantly reduced microglial movement induced by heat. All together these results suggest a role of TRPM4 and TRPV4 in the temperature-mediated microglia movement and *in vivo* microglia movement observed by two-photon imaging is now under investigation in order to discriminate the precise involvement of these ion channels. (COI: No)

1P-418

Characterization of TRPA1 from disease vector mosquitoes

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Mosquitoes are the primary vectors for transmission of malaria and other epidemic diseases. The transient receptor potential channel, subfamily A, member 1 (TRPA1) channel of mosquitoes plays an important role in nociception. However, the physiological characteristics of mosquito TRPA1 have not been systematically studied. Here, TRPA1 from *Anopheles gambiae* (Ag), *Anopheles stephensi* (As), *Aedes aegypti*(Aa) and *Culex pipiens pallens*(Cp) were investigated. The responses of mosquito TRPA1s to heat or chemical stimuli were examined with calcium-imaging and whole-cell patch-clamp methods. Multiple TRPA1 channels have been cloned from disease vector mosquitoes, and several TRPA1 variants were identified. Fourteen amino acids were shown to be added at the N-terminus of TRPA1B, a modification that profoundly affected channel activity. The rates of heat ramps were strictly controlled because it was reported that the density of heat-evoked current was increased with them. While the rates did not affect temperature thresholds for activation. Thermosensitivity of mosquito TRPA1 varied, and CpTRPA1 was found to have a lower temperature threshold for heat-evoked activation. Chemosensitivity of TRPA1 channels revealed differences not only between variants but also among orthologues. Finally, we discovered 3 novel mosquito TRPA1 agonists. Better understanding of the functional properties of mosquito TRPA1 may permit the design of improved control methods for mosquito-borne diseases. (COI: No)

1P-419

Simultaneous intracellular temperature imaging during patch-clamp recording of TRPV1 activity

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Temperature ranges for the activity of thermosensitive transient receptor potential (TRP) channels have been previously determined. The temperature was measured with a physical probe located in the close vicinity of the patch-clamped cell in the extracellular solution. However, the accurate temperature thresholds for activation of thermosensitive TRP channels expressed in intracellular compartments and plasma membrane are not well known. In the last several years, there was a great progress in terms of methods for the measurement of intracellular temperature with various chemical probes, as well as for the heat stimulation of cells. These methods enable us to detect a more accurate temperature difference in a cell so that we can modulate and record the precise temperature at the plasma membrane. We attempt to measure the channel activity and temperature changes on the plasma membrane simultaneously.

We developed a combination system of patch-clamp and fluorescence imaging systems. We attempted to observe the channel activity of rat TRPV1 (rTRPV1) using a patch-clamp method and monitor the intracellular temperature using a genetically encoded ratiometric fluorescent temperature indicator (gTEMP) expressed in HEK293T cells. We would like to report the results of temperature ranges for the activity of rTRPV1 channel obtained from simultaneous measurement of an intracellular gTEMP and an extracellular physical temperature probe. (COI: No)

1P-420

A key interaction for modulation of voltage dependence by phosphoinositides in two-pore channel 3

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Two-pore channels (TPCs) are a family of Na^+ channels that have 2 repeats of 6 transmembrane helices. Each of the repeats is composed of a voltage sensor domain and a pore domain (S5-S6). In our previous report, we analyzed TPC3 from *Xenopus tropicallis* (XtTPC3) and showed that the phosphoinositide (PI) binding to the 1st repeat potentiates the voltage dependent activation which is primarily governed by the 2nd repeat, using two-electrode voltage-clamp in *Xenopus laevis* oocytes expression system. We also identified a key interaction for PI-dependent modulation formed between the 1st and 2nd repeats. The XtTPC3 homology model based on the mouse TPC1 structure suggested the presence of an electrostatic interaction between Arg297 in the 1st S6 and Glu665 in the 2nd S6, which is further reinforced by the hydroxyl group of Tyr293. R297E showed a reduced potentiation of the voltage dependence by PI. E665R did not show detectable current, but R297E/E665R retained a similar extent of potentiation as WT. Y293F and Y293A, in which a hydroxyl group with a bulky side chain of Tyr is removed, generated no detectable currents, while Y293Q and Y293H with polar and bulky side chains showed currents with decreased potentiation. These results suggest that the interaction between Tyr293 and Arg297 in the 1st S6 and Glu665 in the 2nd S6 is important for PI-dependent modulation of the voltage dependence, possibly by coupling the PI-dependent conformational change with the voltage-dependent one. (COI: No)

1P-421

Inhibition of IL-10 transcription by $K_{Ca}3.1$ K^+ channel activation in human T-cell lymphoma

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IL-10 from tumor-infiltrating lymphocytes and macrophages, lymphoma, and carcinoma cells facilitates escape from tumor immune surveillance. The objective of the present study is to elucidate the involvement of Ca^{2+}-activated K^+ channel, $K_{Ca}3.1$ in IL-10 expression and production using the human T-cell lymphoma HuT-78 cells. In HuT-78 cells, IL-10 gene expression and production were significantly reduced by the treatment with the $K_{Ca}3.1$ activator. Western blotting showed that the protein expression ratio of P-Smad2/Smad2 was significantly decreased by the treatment with $K_{Ca}3.1$ activator in HuT-78 cells. Concomitant with this, the nuclear translocation of P-Smad2 was significantly inhibited by $K_{Ca}3.1$ activator. Furthermore, the $K_{Ca}3.1$ activator-induced transcriptional repression of IL-10 disappeared with the pre-treatment with the calmodulin kinase II (CaMKII) inhibitor, KN-62, and $K_{Ca}3.1$ activator-induced decreases in the nuclear translocation of P-Smad2 were also prevented by the pre-treatment with KN-62. Taken together, the $K_{Ca}3.1$ activator-induced transcriptional repression of IL-10 is due to the inhibition of the nuclear translocation of P-Smad2 in HuT-78 cells, resulting in the prevention of P-Smad2/3 complex formation in nuclei, and the activation of CaMKII induced by $K_{Ca}3.1$ activators suppresses the constitutive activation of P-Smad2/3 in HuT-78 cells. $K_{Ca}3.1$ activators have potential as a therapeutic option to suppress the tumor-promoting activities of IL-10. (COI: Properly Declared)

1P-422

Ion Permeation of Voltage Sensor and its Foundation Structure

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Ion transport across the membrane is based on the principle of the membrane protein forming an aqueous pore structures. Voltage-sensing phosphatase (VSP) is the non-conducting membrane protein for ions which consists of four transmembrane domains (S1-S4) and the cytoplasmic PTEN phosphatase. Two crystal structures of the voltage-sensor domain, the activated and resting forms, have been determined, demonstrating the voltage sensor rearrangement across the membrane. These also indicate the existence of the water cavity in the transmembrane region, but in the wild type, it is clogged with amino acid residues in the both states. In an attempt to form a pore in the voltage sensor, we made mutants and analyzed their electrophysiological properties using the two-electrode voltage clamp recording technique. By varying the external medium pH, introductions of aspartate into S1, S2 and S3 yielded H+ currents, and mutations of S4 showed ionic conductance. Next, we evaluated the mechanical movement of S4 of the mutants by analyzing the coupling with the PTEN function at its downstream. Our findings will help validate the proposed aqueous pore structure and ion permeation mechanism for the voltage sensor, as well as the operative mechanism of the voltage sensor. (COI: No) (COI: No)

1P-423

Identification of amino acids involved in the 4-isopropylcyclohexanol action on TRP channels

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Several transient receptor potential (TRP) channels play important roles in the sensory nervous system, including TRP vanilloid (TRPV1), TRP ankyrin 1 (TRPA1), TRP melastatin 8 (TRPM8) and TRPV4. These channels are critical for sensing natural substances. Although their inhibition would be the significant strategy for pain reduction, no channel antagonists are available in the clinical setting. This is in part due to that specific inhibition of these channels can have side effects coincidently. Takayama et.,(2017) revealed that 4-isoproylcyclohexanol (4-iPr-CyH-OH) could inhibit both cation (TRPV1, TRPA1, TRPM8, and TRPV4) and ANO1 channels suggesting that 4-iPr-CyH-OH is the potential component for designing optimal analgesia agents. However, the underlying mechanisms and action sites of 4-iPr-CyH-OH on these channels are unknown. 4-iPr-CyH-OH is a compound with widespread biological effects. Like other compounds such as capsaicin, menthol, allicin, carvacrol, eugenol, and vanillin, 4-iPr-CyH-OH activates and modulates TRP ion channels.

In this study, using a patch-clamp method, the inhibitory effects of 4-iPr-CyH-OH on TRPV1-2, TRPV4, TRPV6, TRPM2, TRPM5 and TRPM8 and activating effect were observed. We found that 4-iPr-CyH-OH acts as a partial agonist of TRPV1, TRPV4, TRPM8, and TRPA1. 4-iPr-CyH-OH also showed prolonged inhibition of TRPV1, TRPV4, TRPA1 and TRPM8 following activation. Here we demonstrated bimodal actions on several TRP channels of 4-iPr-CyH-OH. (COI: No)

1P-424

TRPV1 and ANO1/TMEM16A interaction in inflammatory pain conditions

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Transient Receptor Potential (TRP) channels directly and functionally interact with anoctamin 1 (ANO1, also known as TMEM16A). Most of the TRP channels have high calcium permeability and ANO1 is a chloride channel activated by intracellular calcium. Therefore, directions of chloride movements through ANO1 depend on equilibrium potentials for chloride ions of the cells. Intracellular chloride concentrations of dorsal root ganglia (DRG) neurons expressing ANO1 are kept high. Accordingly, ANO1 activation induces neuronal excitation through chloride efflux-evoked depolarization in DRG neurons. ANO1 was activated by calcium influx through TRPV1 upon activation by capsaicin in HEK293T cells and DRG neurons. Furthermore, ANO1 activation enhanced the action potential generation depending on TRPV1 in DRG neurons. In fact, capsaicin-induced pain-related behaviors were reduced by a concomitant administration of an ANO1 antagonist. Additionally, slight activation of TRPV1 upon phosphorylation by the protein kinase C activator, PMA, significantly induced ANO1 currents in HEK293T cells at room temperature. TRPV1-ANO1 interaction is involved in enhancement of action potential generation in DRG neurons. Moreover, phosphorylated TRPV1 can strongly activate ANO1 although the increase in TRPV1 activity is weak. These results indicate that ANO1 inhibition is effective to reduce pain sensation both in acute and inflammatory pain conditions. (COI: No)

1P-425

DNA origami scaffolds as templates for Kir3.1/3.4 heterotetrameric channels

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In native systems, scaffolding proteins play important roles in assembling proteins into appropriate multimeric complexes to regulate signal transduction. However, this concept is yet to be incorporated in assembling functional transmembrane protein complexes of artificial systems. To address this issue, we employ the DNA origami as templates that arrange proteins at specific positions in nanometer size complexes. Here, we demonstrate that K^+ channel proteins are assembled through zinc-finger protein (ZFP)-adaptors at specific locations on DNA origami scaffolds as functional channels in vitro and in living cells. More specifically, when Kir3.4 K^+ channel proteins fused with ZFP adaptors are targeted at its specific recognition DNA sequences arranged in an optimized distance (17 × 18 nm) on the DNA origami, they assemble into tetrameric channels sensitively recognized by the selective inhibitor tertiapin-Q (TPN_Q). The binding of TPN_Q is directly observed as a reduced nanometer size of single Kir3.4 channel complexes through high-speed atomic force microscopy. Strikingly, electrophysiological experiments reveal that intracellular dialysis of DNA origami scaffolds with targeting sequences significantly elevates functional expression of ionic currents via hetero-tetrameric Kir3.1/Kir3.4 channels in living HEK293T cells. The results demonstrate great potentiality of DNA origami scaffolds as templates to precisely control the oligomerization states of membrane proteins. (COI: No)

1P-426

A tension-modulated modality of the KcsA channel exclusive for acid-activated state

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Proteins embedded in the cell membrane are subjected to membrane's chemical and physical modifications. Among the membrane lipids, sterols have been shown to exhibit regulatory behavior to various membrane proteins. Here, we examined the action of membrane sterols on a prototypical potassium channel, KcsA, using the contact bubble bilayer (CBB) method. Sterols, involving cholesterol, epicholesterol, ergosterol, and lanosterol, were perfused around the KcsA channel embedded in CBB (membrane perfusion). The activation gate of the channel closed upon administration of all the sterols tested. Subsequently, changes in the physical properties of CBB were analyzed in the presence of sterols. We found that the closure of the activation gate correlated well with the sterol-induced reduction of the bilayer tension rather than bilayer thickness. Accordingly, bilayer tension was mechanically manipulated in the CBB, and the channel was closed by reducing the bilayer tension. The above experiments were performed at acid pH-activated state, but at the resting state of the channel at neutral pH, the activation gate never opened upon application of high bilayer tension. This is distinct from conventional stretch-activated channels and accordingly, the KcsA channel is assigned as tension-modulated. This modality has not been recognized since the inherent lipid bilayer tension is high, where the KcsA channel remains open. (COI: No)

1P-427

Determinants of Ba^{2+} sensitivity in zebrafish ROMK channels

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(Purpose)

ROMK (Kir1.1) channels are renal inward-rectifier potassium channels. In human genome, there is only a single ROMK gene (*KCNJ1*), while zebrafish possesses seven ROMK genes in its genome. Three out of seven zebrafish ROMK genes are functional (*kcnj1a.1, kcnj1a.2 and kcnj1b*). They showed various Ba^{2+} sensitivity: kcnj1b channel was the most sensitive to external Ba^{2+}, and kcnj1a.2 channel was the least sensitive. In this study, we tried to identify which part or amino acid residues determine the different Ba^{2+} sensitivity.

(Methods)

We made point mutants of the zebrafish ROMK channels. ROMK channels were expressed in *Xenopus* oocytes and analyzed by two electrode voltage clamp.

(Results)

In Kir2.1, E125 in the outer pore region and T141 close to the selectivity filter are responsible for the Ba^{2+} sensitivity (Alagem, J Physiol, 2001). Both of these amino acids are conserved in *kcnj1b,* while both are changed in *kcnj1a.2*. We made two kcnj1b mutants (E100Y and T117A) and two kcnj1a.2 mutants (Y104E and A121T) to examine if these two amino acid residues determine the Ba sensitivity. T117A of kcnj1b showed slightly reduced Ba^{2+} sensitivity and A121T of kcnj1a.2 showed slightly increased Ba^{2+} sensitivity. On the other hand, E100Y and Y104E did not affect the Ba^{2+} sensitivity at all.

(Conclusions)

While the amino acid residues of the selectivity filter partly affects the Ba^{2+} sensitivity, there should be other amino acid residue(s) which determines the Ba^{2+} sensitivity. (COI: No)

1P-428

Functional Interaction between TRPM8 and ANO1

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Transient receptor potential cation channel subfamily M member 8 (TRPM8), known as a cold and menthol receptor, is expressed in dorsal root ganglion (DRG) neurons. TRPM8 is activated by cold temperature, icilin, or menthol, which could cause cation influx. Several members of the transient receptor potential channel (TRP) superfamily, such as TRPV1 and TRPV4, were reported to have functional and physical interaction with anoctamin 1 (ANO1), a calcium-activated chloride channel. We found the functional interaction between TRPM8 and ANO1 in HEK293T cells. We observed chloride currents and shrinkage of HEK293T cells after applying a TRPM8 agonist, icilin. Moreover, the icilin-evoked chloride currents was significantly decreased by treating with an ANO1-specific antagonist (Ani9). These results indicate TRPM8 and ANO1 have functional interaction and the interaction could modulate the water transport in HEK293T cells. We will do immunostaining and patch-clamp experiments to further confirm the TRPM8-ANO1 interaction in HEK293T cells and DRG neurons. (COI: Properly Declared)

1P-429

Analysis of dynamic structural rearrangements of Two-Pore Na^+ Channel 3 by voltage clamp fluorometry

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Two-Pore Channel 3 (TPC3) is a voltage-gated Na^+ channel. TPC3 single polypeptide has two repeats of canonical units of voltage-gated cation channel, 6 transmembrane helices (6TM). We previously showed that the 2nd 6TM functions as the principal voltage sensor, whereas the 1st 6TM contains phosphoinositide (PI) binding site for potentiation of voltage sensitivity. We also showed that a functional interaction between the 1st and 2nd 6TMs is critical for the PI-dependent potentiation. In the present study, we aimed to elucidate the dynamic structural rearrangements of TPC3 upon PI binding and/ or voltage changes. We expressed *Xenopus tropicallis* TPC3 in *Xenopus* oocytes and detected local structural rearrangements, by labeling the critical positions for PI-dependent potentiation with fluorescent molecules. We used Cys-reactive fluorescent molecule to label the extracellular region. For the intracellular regions, we used fluorescent unnatural amino acid, which can be incorporated at any aimed position at the step of protein translation. We successfully detected voltage-dependent fluorescent changes at Ser506 and Gln507 of the voltage sensor in the 2nd 6TM and at Asn52 in the putative PI binding site. We also detected a change at Lys301 in the cytoplasmic linker region between the 1st and 2nd 6TMs. (COI: No)

1P-430

Mechano-gating of Piezo1 mutants identified in patients affected by Hereditary Xerocytosis

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Hereditary Xerocytosis (HX), or dehydrated stomatocytosis, is caused by the mutation of Piezo1, known as a non-selective cationic stretch-activated channel (SAC). The red blood cells (RBC) are compressed when they enter capillaries and the resulting mechanical forces activate Piezo1, leading to Ca^{2+} influx. The increase in internal Ca^{2+} concentration activates Ca^{2+}-activated K^+ channels, leading to the loss of H_2O caused by K^+ efflux, decreasing RBC volume. Additional mutations of hPiezo1 found in different patients having HX have been identified but the alteration of the channel gating properties has not been characterised. Using the patch-clamp technique, we characterised the ion channel activity of five hPiezo1 mutants (G782S, R808Q, G782S/R808Q, F681S, V598M) expressed in HEK293T cells. Upon mechanical stimulation with brief (500 ms) negative pressure pulses of 10 mm Hg increments, applied through the recording electrode in the cell-attached configuration, we obtained a significant increase of the current from G782S and G782S/R808Q mutants. This was also associated with a reduction of the pressure for the half-maximal activation. Meanwhile, R808, F681S, and V598M mutants remained unchanged compared to the WT channel. Furthermore, G782S and G782S/R808Q desensitize faster than the WT channel upon repeated stimulations at 1.0 Hz. These results show that G782S and G782S/R808Q mutations are critical for the mechano-gating of Piezo1. (COI: Properly Declared)

1P-431

Involvement of TRPA1 channel in FK506-incuced pain sensation

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FK506 (tacrolimus) is an immunosuppressant widely used as an ointment in the treatment of atopic dermatitis. However, local application of FK506 evokes burning sensation in atopic dermatitis patients, and its mechanism are unknown. In this study, we found that FK506 activate transient receptor potential ankyrin 1 (TRPA1) channels. In Ca^{2+}-imaging experiments, increases in intracellular Ca^{2+} concentrations by FK506 were observed in HEK293T cells expressing TRPA1. FK506-induced currents were observed in HEK293T cells expressing TRPA1 using a whole-cell patch-clamp technique. FK506 also evoked single-channel opening of TRPA1 in an inside-out configuration. Moreover, intraplantar injection of FK506 evoked licking or biting behaviors and these behaviors were almost abolished in TRPA1 knockout mice. These results indicate that FK506 might cause pain sensation through TRPA1 activation. (COI: No)

1P-432

What is the pH-gradient Sensing in the Voltage-Gated H^+ Channel?

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Voltage-gated H^+ channels (Hv channels) are responsible for sensing external pH and regulating the homeostasis in many organs. The sensor characteristics are unique; Hv channels do not sense the absolute pH value but sense the differential pH value (ΔpH) between outside and inside of a cell membrane. Extracellular alkalization relative to cytoplasm shifts the threshold of the voltage-dependent gating toward the hyperpolarization direction, while extracellular acidification shifts the threshold toward the depolarization direction, which result in the functional outward rectification of H^+ current. The molecular structure of Hv channel is also unique, where it has no canonical pore domain; the voltage sensor domain (S1-S4) bears both voltage sensing and H^+ permeation. There have been a lot of attempts to find a pH sensor, but no study can reach the molecular basis of its existence. Toward the understanding the ΔpH dependent gating, in this study, we performed patch-clamp recordings for mouse Hv channel mutants under a variety of extracellular/intracellular pH conditions. We observed that the introduction of Arg into the intracellular half of S4 decreased the sensitivity of the extracellular pH. We also analyzed various Arg mutants, and observed that they changed the balance between extracellular and intracellular pH sensitivity. In this poster I will discuss how Hv channels sense the pH-gradient. (COI: No)

1P-433

withdrawn

1P-434

Magnesium ion influx in H9c2 cells with TRPM7 gene silencing

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To study physiological roles of TRPM7, we measured cytoplasmic free Mg^{2+} concentration ($[Mg^{2+}]_i$) in myocytes with TRPM7 gene silencing.

H9c2 cells derived from the rat heart were transfected by lipofection with shRNA of TRPM7 or non-targeting shRNA (control) with green fluorescence protein (GFP) as a marker. The gene transfer efficiency was about 50%. Real-time PCR detected the decrease in the expression of TRPM7 mRNA to approximately 40% after 72 hours of the transfection. We measured $[Mg^{2+}]_i$ of a cluster of 4-10 cells (including 2-5 GFP-positive cells) loaded with a fluorescent indicator, mag-fura-2. In Ca^{2+}-free Tyrode's solution that contained 1 mM Mg^{2+}, $[Mg^{2+}]_i$ of TRPM7 knockdown cells was, on average, 1.00±0.05 mM (n=9), which was not significantly different from that of control cells (1.01±0.06 mM, n=10). The addition of extracellular Mg^{2+} (91.5 mM) raised $[Mg^{2+}]_i$ to 1.48±0.14 mM in 30 min in control cells, and to 1.14±0.07 mM in TRPM7 knockdown cells. The increment in $[Mg^{2+}]_i$ of TRPM7 knockdown cells (0.14±0.02 mM) was significantly smaller than that of control cells (0.47±0.14 mM).

In conclusion, it is suggested that TRPM7 knockdown decreases the rate of Mg^{2+} influx in H9c2 myocytes, although cytoplasmic Mg^{2+} homeostasis appears to be still maintained at normal $[Mg^{2+}]_o$. (COI: No)

1P-435

The role of TRPM4 in immune responses in keratinocytes and the novel TRPM4 agonist

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Regulation of the immune system in skin is important because skin disorders decrease the quality of life. The immune regulating mechanism in skin has not been fully clarified yet. Keratinocytes are known to be major sources of the interleukin-1a which triggers the inflammatory responses in skin. TRPM4, a calcium-activated non selective cation channel, is expressed in several types of immune cells, and regulate the secretion of cytokines and inflammatory mediators. The expression and role of TRPM4 in skin cells has not been studied so far.

In this study, we confirmed the TRPM4 protein expression in HaCaT and normal human epidermal keratinocytes. The treatment with a known TRPM4 agonist BTP2 reduced the cytokine expression induced by TNFa in HaCaT. This reducing effect was not observed in TRPM4-knockout HaCaT. These results indicate TRPM4 regulates the immune responses in keratinocytes. To develop a novel treatment for skin inflammation through TRPM4 activation, we performed the screening of TRPM4 agonist. TRPM4 activation is consider to lead membrane depolarization and reduces Ca^{2+} influx. We found that aluminum potassium sulfate reduced the Ca^{2+} influx with a Ca^{2+}-imaging method. Furthermore, we confirmed aluminum potassium sulfate evoked the TRPM4 currents using a patch-clamp method.

It is considered that aluminum potassium sulfate directly activate TRPM4, and be effective as a new skincare product which regulates the immune system in human skin through TRPM4 activation. (COI: No)

1P-436

Analysis of chondrocytes anion channel activity in vitro model of osteoarthritis

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Purpose: Osteoarthritis (OA) is difficult to treat with drugs and patients frequently require surgery. Elucidation of physiological function and construction of detailed cartilage models may facilitate future OA preventative treatments. In this work we use an *in vitro* model of inflammatory OA with isolated canine and rat chondrocytes.

Methods: Canine and rat cartilage was isolated with type-II collagenase. Cytokine treatment was TNF-α and IL-1β. Single-channel activity was recorded using cell-attached patch clamp. Ion channel expressions were analysed by functional properties and molecular identification.

Results: Single-channel analyses of both control and cytokine treated canine chondrocytes show the presence of a large number of phenotypically distinct ion channels upon hyposmotic shock. In untreated patches, they exhibited clear channel activity reversing near to E_{Cl^-} and the mean slope unitary conductance for these channels were 78.74 pS in isosmotic condition and 101.28 pS in hyposmotic condition. Following cytokine treatment, the proportion of patches exhibiting chloride-like ion channel activity was higher. In qPCR experiments, ClC-2 and ClC-7 showed over 2-fold difference in expression.

Conclusions: In this simple OA model, we have discovered that an additional phenotype of ion channel becomes evident following cytokine treatment. It is important concept of the complex etiology that these anion channels accompany OA progression. (COI: No)

1P-437

The Ca^{2+}-permeable cation TRPV3 channel: an emerging pivotal target for itch and skin diseases

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Itch or pruritus is an unpleasant cutaneous sensation that evokes the urgent desire to scratch. Itching can intensify when scratching aggravates lesions of the skin. Although acute itch is considered to be a defense mechanism that alerts the body to remove irritants, transient itching can progress to become persistent and chronic. Chronic itch can be a widespread symptom of systemic diseases including atopic dermatitis, liver disease, kidney failure, cholestasis, diabetes, and cancers. Chronic itch as an unmet medical needs due to its complex underlying mechanism presents a serious health issues. Temperature-sensitive transient receptor potential (thermo-TRP) channels have been indicated as downstream ion channel targets in transduction of itch. As a member of thermo-TRPs, the Ca2+-permeable nonselective cation channel TRPV3 is expressed abundantly in the skin keratinocytes. Recent identification of gain-of-function mutations of human TRPV3 from patients with Olmsted Syndrome characterized by severe itching and palmoplantar and periorificial keratoderma unveils its crucial role in chronic itch and skin diseases. In this presentation, I will focus on recent progress made in the understanding of TRPV3 that emerges as an attractive target for developing effective anti-pruritic therapy for chronic itch or skin-related diseases. (COI: No)

1P-438 (AP-3)

Cytoplasmic conformational changes of VSP detected by voltage clamp fluorescence spectroscopy

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Voltage-sensing phosphatase (VSP) consists of the voltage sensor domain (VSD) and the cytoplasmic catalytic region (CCR) which acts as an enzyme that dephosphorylates $PI(4,5)P_2$ regulated by membrane potential change (Murata et al. 2005). The voltage-induced regulation mechanism of the CCR has not been fully understood. We previously reported the conformational changes of the CCR by voltage clamp fluorometry with environment-sensitive unnatural fluorescent amino acid (Anap). This powerful method shed light on the cytoplasmic conformational changes of membrane proteins in living cells, but the obtained information is so far limited because detailed molecular mechanisms of change of fluorescence intensity still remain unknown.

To gain more insights, we combined a spectrometer and inverted fluorescence microscope with electrophysiological instrument. This system can observe the absolute fluorescence spectra from selected region of a cell under voltage clamp condition. Using this method, we measured the fluorescence spectra of Anap incorporated at His-237 in the cytoplasmic region of *Ciona intesinalis*-VSP expressed in HEK293T. When we applied voltage step pulses from -80 to 100 mV in 20-mV increment to Ci-VSP H237Anap expressed cell, the fluorescence spectra of Anap showed the voltage-dependent changes, which may reflect the alteration of the local hydrophobic environment around incorporated Anap due to the conformational changes. (COI: No)

1P-439

The regulation of TRPV1 channel gating by intracellular ATP

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Transient receptor potential vanilloid 1 (TRPV1) is a non-selective cation channel activated by capsaicin, noxious heat, and proton. Here, we found that intracellular ATP is essential for generating TRPV1 currents in the absence of capsaicin, and the mechanism of ATP-dependent regulation of TRPV1 currents was investigated. In the whole-cell patch-clamp recordings in HEK293T cells overexpressing human TRPV1, the cells exhibited strong outwardly rectifying currents with time-dependent activation in the absence of capsaicin. The TRPV1 outward currents were increased by elevating intracellular ATP concentration. Although TRPV1 is reported to have intracellular ATP binding sites, AMP-PNP, a non-hydrolyzable ATP analogue, caused rundown of TRPV1 outward currents. Interestingly, rundown of the currents were observed in the presence of LY294002 at 300 μM, which inhibits phosphatidylinositol 4-kinase (PI4K), but not in the presence of the drug at 10 μM, which inhibits PI3K. The results suggest that intracellular ATP might regulate TRPV1 channels via PIP_2 production. On the other hand, we found that an increase of intracellular ATP accelerated time-dependent activation of TRPV1 outward currents. In addition, voltage dependence of TRPV1 current activation was negatively shifted by elevating intracellular ATP concentration. Our present study suggests that intracellular ATP indirectly regulates the gating of TRPV1 channels. (COI: No)

1P-440

Recognition of capsaicin via transient receptor potential channel and transmembrane protein

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Purpose : Capsaicin enhances energy metabolism, such as elevating blood pressure, by increasing the inflow of Ca^{2+} into the cell via mediating TRPV1 present in the sensory nerve. It has been reported that TRPV1 frequently co-expresses in sensory nerves with TRPA1, TMEM100 and TMEM 16A (ANO1). Thus, capsaicin may exert physiological functions not only via TRPV1, but also via the TRP channel and TMEM.

Methods and Results : First, considering that TRP channels and TMEM proteins interact, we compared mRNA expression levels of each gene using *Trpv1* deficient mice. In *Trpv1* deficient mice compared with wild type mice, mRNA expression levels of *Trpa1*, *Tmem100*, and *Ano1* showed a tendency to decrease. In addition, intracellular Ca^{2+} increased by establishing primary dorsal root ganglion neuronal cultured cells reported to express TRP and TMEM using capsaicin treatment. Next, HEK293T cells expressing TRP and TMEM were established to clarify whether capsaicin cooperatively promotes Ca^{2+} influx via the TRP channel and TMEM. HEK293T cells co-expressed with TRP and TMEM showed a marked Ca^{2+} inflow increase with capsaicin treatment as compared with cells expressing only TRPV1. These studies suggested that capsaicin may exert strong physiological effects not only through TRPV1, but also through TRPA1 and TMEM.

Conclusions : In the future we will also consider other compounds that may have physiological functions through the TRP channel and TMEM using this experimental system. (COI: No)

1P-441

Regulation of TRPM7 channel activity by its kinase domain

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TRPM7 is a bifunctional protein comprised of both a channel and kinase domains. TRPM7 channel activity is regulated by intracellular Mg^{2+}, of which increase inhibits the current. We have reported that TRPM7 channel activity is inhibited by hydrogen peroxide (H_2O_2) due to increased Mg^{2+} sensitivity. In the present study, we demonstrated that deletion of the kinase domain after 1510 induced an increase of Mg^{2+} sensitivity in the channel domain (1-1509; TRPM7-CD). Since it has been reported that TRPM7 can be cleaved by caspases at 1510 and H_2O_2 can activate caspases, it was possible that the inhibition of the current by H_2O_2 was resulted from cleavage of TRPM7 by caspases. To test this possibility, the effect of H_2O_2 on a caspase-cleavage resistant mutant, TRPM7-D1510A was assessed. Similar to wild-type TRPM7, H_2O_2 inhibited the current of TRPM7-D1510A. Thus, caspases-dependent TRPM7 cleavage is not likely involved in the inhibition by H_2O_2. Interestingly, the increased Mg^{2+} sensitivity of TRPM7-CD was restored by co-expression of its kinase domain (1510-1863; TRPM7-KD) as a separate protein. Mutations in the zinc finger motif of TRPM7-KD (C1809S, C1813S, and delta Zn) failed to restore the current. These results suggest that H_2O_2 oxidizes cysteines in the zinc finger motif and thereby interrupts the proper interaction between the channel domain and the kinase domain to increase its Mg^{2+} sensitivity. (COI: No)

1P-442

Mapping the agonist binding site of the FMRFamide-gated Na^+ channel

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FMRFamide-gated Na^+ channel (FaNaC) is a homo-trimeric peptide-gated sodium channel and a member of DEG/ENaC family. The crystal structures of the acid-sensing ion channel which is also a member of this family show that the extracellular domain of DEG/ENaC channels is subdivided into five subdomains, i.e., Finger, Knuckle, Thumb, Palm, and beta-ball domains. The palm domain is connected to the transmembrane helices which construct the channel pore. FaNaC is activated by a molluscan cardioactive peptide, FMRFamide. Some orthologs of FaNaC identified from different species show different sensitivity to FMRFamide. Earlier studies on the chimeric channels of such orthologs identified a region which determines the sensitivity of FMRFamide (Cottrell et al, 2001). In the 3D-model of FaNaC made by homology modeling, the region is in the Finger domain of FaNaC. To better understand the binding site of FMRFamide in FaNaC, we carried out mutagenic experiments in the Finger domain of *Aplysia* FaNaC. We hypothesized that phenylalanines of FMRFamide may interact the aromatic moieties of FaNaC. Here, we show the results of mutagenic experiments directed to the aromatic amino acids which are within or close to the presumed binding site identified by Cottrell et al (2001). Our results show that the aromatic moieties in the Finger domain may construct the binding site of FMRFamide. (COI: No)

1P-443

Development of tonotopic differentiation of axon initial segment in avian nucleus magnocellularis

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The axon initial segment (AIS) is a specialized neuronal subregion enriched with voltage-gated Na^+ channels, and is the most critical site to determine the output of neurons. Recently it was revealed that the AIS differs in its distribution in a cell-specific manner. However, how this distribution is determined in individual neurons remains elusive. In this study, we addressed this issue in neurons of avian cochlear nucleus (nucleus magnocellularis, NM), which show tuning-frequency-specific differentiation in the length of AIS in mature animals; shorter AIS in neurons with higher tuning frequency. We first examined AIS length in NM during development with immunostaining of Na^+ channels, and found that the AIS was shortened to a larger extent in neurons with higher tuning frequency between embryonic day 15 and posthatch day 4, creating the tonotopic differentiation of AIS. We then recorded action potential and Na^+ current with patch clamp technique in slice preparations, and found that the biophysical features of NM neurons correlated with the structural differentiation of AIS. To further explore the involvement of activity-dependent mechanism in the differentiation, we are now trying to modulate the activity by different methods, like removal of otocysts in embryos and cochlea in posthatch animals. (COI: No)

1P-444

A calcium-binding protein S100A10 is a regulator of Maxi-Cl channel activity

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Maxi-Cl is characterized by its large single-channel conductance (300-400 pS) and known to serve as a pathway for ATP release from many cell types in response to osmotic and ischemic/hypoxic stress. We identified SLCO2A1 as the core molecule of Maxi-Cl by applying unbiased genome-wide approaches (Sabirov et al. 2017). Based on microarray analysis between Maxi-Cl-rich C127 and -deficient C1300 cells, we found that annexin A2 (Anxa2) is a modulator of Maxi-Cl activity, as reported at the last congress (Islam et al. 2015). Since Anxa2 is known to form a heteromeric complex with a calcium-binding protein S100A10, we here tested a possibility that S100A10 is also involved in regulation of Maxi-Cl activity. *S100a10* gene expression in C127 cells was over three times more prominent than that in C1300 cells. Maxi-Cl currents in C127 cells were suppressed by *S100a10* gene-specific siRNA and enhanced by increases in the intracellular Ca^{2+} concentration. Thus, it is concluded that Maxi-Cl/SLCO2A1 channels are regulated by S100A10, in a manner dependent on intracellular Ca^{2+}, presumably by forming the Anxa2-S100A10 complex. (COI: No)

1P-445

Toward the understanding of hexose specificity of Na^+D-glucose cotransporters SGLT1 and SGLT2

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Na^+/D-glucose cotransporters (SGLTs) utilize transmembrane sodium gradients for cellular D-glucose uptake. SGLT1 is abundant in small intestine where it contributes to the D-glucose absorption. Meanwhile SGLT2 largely mediates the reabsorption of glucose in the kidney. Both SGLTs are highly expressed in tumor cells to facilitate cellular D-glucose uptake and glycolysis, resulting in the tumor growth. Considering these physiological functions, SGLT1 and SGLT2 could be the targets for treatment of diabetes and cancer, and some SGLT2 inhibitors have been already approved as anti-diabetic drugs. Despite their physiological and clinical significances, molecular and structural basis of SGLT1 and SGLT2 hexose specificities remain unclear.

Here we employed the two-electrode voltage-clamp method in Xenopus oocytes expressing human SGLT1 or SGLT2. D-glucose induced an inward current in the presence of Na^+, showing the cotransport of Na^+ and D-glucose. We analyzed transport capacity of various hexoses including rare sugars D-allose, and D-allulose. The results clarified the substrate specificity of these SGLTs. Further we performed homology modeling using the structure of *V. parahaemolyticus* Na^+/D-galactose cotransporter as a template. Mutation analyses based on the structure model supported to understand the structural basis of their hexose specificity. Present analyses would contribute to the clinical strategies targeting SGLTs, on the points of controlling efficacy and side effects. (COI: No)

1P-446

The comparison of sensitivity between NaPi-IIa and NaPi-IIb activity to phosphoinositides

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Inorganic phosphate (Pi) is an essential constituent for maintenance of cell activity. Although Na-Pi cotransporter (SLC34) is the main molecule for Pi homeostasis, the regulation mechanisms of its activity on plasma membrane are largely unknown. Because the activity of various ion channels and some transporters is regulated by phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), the activity of Na-Pi cotransporter could also depend on $PI(4,5)P_2$. Therefore, we investigated $PI(4,5)P_2$ dependence of two types of NaPi, IIa or IIb, by two different approaches. One is the Xenopus oocyte system with depletion of $PI(4,5)P_2$ by using *Ciona intestinalis* voltage-sensing phosphatase (Ci-VSP) and the other is cultured cell line N2a using Pseudojanin (PJ), which has both activities of 5-phosphatase and 4-phosphatase induced by rapamycin. During current recording, depolarization pulse was applied to activate Ci-VSP to induce reduction of $PI(4,5)P_2$ on the plasma membrane. After depolarization, mNaPi-IIb current significantly decreased compared to before depolarization, suggesting $PI(4,5)P_2$ dependence of mNaPi-IIb. On the other hand, mNaPi-IIa current did not change before and after $PI(4,5)P_2$ depletion. We also confirmed that mNaPi-IIb current decreased after $PI(4,5)P_2$ depletion by PJ. These results indicate that mNaPi-IIb activity is regulated by $PI(4,5)P_2$. We are now trying to identify sites responsible for $PI(4,5)P_2$ sensitivity of mNaPi-IIb. (COI: No)

1P-447

An endosome-resident zinc transporter negatively regulates systemic dsRNA spreading in *C. elegans*

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Functional RNAs, including double-stranded RNA (dsRNA), regulate gene expression in base-paring mechanisms. In *C. elegans*, dsRNA spreads from one cell to another and leads to RNA silencing in a cell nonautonomous manner. This phenomenon is called systemic RNAi and can be used as a model for systemic RNA spreading. Previous studies suggested that systemic RNAi depends on vesicle trafficking. However, the molecular mechanisms by which cells export and import dsRNA are still poorly understood. We previously reported that *rsd-3*, a gene encoding conserved ENTH domain protein, is required for efficient import of silencing RNA. Here, from a genetic screen for mutations able to suppress the defective RNAi phenotype of*rsd-3* mutants, we identified an allele of a zinc transporter gene. This zinc transporter is widely expressed during development, acts in a cell nonautonomous manner, and is mainly localized to late endosome. The mutant showed the enhanced RNAi phenotype. We tested whether this phenotype is caused by downregulation of endogenous RNAi or upregulation of exogenous RNAi, which are antagonistic. We found that certain genes targeted by the endogenous RNAi pathway showed normal expression. Instead, its null mutants showed altered RAB-11 positive vesicle size in intestine, suggesting its potential involvement in membrane trafficking. Our data uncovered the zinc transporter as a novel cellular factor acting in regulation of systemic RNAi. (COI: No)

1P-448

Evaluation of effects of empagliflozin on mouse ventricular myocytes

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Purpose: Empagliflozin (EMPA), an inhibitor of sodium-glucose cotransporter 2, reduces the risk of cardiovascular death in patients with type 2 diabetes. It was reported that EMPA directly inhibits Na^+/H^+ exchanger (NHE) in rabbit and rat cardiomyocytes, lowering cytoplasmic Na^+ (Na_i), cytoplasmic Ca^{2+} and increasing mitochondrial Ca^{2+} (Ca_m) concentrations. We aimed to study the effects of EMPA on mouse ventricular myocytes. **Methods**: The experimental protocol was approved by the Institutional Animal Care Committee in University of Fukui. Ventricular myocytes were enzymatically isolated from mice using Langendorff perfusion system. Na_i was measured as SBFI fluorescence ratio (excitation 340/380 nm, emission 510 nm) using a fluorescence microscope and an EMCCD camera, and mitochondrial NADH fluorescence was measured with excitation 340 nm and emission 460 nm. Experiments were performed at 30-35°C, superfusing myocytes with HEPES-buffered Tyrode solution (pH 7.4). **Results**: Superfusion of myocytes with 1 μM EMPA did not significantly decrease Na_i, but rather increased it. Similar results were obtained using a Tyrode solution with pH 7.0 to enhance NHE activity. If EMPA could increase Ca_m, then mitochondrial NADH should increase. However, 1 μM EMPA did not increase NADH fluorescence. **Conclusions**: These data suggest that the proposed mechanism of action of EMPA is not applicable to mouse ventricular myocytes. (COI: No)

1P-449

A united chemotherapy to reverse drug resistance in ovarian cancer

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The adaptive drug resistance has been the main obstacle for chemotherapy in ovarian cancer patients. Many studies have proved the increased drug efflux by the ATP-binding cassette (ABC) transporters was the main cause of drug resistance acquisition. We have detected that several transporter genes, especially P-gp, in PTX-resistant A2780 cell line were dramatically higher than the A2780-WT. These results indicate that finding a promising way to block ABC transporters and reverse the drug resistance in tumor cells is the key point for successful ovarian cancer chemotherapy.

Zebularine was reported to be the most effective methyltransferase inhibitor with less cytotoxicity, which can repress the transcription of ABC family. First, with the treatment of zebularine, the expression of P-gp was significantly reduced in the PTX-resistant A2780 cells. Accordingly, the drug sensitivity was increased. Then BAPTA-AM, a cell permeable Ca^{2+}chelator, was used to block the function of existed transporter pumps. Results showed that BAPTA-AM could not only inhibit the function of transporter genes with less drug expelled into the medium, but also inhibit the expression of P-gp. Furthermore, with the combination of zebularine and BAPTA-AM, the drug resistant A2780 cells showed much higher sensitivity to PTX. Therefore, this combined therapy may be applicable in the clinical treatment of PTX resistant patients. (COI: No)

1P-450

AMP-activated protein kinase dissociates vesicle association of clathrin heavy chain CHC22

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Clathrin heavy chain CHC22 is essential for formation of the GLUT4 storage vesicle (GSV) in human skeletal muscle. CHC22 controls protein sorting from late endosomes to the trans-Golgi network and is associated with expanded GLUT4-containing compartments that accumulate in the muscle of patients with insulin-resistant type 2 diabetes. CHC22 has been lost in mice, which form their GSV using the ubiquitous CHC17 clathrin isoform. We have compared the biochemical properties of CHC22 to those of CHC17 to define species-restricted characteristics of the membrane traffic pathway that leads to human GSV formation. CHC22 is missing the binding sequence for the uncoating ATPase Hsc70, which interacts with auxilin to uncoat CHC17, and was not disassembled by this protein complex. However, cell starvation caused reduction in membrane-associated CHC22 along with its interacting adaptor molecules. Stimulation of AMPK activity by AICAR phenocopied this effect, but treatment of cells with rapamycin, a mTOR antagonist did not. Thus, CHC22 membrane dissociation results from AMPK activation, a pathway that increases GLUT4 availability. Notably however, insulin treatment did not affect CHC22 membrane association. Due to these observations, we hypothesize that due to its membrane association properties CHC22 contributes to forming a human GSV that is more stable than the murine GSV, and consequently more prone to insulin resistance. (COI: No)

1P-451

Function analysis of NHE1 using a strategy of cardiomyocyte differentiation from human iPS cells

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There are still many functionally unknown genes expressed in hearts. One of promising approaches is to analyze the phenotype of genes in differentiated cardiomyocytes after genetic engineering in pluripotent stem cells (iPSCs) that multiply infinitely. As the first target, we chose the gene coding the plasma membrane Na^+/H^+ exchanger (NHE1) which regulates the intracellular pH and Na^+ concentration. Knockdown (KD) and overexpression (OE) of NHE1 were performed by CRISPR interference (CRISPRi, modified method of CRISPR/Cas9) (>95% KD efficiency) or by stable transfection of vector under the control of CAG promoter (>100 times expression, respectively. We used doxycyclin (Dox)-inducible systems which induce KD and OE. (1) Interestingly, nearly all iPSCs died within 48 hours after OE of NHE1. This reaction was found to be due to the secondary necrosis following apoptosis. Such cell death did not occur in mesendoderm cells 2 days after start of differentiation and was suppressed by loss-of-function mutation of NHE1 (E262I), suggesting that undifferetiated iPSCs are particularly sensitive to ionic changes caused by NHE1. (2) While KD of NHE1 did not apparently affect the contraction of cardiomyocytes, OE of NHE1 often produced the irregular beating with prolonged relaxation. (3) KD of NHE1 reduced the cell size of cardiomyocytes. These results suggest that cell death of iPSCs, cardiomyocyte contraction and hypertrophy are substantially affected by ionic changes by NHE1. COI:None (COI: No)

1P-452

Developmental regulation of KCC2 phosphorylation is essential for GABA signaling and survival

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A post-natal decrease in neuronal Cl^- concentration ($[Cl^-]_i$) dependent on the Cl^--extruding K^+-Cl^- cotransporter (KCC2) establishes GABA-mediated fast synaptic inhibition. However, the mechanisms that regulate KCC2 activity during development are unknown. We showed that KCC2 phosphorylation at two threonines (T906/T1007), a key regulatory switch of KCC2 activity *in vitro*, decreased in parallel with the developmental increase in KCC2 activity and the lowering of neuronal $[Cl^-]_i$ *in vivo*. Mice engineered with homozygous glutamate (E) substitution at T906/T1007 (*Kcc2*$^{E/E}$), modeling constitutive phospho-mimetic inhibition of KCC2, died shortly after birth due to apparent spontaneous respiratory arrest. *Kcc2*$^{E/E}$ mice exhibited diminished GABA-dependent neuronal Cl^- extrusion capacity, lacked spontaneous respiratory discharges from cervical spinal cord neurons (C4), and displayed severely altered locomotor rhythm recordings from lumbar spinal cord neurons (L2). *Kcc2*$^{E/E}$ mice exhibited touch-evoked generalized seizures and an anomalous neuronal distribution but normal dendritic spine morphology. These results showed that regulated KCC2 T906/T1007 phosphorylation, via effects on neuronal Cl^- homeostasis, is essential for developmental GABA signaling and rhythmogenesis, excitation-inhibition balance, and survival. (COI: No)

1P-453

Characterization of transgenic mice overexpressing dominant negative TRPM7 mutant

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Magnesium ion (Mg^{2+}) is an essential divalent cation and intracellular Mg^{2+} concentration is tightly controlled by various Mg^{2+} transporters. Therefore, Mg^{2+} transporter dysfunction may lead to a variety of diseases, such as cardiovascular diseases. Recently, several molecules of Mg^{2+} transporters have been identified. However, the regulation of Mg^{2+} homeostasis and the pathophysiological significance of Mg^{2+} metabolisms have not yet been precisely elucidated. To clear these issues, we focused on TRPM7, which is non-selective, but predominantly permeates Mg^{2+} under physiological conditions, and constructed the dominant negative TRPM7 mutant as an experimental tool. Whole-cell patch-clamp recordings revealed that TRPM7 currents in HEK293 cells were almost completely attenuated by co-expression of the dominant-negative TRPM7 mutant. Next, we generated transgenic mouse model overexpressing the dominant negative TRPM7 mutant (M7DN-Tg). Renal specific M7DN-Tg exhibited dysregulation of serum Mg^{2+} level and urinary Mg^{2+} excretion. Interestingly, we found that vascular contractile responses in renal specific M7DM-Tg was significantly attenuated compared to the responses in wild-type mice. In renal specific M7DM-Tg, Mg^{2+}-enriched diet reversed these abnormal responses to the normal level. These results suggest that TRPM7 is involved in the regulation of Mg^{2+} homeostasis. Renal specific M7DN-Tg will be a useful animal model for studying magnesium disorders. (COI: No)

1P-454

Characterizations of the HCO_3^- transport activities of a choroid plexus-specific variant of NBC4

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Secretion of HCO_3^- at the apical side of the epithelial cells of the choroid plexus is an essential step in the formation of cerebrospinal fluid. We previously reported that a novel variant of the Na^+/HCO_3^- cotransporter 4 (NBC4g/Slc4a5-g), an electrogenic member of the NBC family, is almost exclusively expressed in the apical membrane of rat choroid plexus epithelium at exceptionally high levels. Since NBC4g has the same structure as NBC4c, the general form of NBC4, in the membrane-inserted regions consisting of 12–14 transmembrane spans and the C-terminal tail, it is expected to retain most of the electrophysiological properties previously characterized for NBC4c including electrogenicity. However, the differences between the HCO_3^- transport activity of NBC4g and NBC4c remain unclear. In the present studies, we revealed a marked difference in the cAMP dependency between NBC4c and NBC4g, suggesting the possible involvement of the N-terminal cytoplasmic tails in defining their characteristic electrophysiological profiles. (COI: No)

1P-455

Glycative stress influences skeletal muscle growth and cell growth signaling in mice

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[Aims] Glycative stress are related with deterioration of skeletal muscle functions. In this study, we aimed to examine the effect of advanced glycation end products (AGEs) on skeletal muscle growth and cellular signaling in skeletal muscle. [Methods] Male ICR mice (5-week-old) were divided into two groups: low-AGEs fed group (L-AGEs, n=10) and high-AGEs fed group (H-AGEs, n=10), and treated with standard-diet and a heat (160 °C for 1 h) treated diet for 16 weeks, respectively. Cellular signaling change was analyzed by Reverse Phase Protein Array (RPPA) assay in C2C12 cells. [Results] There were no changes of initial body weight, final body weight, and food intake (kcal/day) between L-AGEs and H-AGEs groups. Normalized muscle weight (soleus, extensor digitorum longus, plantaris) by body weight were tended to be lower in H-AGEs group compared with L-AGEs group. Furthermore, grip strength of four limbs and wire hanging time were lower in H-AGEs group than L-AGEs group. The phosphorylation levels of 72 proteins were changed by AGEs treatment; 8 proteins were decreased and 64 were increased. The cluster analysis revealed that the cell growth signaling were affected by AGEs treatment. [Conclusions] The findings suggest that glycative stress suppresses skeletal muscle growth in mice, partly through inhibiting cell growth signaling. (COI: No)

1P-456

Intracellular cAMP induces Ca^{2+} influx in odontoblasts

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Purpose: In our previous study, we revealed functional crosstalk between cannabinoid 1 receptors and TRPV1 channels, mediated by cAMP in odontoblasts. The results suggested that cAMP might play important roles in dentin formation and/or sensory transduction mechanisms for tooth pain. However, the detailed intracellular cAMP signaling pathway, and the involvement of cAMP in Ca^{2+} signaling in odontoblasts remain unclear. We, thus, examined crosstalk between intracellular cAMP and Ca^{2+} signaling in odontoblasts. Methods: We simultaneously measured fluorescence intensities from mNeonGreen-based cAMP sensor and fura-2 in human odontoblast cells. Results: In the presence of extracellular Ca^{2+}, the application of forskolin (an adenylyl cyclase (AC) activator) or isoproterenol (an agonist of the Gs protein-coupled beta-2 adrenergic receptors) dose-dependently increased intracellular cAMP level. The increases were suppressed by application of AC inhibitor. In the presence of extracellular Ca^{2+}, the application of forskolin increased both intracellular Ca^{2+} concentration and cAMP level. In the absence of extracellular Ca^{2+}, application of forskolin increased intracellular cAMP level, but not intracellular Ca^{2+} concentration. Conclusion: These results suggested that activation of Gs protein-coupled receptors increased intracellular cAMP by stimulating AC. These increases in intracellular cAMP level is capable to evoke Ca^{2+} influx from extracellular medium in odontoblasts. (COI: No)

1P-457

P2Y6 receptor antagonist MRS2578 induces atypical signaling

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Background- P2Y6 receptor (P2Y6R) is a member of G protein-coupled receptors (GPCRs) and activated by extracellular uridine nucleotides. We previously reported that MRS2578, an antagonist for P2Y6R, suppressed cardiac remodeling induced by pressure overload (**EMBO J.** 2008). We thus hypothesized that $P2Y6R^{(-)}$ mice would show same phenotype, and investigated whether pressure overload-induced heart failure was attenuated in $P2Y6R^{(-)}$ mice.

Methods & Results- We performed transverse aortic constriction (TAC) to induce cardiac pressure overload to $P2Y6R^{(-)}$ mice. Contrary to our expectation, P2Y6R deficiency exacerbated cardiac remodeling induced by TAC. Therefore, we speculated that MRS2578 could activate P2Y6R-dependent signaling pathway in canonical purinergic signaling-independent manner. We administrated MRS2578 to WT or $P2Y6R^{(-)}$ mice, and found that MRS2578 induced upregulation of superoxide dismutase 2 (SOD2) in WT mice and this upregulation was not observed in $P2Y6R^{(-)}$ mice. We further examined localization of P2Y6R during treatment of MRS2578 in FLAG-P2Y6R-transfected HEK 293 cells and revealed that P2Y6R was internalized by MRS2578. We further found that MRS2578 induced oligomerization of P2Y6R proteins.

Summary- Our data suggest that MRS2578-induced oligomerization increased SOD2 expression. MRS2578-induced changes in localization and protein quality of P2Y6R might trigger the activation of cardioprotective signaling pathways such as SOD2. (COI: No)

1P-458

PDGF signals contribute to proliferation and migration of human prostate cancer cell

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Purpose:Prostate cancer(PC) is a common malignancy in men.Due to high proliferation, migration, invasion and metastasis rate of prostate cancer cell, it has become a major challenge to treat the PC.The androgen receptors(ARs) play important role in PC progression.A metastatic prostate cancer cell line, PC-3 cell growth and proliferation is independent of AR.The Platelet-derived growth factor(PDGF) signaling is closely related with cancer cell growth, proliferation, migration and survival.However, role of PDGF signaling in PC is not well established yet.In this study, we examined effects of imatinib, a tyrosine kinase inhibitor(TKI) on proliferation,migration and PDGFR expression in PC-3 cells.

Results and Methods: Expressions of PDGF receptors in PC3 cells were examined by western blotting, indicating PDGFRα and PDGFRβ were clearly expressed in PC3 cells.The effect of imatinib on viability of PC3 cells was quantified using Cell Counting Kit.Excessive proliferation of PC-3 cells was blocked by imatinib in a concentration dependent manner(10μM, 83.8 ±4.6%,$p<0.01$,n=7).Furthermore,migration of PC3 cells was examined using the Trans-well system. Imatinib(10μM) also inhibited PC-3 cell migration(58.5 ±10.2%,$p<0.01$).We also discuss effects of PDGFRs knockdown on growth and migration of PC-3 cells.

Conclusion: Imatinib inhibited proliferation and migration of PC-3 cells.These findings indicate that therapy of TKI may be useful as a novel therapeutic approach against metastatic PC. (COI: No)

1P-459

Single-cell imaging analysis of inflammatory JNK signaling

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«Purpose» JNK (c-junN-terminal kinase) responses to a wide range of inflammatory stimuli and mediates important physiological cellular processes such as gene expression, cytoskeletal rearrangement, and cell death. In this study, we aimed to elucidate JNK dynamics in living cells at higher resolution in time to understand its regulatory mechanism in response toinflammatory cytokine stimulation.

«Methods» Fluorescence protein-based JNK FRET reporter was engineered to analyze JNK dynamics in living HeLa cells. Periodic pulsatile IL-1β_stimuli to cells at variable frequencies were employed to analyze the frequency-responseof JNK activation, which is subjectedto systems-analysis.

«Results» We found that IL-1β-induced JNK activity was governedby a negative feedback regulation caused by a MAPK phosphatase, MKP-1. Actually,the duration of JNK activity was restricted within about an hour even when IL-1βwas given continuously to cells, and MKP-1 expression mirrored the JNK inactivation. Furthermore, JNK was repeatedly activated when IL-1β pulses were given periodically at an intervalmore than 4hr which is about as long as the lifetime of MKP-1 in cells.

«Conclusion» Our novel approach combining quantitative FRET imaging and systems-analysis revealed a key mechanism that governs JNK dynamics in livingcells. Such amechanismwould be required to avoid excessive inflammatory response while properly maintaining the response to periodic cytokine stimuli. (COI: No)

1P-460

LMHFV promotes BMSCs to Differentiate into osteoblast via a Novel lincRNA-7140 in osteoporosis rat

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Purpose Low-magnitude high-frequency vibration (LMHFV), a mechanical factor that has been proved to prevent osteoporosis, but the underlying mechanisms is poorly understood. Here we want to figure out if lncRNAs involved and what the mechanisms underlying in this process.

Methods The osteoporotic model was established using ovariectomy (OVX) SD rats, BMSCs were obtained from the control and OVX rats, then RNA–seq was performed followed by a bioinformatics analysis. BMSCs were treated with a optimal LMHFV condition. Overexpression vectors and siRNAs were transfected into BMSCs. qPCR and western blot were utilized to testify the expression.

Results A Novel lncRNA lincRNA-7140 and its potential target integrin β 2(ITGB2) were selected, and they are significantly decreased in the OVX rats' BMSCs, however, they are up-regulated in LMHFV situation both in vitro and in vivo. When overexpressed lincRNA-7140, ITGB2 increased, followed by the osteogenic differentiation markers ALP, Runx2, OCN,and ColI increased in BMSCs, as the same effects with LMHFV. Conversely, lincRNA-7140 knockdown showed the opposite results. Moreover, overexpress ITGB2 induced the upregulation of the osteogenic genes, and interestingly the expression of Wnt3a and β-catenin.

Conclusions Taken together, our results showed LMHFV can promote BMSCs to differentiate into osteoblast, probablely through lincRNA-7140 which regulate the ITGB2, in response to the mechanical stimuli, LMHFV, induces the bone formation. (COI: No)

1P-461

Effect of hydrogen sulfide and L-cysteine on the principal cells of rat cortical collecting ducts

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Hydrogen sulfide (H_2S) has been reported to be act as a gaseous signaling molecule in different mammalian cells. In the kidney, it has been demonstrated that H_2S was produced through endogenous enzymatic reaction and affected renal blood flow and glomerular filtration rate.

However, there are no reports examining cellular responses to H_2S in renal tubular epithelial cells. In this study, the effects of NaHS, an H_2S donor and L-cysteine, a substrate for H_2S production on the principal cells of rat cortical collecting ducts (CCDs) were investigated with Fura-2 fluorescence imaging and cell-attached patch-clamp current recording.

Application of NaHS increased the intracellular Ca^{2+}concentration($[Ca^{2+}]_i$) in the principal cells. This $[Ca^{2+}]_i$ response was markedly attenuated by removal of extracellular Ca^{2+}. The increase in $[Ca^{2+}]_i$ induced by NaHS was significantly inhibited by non-selective cation channel blockers but not by a voltage-gated Ca^{2+} channel blocker. Extracellular application of L-cysteine also evoked $[Ca^{2+}]$i increase in principal cells. After pretreatment of CCDs with AOAA, an inhibitor of H_2S production enzyme, L-cysteine induced $[Ca^{2+}]_i$response was significantly reduced. In addition, NaHS markedly increased single-channel activity of large conductance Ca^{2+}-activated K channel (BK channel) in apical membrane of principal cells.

These results suggest that H_2S produced by endogenous enzymes activates extracellular Ca^{2+}entry pathway in the principal cells of rat CCDs. (COI: No)

1P-462

Voltage-dependent Ionic Channels in Human Cementoblast

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Purpose: Cementoblasts are cementum forming cells. Although transmembrane signaling associated with ionic transport regulates various physiological processes of the cells, there is no report on the expression of ionic channels in human cementoblasts, based on our knowledge The present study investigated functional expression of ionic channels in human cementoblast cell line (HCEM) by recording membrane currents.

Methods: We measured ionic currents using whole-cell patch-clamp recording. Krebs solution was used as a standard extracellular solution (ECS). Standard intracellular solution (ICS) was composed followings (in mM) ;140KCl, 10NaCl and 10HEPES. To eliminate contribution of $K^+$and Cl^-conductance to the currents, we prepared solution by equimolary replacing $K^+$and Cl^- in the ECS with $Cs^+$and gluconate$^-$, respectively (Cs-gluc-ECS/ICS).

Results & Conclusion: Depolarizing steps from holding potential (Vh) of -70 mV with 10 mV increments evokedoutward and inward currents under the ECS/ICS condition. Under the condition of Cs-gluc-ECS/ICS, outward current amplitudes were decreased when we recorded the currentsat Vh oh -70 mV, while inward currents were developed when the currents were measured at Vh of -100mV. Application of non specific K+ channel blocker, TEA, had no effects on the outward currents. (COI: No)

1P-463

Insulin Regulates Adrenal Steroidogenesis by Stabilizing SF-1 Activity

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Development of metabolic syndrome is associated with hyperactivity of the HPA axis characterized by elevated levels of circulating adrenal hormones including cortisol and aldosterone. However, the molecular mechanism leading to the dysregulation of the HPA axis is not well elucidated. In this study, we found that insulin regulates adrenal steroidogenesis by increasing the expression and activity of steroidogenic factor 1 (SF-1) both in vitro and in vivo and this insulin effect was partly through inhibition of FoxO1. Specifically, insulin increased the protein and RNA levels of SF-1 and steroidogenic target genes. Further, adrenal SF-1 expression was significantly increased by hyperactivation of insulin signaling in mice. Together with the elevated SF-1 expression in adrenal glands, hyperactivation of insulin signaling led to increased aldosterone and corticosterone levels. On the other hand, suppressing the insulin signaling using streptozotocin markedly reduced the expression of adrenal SF-1 in mice. In addition, overexpression of FoxO1 significantly suppressed SF-1 and its steroidogenic target genes implying that the positive effect of insulin on SF-1 activity might be through suppression of FoxO1 in the adrenal gland. Taken together, these results indicate that insulin regulates adrenal steroidogenesis through coordinated control of SF-1 and FoxO1. (COI: Properly Declared)

1P-464

The 2nd Residue of GPCR Helix 8 May Control Transient and Specific Interaction with its G Protein

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G protein-coupled receptors (GPCRs) specifically activate one or a few of four subtypes of G proteins (G_s, $G_{q/11}$, $G_{i/o}$, and $G_{12/13}$) for detection of various extracellular signals. Crystal structures of GPCR complexes have revealed shared and extensive interactions between the conserved GPCR DRY motif and the G-protein C-terminal region. However, the specific GPCR–G protein interactions remain unclear. Alanine scanning mutagenesis of an olfactory receptor revealed that the 2nd residue of helix 8 is responsible for initial, transient, and specific interaction with chimeric $G\alpha_{15\_olf}$, resulting in 2.2-fold rapidity and 1.7-fold robustness of responses compared to those of $G\alpha_{15}$, as well as the importance of the hydrophobic core buried between helix 8 and TM1–2. Based on agonist groups and subtypes of G proteins, an exemplified 178 human non-olfactory GPCRs were classified into 88 subclasses, 64 (73%) of which conserved single types of residues at the 2nd position of helix 8. This result suggests that the 2nd residue of helix 8 enables functional sub-classifications of GPCRs for single types of G proteins, via specific interactions and the resultant signal detection rapidity in GPCR parallel signaling pathways. Moreover, a significant association between weaken hydrophobic core of chemokine receptor genetic variant CX3CR1-A55T and schizophrenia and autism spectrum disorders was reported. These results suggest that the helix 8 and the 2nd residue are novel therapeutic targets. (COI: No)

1P-465

The role for *O*-linked *N*-acetylglucosamine cycling in macrophage Toll-like receptor signaling

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[Purpose] *O*-linked *N*-acetylglucosamine (*O*-GlcNAc), which is reversibly catalyzed by *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA), interplays with *O*-phosphorylation and modulates intracellular signaling. Macrophage pro-inflammatory responses mediated by Toll-like receptor (TLR) signaling is physiologically essential for host defense against bacterial infection. This study aimed to clarify the roles of *O*-GlcNAc cycling in macrophage TLR signaling.

[Methods] Murine peritoneal exudate macrophages (PEMΦ) or RAW264.7 cells were stimulated with lipopolysaccharide (LPS) after treatment with OGT inhibitor BADGP, OGT siRNA, OGA inhibitor PUGNAc, or high glucose. *O*-GlcNAc and *O*-phosphorylation levels on TLR signal proteins were analyzed by western blotting. Pro-inflammatory cytokine productions were analyzed by real-time PCR and ELISA.

[Results] PEMΦ treated with BADGP showed globally decreased *O*-GlcNAc levels and augmented LPS-stimulated production of tumor necrosis factor-α and interleukin-6 in both mRNA and protein levels. RAW264.7 cells transfected with OGT siRNA also showed similar phenotypes and augmented TLR signaling, such as inhibitor of κBα degradation and p65 phosphorylation. On the other hand, both PUGNAc and high glucose induced small increases in *O*-GlcNAc levels and did not influence LPS-stimulated pro-inflammatory cytokine production in PEMΦ.

[Conclusions] Macrophage TLR signaling is negatively regulated by OGT activity. (COI: No)

1P-466

Hypotonic Stress Induces ATP Release via Volume-regulated Anion Channels in Breast Cell Lines

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The high interstitial ATP concentration in the cancer microenvironment plays a wide range of roles in cancer including as a major source of adenosine, a strong immune suppressor. However, the source of ATP has not yet been elucidated. We measured the ATP release during hypotonic stress using a real-time ATP luminescence imaging system and found that two completely different releasing manners exist in primary cultured mammary cells and in breast cell lines. In primary cultured cells, ATP was intermittently released with transient-sharp peaks, while in breast cell lines ATP was released with a slowly rising diffuse pattern. DCPIB, an inhibitor of volume-regulated anion channels (VRACs), only suppressed the diffuse pattern. Cholera toxin treatment of breast cell lines changed the ATP response from the diffuse to the transient-sharp pattern. The inflammatory mediator sphingosine-1-phosphate induced ATP release with a diffuse pattern isovolumetrically in breast cell lines. In addition, treatment with TGFβ changed the ATP release pattern from transient-sharp to diffuse in the primary cultured cells. A real-time PCR analysis indicated that among the isoforms of leucine-rich repeat-containing protein 8 (LRRC8), the molecular entities of VRAC, LRRC8A and 8C were expressed substantially in breast cell lines, and the expression increased with TGFβ. These results suggest that abundantly expressed VRACs are a conduit of ATP in undifferentiated cells including cancer cells.

(COI: No)

1P-467

Estrogen deficiency compromised the β_2AR-Gs/Gi: implications for arrhythmia and cardiac injury

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Purpose: Estrogen and β_2-adrenergic receptors (β_2AR) play important protection roles in the processes that against stress induced cardiac injury. Here, we investigated how ovariectomy influenced the β_2AR downstream pathways in the context of catecholaminergic stress.

Methods:*In vivo* and *vitro* stress models were developed in female Sprague-Dawley (SD) rats by epinephrine (Epi) treatments. Functional and molecular experiments were performed at *in vivo* and *in vitro* levels in terms of contraction, rhythm, injury and protein expression.

Results: Ovariectomized (OVX) rats shows higher incidence of arrhythmia during Epi treatments. In normal state, myocardial contractility was not altered between Sham and OVX group, the levels of cAMP and activated Gs were increased in OVX group comparing to Sham. In Epi induced stressed state, myocardial contractility was decreased, and abnormal rhythms were increased in OVX group, the levels of activated Gi, activated Gs and cAMP were decreased in OVX group comparing to Sham. Furthermore, inhibition of the β_2AR-Gi-PI3K/p38MAPK pathway with ICI118-551, PTX or LY294002 aggravated Epi induced injury on cardiomyocytes but obliterated significantly differences between Sham and OVX groups.

Conclusions: Results suggested that estrogen protect the cardiomyocytes against Epi induced stress via β_2AR-Gi/Gs pathways. estrogen deficiency impaired the β_2AR-Gs/Gi coupling during stress which compromised cardiac contractility and increased abnormal rhythms. (COI: No)

1P-468

Inhibition of HSC activation by caffeine is elicited by antagonizing adenosine receptor-Akt1 pathway

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Hepatic stellate cells (HSCs), one of liver nonparenchymal cells, exist in the space of Disse in the liver. During liver injury, HSCs are activated by various cytokines and transdifferentiated into myofibroblast-like form, so-called activated HSCs. As compared with quiescent HSCs, activated HSCs are large and show increases in proliferation potency, α-smooth muscle actin expression, and collagen production, leading to liver fibrosis. Therefore, **the suppression of HSC activation is expected to be a therapeutic target for liver fibrosis**. Several epidemiologic studies have suggested that intake of caffeine decreases the risk of liver disease. Thus, in the present study, we investigated the mechanism underlying the inhibition of HSC activation by caffeine in primary HSCs isolated from mice.

Caffeine (0.3–10 mM) suppressed the activation of HSCs in a concentration-dependent manner. BAPTA-AM, an intracellular Ca^{2+} chelator, had no effect on the caffeine (3 mM)-induced suppression of HSC activation. None of the isoform-selective inhibitors of phosphodiesterase 1 to 5 suppressed the activation of HSCs unlike caffeine, whereas CGS-15943, an adenosine receptor antagonist, inhibited it. Caffeine (3 mM) did not increase intracellular cAMP levels or the phosphorylation of ERK1/2. In contrast, caffeine (3 mM) significantly decreased the phosphorylation of Akt1. These results suggest that **caffeine inhibits HSC activation by antagonizing adenosine receptors that stimulate the Akt1 pathway**. (COI: No)

1P-469

Phosphorylation analysis in renal arterioles by advanced phos-tag SDS-PAGE method

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Purpose: The pre-glomerular afferent and post-glomerular efferent arterioles exhibit marked differences in regard to excitation/contraction coupling. Unfortunately, however, the detailed molecular mechanisms underlying the regulation of arteriolar contractions are still unknown because they are too small to analyze signaling pathways by conventional biochemical techniques. In this study, we applied the advanced phos-tag SDS-PAGE method (Takeya et al, Electrophoresis, 2017) to measure phosphorylation in isolated renal arterioles. **Methods:** The renal arterioles were isolated from rat left kidneys by utilizing agarose fixed method. Each isolated vessel was stimulated with angiotensin II and endothelin-1 at various concentrations. After termination of the reactions by addition of TCA/acetone, precipitated vessels were dissolved in advanced urea/LDS sample buffer. Phosphorylation was measured by the combination of phos-tag SDS-PAGE and 3-step western blotting. **Results:** By utilizing the advanced phos-tag SDS-PAGE methods, we were able to detect and quantify myosin light chain phosphorylation in the tiny isolated renal arterioles. Various phosphorylation patterns in the responses to angiotensin II and endotelin-1 were observed, suggesting unique signaling pathways were involved in each response. **Conclusions:** The advanced phos-tag SDS-PAGE method allowed us to easily measure phosphorylation in both the afferent and efferent arterioles. (COI: Properly Declared)

1P-470

IL-6 promotes CDK5-induced STAT3/androgen receptor activation in prostate cancer cells

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Interleukin-6 (IL-6), a cytokine, can activate prostate cancer cell proliferation. On the other hand, in our findings suggest that CDK5 can promote prostate cancer cells growth though STAT3 and androgen receptor (AR) regulation. However, the relationship between IL-6 and CDK5 remains unknown. Purpose: We investigate whether IL-6 may regulate CDK5 in prostate cancer. Methods: The phosphorylation level of S727-STAT3 and S81-AR were investigated under IL-6 treatment by Western blotting. Moreover, the interaction ability between pS727-STAT3 and AR were observed by Immunoprecipitation. Result: We found that IL-6 stimulates S727 phosphorylation of STAT3, and furthermore up-regulates AR expression, especially S81 phosphorylate. Moreover, we also found that CDK5 is involved in the IL-6 regulatory pathway as well as its phosphorylation site Y15 was activated by IL-6 to affect downstream S727-STAT3 and S81-AR proteins. Conclusion: IL-6 treatment can activate S727-STAT3, and then promote AR phosphorylation. Therefore, AR could be more stability in order to increase its transcriptional activity as well as prostate cancer cell growth. IL-6 can also increase the phosphorylation of Y15-CDK5; therefore, S727-STAT3 and S81-AR were increased and enhanced AR and STAT3 protein interaction ability. (COI: No)

1P-471

Acute exposure to PRMT1 inhibitor can regulate contraction in isolated mouse ventricular myocytes

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Recent study showed that cardiac-specific deletion of PRMT1 resulted in dilated cardiomyopathy and heat failure within 2 month of age via CaMKII dysregulation. Although PRMT1 deficiency caused a reduction in ejection fraction of heart, however, it is still unclear whether and how PRMT1 regulates contraction of cardiac myocytes. Here we investigated the role of PRMT1 in contractility of ventricular myocytes. Single ventricular myocytes were isolated from mouse hearts. The Ca^{2+} fluorescence and sarcomere length were simultaneously measured in myocytes that were stimulated to contract by external Pt electrodes at a frequency of 1Hz. Sarcomere length was measured using a video image of the cell (IonOptix). The inhibition of PRMT1 with a PRMT1 specific inhibitor, furamidine reduced the sarcomere length shortening of ventricular myocytes. The pretreatment of cells with a CaMKII inhibitor, KN93 prevented the furamidine-induced decrease in sarcomere length shortening. These data suggest that PRMT1 inhibition could be detrimental to the control of ventricular myocyte contractility likely, via CaMKII activation. Further study to identify the targets of CaMKII in contractility control is in progress. (COI: No)

1P-472

Conditional deletion of PRMT1 in adult brain reveals its neuronal cell type-specific roles

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Protein arginine methyltransferases (PRMTs) are enzymes that catalyze the transfer of a dimethyl group to arginine residues of target proteins. Among nine PRMTs, PRMT1, originally identified as a histone H4 methyltransferase, methylates also several non-histone proteins and implicated in diverse cellular processes. In a previous study, we demonstrated the physiological importance of PRMT1 in the central nervous system, showing that PRMT1+/- mice exhibit epileptic seizures. In the present study, we further investigated the function of PRMT1 in adult brain. We generated conditional PRMT1-deficient mice using the Cre-loxP recombination system to delete PRMT1 in cells expressing CaMKIIalpha in the brain several weeks after birth. Immunoblot analysis demonstrated that PRMT1 proteins were highly expressed in hippocampus and cortex from control mice but significantly reduced in those regions of PRMT1 cKO brain. Patch clamp analysis showed that hippocampal dentate gyrus (DG) granule cells showed increased excitability and abnormal responses to XE991, a KCNQ channel blocker. In contrast, intrinsic excitability of prefrontal cortex (PFC) layer V pyramidal neurons in cKO mice was not greatly different from that observed in control mice. Taken together, these data show the neuronal cell type-specific role of PRMT1 in postnatal brain and also suggest that the Prmt1 signaling in DG granule cells regulates neuronal excitability possibly via KCNQ channels. (COI: No)

1P-473

Procathepsin B without mannose-6-phosphaste is released from secretory granules

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PURPOSE: In rat parotid acinar cells, secretory granules (SGs) are generated from Golgi complex. After the generation, membrane proteins of SGs are rearranged by transport vesicles. Amylase, which is a secretory protein, has no known sequence of transport signal to maintain in SGs. However, it is retained in SGs during granule maturation by membrane rearrangement. Procathepsin B (proCB) is a precursor of lysosome enzyme. It is considered that proCB is transported from Golgi to lysosome through immature SGs after modification of mannose-6-phosphate (M6P), which is the transport signal to lysosome. To confirm whether secretory proteins need no transport signal, we examined localization of proCB without M6P.
MATERIAL&METHODS: Acinar cells were isolated from glands by treatment with collagenase. After stimulation with 1 μM isoproterenol, secretions of amylase and proCB into incubation buffer were measured by immunoblotting analysis. M6P modified proteins were collected by concanavalin A-biotin and Dynabeads® M-280 Streptavidin.
RESULTS&CONCLUSION: We detected secretion of amylase and proCB from acinar cells. To confirm whether released proCB had M6P, we collected M6P modified proteins by concanavalin A-biotin. Released proCB had no M6P. Our results indicate that proCB without M6P is maintained in SGs during maturation. And it is suggested that secretory proteins do not need a specific signal to be retained in SGs. (COI: No)

1P-474

Pathophysiological roles of an actin-binding protein ezrin in the kidney

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Ezrin, a member of ERM proteins, is an actin-binding protein which links plasma membrane proteins with actin cytoskeleton, and stabilizes the expression of transporters and channels on the membrane. It is highly expressed in the stomach, small intestine, and kidney. In the kidney, it is expressed on the apical membrane of proximal tubules and podocytes in the glomeruli. However, its physiological roles have not been clear yet. Here, we focused on the physiological roles of ezrin in the kidney by using transgenic ezrin knockdown ($Vil2^{KD/KD}$) mice. In the proximal tubule, ezrin is assembled with a Na^+/P_i co-transporter (Npt2a) through a scaffold protein, NHERF1 at the apical membrane. The $Vil2^{KD/KD}$ mouse kidney was apparently intact. However, the cell surface expression of Npt2a was impaired, and urinary loss of P_i and hypophosphatemia were observed. In the podocytes, ezrin is assembled with a sialoprotein, podocalyxin through a scaffold protein, NHERF2 at the apical membrane. The negative charges on podocalyxin are important for the structure of foot process. In the $Vil2^{KD/KD}$ mice, podocytes were apparently intact without foot process effacement. Proteinuria was not observed. The expression of podocalyxin at the apical membrane was observed. These results suggest that ezrin is indispensable for P_i reabsorption at the proximal tubules through cell surface expression of Npt2a. However, it is dispensable for the expression of podocalyxin and structural integrity of foot process. (COI: No)

1P-475

The efflux characteristics of mitochondrial calcium

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Regulation of $[Ca^{2+}]_m$ is related to various physiological and pathophysiological phenomena. In a recent review, several Ca^{2+} transporting mechanisms were existed in mitochondria. However, the regulating mechanisms were not clear, yet. In this study, we would like to elucidate Ca^{2+} removing mechanisms. We clearly showed Na^+ dependent Ca^{2+} efflux (NDCE) by NCX_{mito} is a major mechanism. Interestingly its activity was regulated in a $[K^+]_c$ dependent manner. There were an inflection in the course of Ca^{2+} efflux and from that, we tried to calculate the mitocondiral Ca^{2+} buffering power and it was about 13mM. The increase of $[Ca^{2+}]_m$ by applying cytosolic Ca^{2+} in the absence of Na^+ was not maintained but transient. Ca^{2+} efflux was still occurred by Na^+ independent manner(NICE). The treatment of RU360, MCU inhibitor, clearly delayed Ca^{2+} efflux. When we changed pH_c from 6 to 8, NICE was faster as pH became alkaline. Ca^{2+} flux also did not change pH_m. During $[Ca^{2+}]_m$ decrease, the reduction of NADH and Ψ_m depolarization were accompanied. In the presence of ATP or ADP, NICE was clearly prevented while NADH and Ψm were maintained. It suggested that the similar mechanisms of Ca^{2+}/Pi-induced Ψ_m depolarization may be responsible for NICE.(G#;2016M3C1A6936605&2018R1A6A3A01011832) (COI: Properly Declared)

1P-476

Role of mito-K_{ATP} channel in Formation of the De-energized Mitochondrial Membrane Potential

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Mitochondria are organelles which play a critical role in the generation of metabolic energy in cells. In previous study, we showed de-energized mitochondria in absence of mitochondrial substrates still have a membrane potential. To pursue the mechanism of that, firstly we tried the possibility of the involvement of mito-K_{ATP} channel. The $\Delta\Psi_m$ of de-energized mitochondria was about 22mV. When we add both Pi and ATP, $\Delta\Psi_m$ was dramatically hyperpolarized to about 65mV and the removal of ATP returned $\Delta\Psi_m$ to the initial value. All agents of blocking K_{ATP} channel did not show any significant effects. Interestingly, DZX, K_{ATP} channel opener, itself could depolarize the Ψ_m in basal condition to about 0 mV, however, the addition of Pi could return Ψ_m to basal level. Interestingly, OligoA, F_1F_0-ATPase inhibitor, showed the similar effects as DZX, depolarizing Ψ_m and reversing by Pi. However, ATP induced hyperpolarization was not affected by DZX but fully inhibited by OligoA. K^+ replacement with NMDG did not show any change on the basal Ψ_m but slowed down the speed of ATP induced hyperpolarization and made it transient. However, the change NMDG to K^+ could reverse and showed the full effect of ATP on Ψ_m. From these results, the mechanism of formation of the resting Ψ_m is still not clear, however, the reverse mode of F_1F_0-ATPase might contribute it but the present results suggest the participation of the other mechanism. It still need further study on them. (2016M3C1A6936605) (COI: Properly Declared)

1P-477

Multistep adaptation of nuclear transport system depending on varying heat stress

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Appropriate cell growth conditions are limited to a narrow temperature range. Once the temperature is out of this range, cells respond to protect themselves, but temperature thresholds at which various intracellular responses occur, including nuclear transport systems, remain unclear. Using a newly developed precise temperature shift assay, we found that individual transport pathways have different sensitivities to a rise in temperature. Nuclear translocations of molecular chaperone HSP70s occur at a much lower temperature than the inhibition of Ran-dependent transport. Subsequently, importin (Imp) α/β–dependent import ceases at a lower temperature than other Ran-dependent transport, suggesting that these are controlled by independent mechanisms. In vitro research revealed that the inhibition of Imp α/β–dependent import is caused by the dysfunction of Imp α1 specifically at lower temperature. Furthermore, we examined thermo-stabilities of six human Imp α isoforms, and found only Imp α3 and Imp α4 are remarkably thermostable. Thus, the various thermo-sensitivities of Imp α family modulates transport balances and enables the multistep shutdown of Ran-dependent transport systems according to the degree of heat stress. (COI: No)

1P-478

Physiological functions of Hikeshi, a nuclear import carrier of molecular chaperone HSP70

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In eukaryotic cells, molecular trafficking between the nucleus and cytoplasm is a highly-regulated process. However, various cellular stresses induce the perturbation of conventional nucleocytoplasmic transport pathways. Therefore, in the stressed cells, appropriate rearrangement of the nucleocytoplasmic transport system is required.

HSP70 is a highly-conserved member of molecular chaperones that play central roles in protein homeostasis. Two major cytosolic HSP70 proteins, HSPA1 and HSPA8, rapidly and efficiently translocate into the nucleus from the cytoplasm in response to heat stress. Previously, we identified Hikeshi (human C11orf73 gene) as a nuclear import carrier of HSPA1/HSPA8 under the heat stress condition (*Kose et al. Cell 2012*). Hikeshi is required to protect cells from heat shock damages and promotes the attenuation and reversion of multiple heat-shock-induced nuclear phenotypes.

Meanwhile, dysfunction of Hikeshi has been found to influence various biological events. Hikeshi-knockout mice died within 48 hours after birth. A missense mutation (V54L) in human Hikeshi gene is linked to human inherited leukoencephalopathy (*Edvardson, Kose et al. J. Med. Genet. 2016*). These results suggest that Hikeshi functions not only under heat stress conditions, but also in various physiological processes.

In this meeting, we will discuss about various physiological functions of Hikeshi molecule. (COI: Properly Declared)

1P-479

Palmitate induces ER Ca^{2+} depletion and defective lysosomal Ca^{2+} release in insulin-secreting cells

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Oxidative stress and Ca^{2+} dysregulation by saturated fatty acids have been observed in insulin secreting cells. We investigated the alteration in organellar Ca^{2+} regulation by palmitate and its functional consequences in mouse clonal beta cells, MIN-6. Palmitate induced cytosolic and mitochondrial reactive oxygen species (ROS) production and apoptosis, which were relieved by low extracellular Ca^{2+}. Palmitate depleted luminal ER Ca^{2+} content and elicited apoptotic ER stress responses, all of which were prevented by mitoTEMPO, a mitochondrial ROS scavenger. Suppression of mitochondrial Ca^{2+} uniporter (MCU) expression completely abrogated mitochondrial ROS production, but affected neither palmitate-mediated ER stress nor cell death. As regulators of autophagy, both AMPK and mTOR are concomitantly activated by palmitate. Palmitate increased LC3 formation but reduced autophagolysosomal degradation. Intriguingly, perilysosomal Ca^{2+} increase by ML-SA1, an activator of Trp-MLs, were abolished in palmitate-pretreated cells. MitoTEMPO recovered defective perilysosomal Ca^{2+} signaling by palmitate. ML-SA1 triggered ER Ca^{2+} release, consistent to the findings that ER Ca^{2+} depletion by cyclopiazonic acid prevented ML-SA1 response. Taken together, we suggest that ER Ca^{2+} depletion by palmitate-induced oxidative stress impairs perilysosomal Ca^{2+} signaling and autophagic flux, which may contribute to lipotoxic mechanism in insulin-secreting cells. (COI: No)

1P-480

Direct Fyn-paxillin binding controls migration of coronary artery smooth muscle cells

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Rho-kinase (ROK)-mediated migration of vascular smooth cells plays a crucial role in cardiovascular diseases. Previously we demonstrated Fyn as an upstream molecule of ROK to mediate sphingosylphosphorylcholine (SPC)-induced actin stress fibre formation, but molecular mechanisms between the two kinases are unclear. Here, we identified paxillin as a novel signalling molecule acting downstream of active Fyn by combined use of pulldown assay and mass spectrometry. Direct binding between constitutively active Fyn (CA-Fyn) and paxillin through the N-terminus of paxillin (N-pax) was demonstrated by surface plasmon resonance assay and further confirmed by co-localization of CA-Fyn and paxillin at the ends of actin stress fibre in vascular smooth muscle cells. SPC-induced ROK activation, actin stress fibre formation and cell migration were inhibited by paxillin knockdown and rescued by full-length paxillin. Surprisingly, N-pax failed to rescue those actions although it showed binding activity to Fyn. Overexpression of N-pax inhibited SPC-induced actin stress fibre formation and cell migration. Co-localization of CA-Fyn and N-pax at the cytosol blocked Fyn-paxillin direct binding at the ends of actin stress fibre and thus inhibited SPC-induced cell migration. In conclusion, paxillin, as a novel signalling molecule acting downstream of Fyn, mediates SPC-induced migration in vascular smooth muscle cells via the Fyn/paxillin/ROK signalling pathway by direct binding to active Fyn. (COI: No)

1P-481

Fascia related muscle contracture

Akihiro Kaizu; Yoshiyuki Tsuboi (*Department of Physiology, Nihon University School of Dentistry, Japan*)

People with musculoskeletal disorders often have contractures of muscles. In recent years, it has been pointed out that densification of fascia is one of causes of pain. However, the nature of the fascia is still unknown. In this study, we report on the effect of fascia using rat lower leg muscle contraction model. In this experiment, we created an original system for measuring muscle tension. 7-week-old SD rats (average 250 g) tibial nerve was stimulated at 50Hz, duration 100 μsec. The lower leg muscle tension was record Spike 2. Elastica van Gieson staining was performed to observe fascia(collagen fibers) under microscope.

These experiments showed that rat lower leg muscle which was contracted by tetanic contraction increased viscosity. Observation with a microscope showed disturbance in the muscle fibers and fascia.

Since this experiment is a short-time contraction, muscle fibrosis does not occur. And another blood flow disorder model revealed no association between blood flow disturbance and contracture. These results suggest that Muscle contracture might be caused by not only fibliosis or Ischemia but also muscle fiber and fascia disorder. (COI: No)

1P-482

Inhibitory effects of chloride intracellular channel protein 2 on distant metastasis of tumor cells

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Distant metastasis is the most aggravating determinant for survival rates of patients with cancer, its prevention is, therby, the critical goal in cancer research. We have established a distant metastasis model using immunocompetent rats. In this model, we subcutaneously transplanted rat C6 glioma cells to the back of neonatal Wistar rats. Rats developed one or more visible tumor masses in the lung by 5 weeks after the transplantation. To investigate the characteristic distinctions between metastatic and non-metastatic C6 glioma cells, cDNA was prepared from cells from the metastatic lung and the primary back tumors for analyses by next generation sequencer. Among many characteristic changes in mRNA expression, we addressed chloride intracellular channel protein 2 (CLIC2) expressed prominently in primary back tumors. Compared to the non-cancer tissues, CLIC2 expression was suppressed in tissues of hepatic cell cancers, colorectal cancers and metastatic colorectal cancers in the liver. Furthermore, the incidence of the lung metastasis was much decreased in the rat model, when transplanted with C6 glioma cells that had been transfected with CLIC2 gene. In the primary back tumor formed by the CLIC2-expressing C6 cells, there was less tumor-associated macrophages, of which tumor-accelerating roles are well known. Collectively, the expression of CLIC2 may be related to the suppression of distant metastasis of cancers. COI; no. (COI: No)

1P-483

Gelatin alters the TGF-beta signaling for RANKL induced osteoclastogenesis

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Evidence has increased to show that extracellular environment is important for a balance between bone resorption by osteoclasts (OCs) and bone formation by osteoblasts (OBs) in order to maintain bone strength and integrity. Here, we examined the effects of extracellular gelatin concentrations on RANKL induced osteoclastogenesis. Increased extracellular gelatin coating concentrations from 0%, 0.5% to 2 % was found to increase the adhesion force from 1.04±0.07, 2.92±0.75, to 6.95±0.13 nN, respectively, and to alter the morphology of RANKL-induced OC cells with the decreased cell fusion index but no changes of TRAP-positive multinucleate OC number. Confocal fluorescence microscope also revealed alterations of the actin ring structure associated with the decreased vinculin density in the RANKL-induced OCs accompanied by increases in gelatin concentrations. Western blot analysis indicates that increased gelatin concentrations caused decreases in the protein level of vinculin in concomitant with the decrease in protein level of TGF-beta in RANKL-induced OC differentiation. In addition, the expression of TGF-beta and its receptors, TGF-beta R1 and R2 was found to decrease in the RANKL-induced OCs with increased gelatin. Taken together, changes of extracellular gelatin concentrations affect the TGF-beta signaling for alterations of vinculin-structured actin ring formation that may lead to alter the morphology of differentiating OCs during RANKL-induced osteoclastogenesis. (COI: No)

1P-484

Evaluation of cell damage during cold-stress and re-warming

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In the case of organ transplantation, isolated organ generally have preserved under an ice-cold condition. It is thought that the ice-cold condition suppressed organ tissue metabolisms, which consume energy and oxygen; however, how to healthy recovery of organ tissues from ice-cold condition is still unknown. In this study, we evaluated mitochondria condition as an indicator of bioactivity of cells.

Human cervical cancer cell line, HeLa cells, were cultured in DMEM at 37℃, subsequently the cells transferred at 4, 15, 27, and 37℃and cultured for 24 hr (cold stress). After cold treatment, the cells were rewarmed at 37℃for 24 hr. After cold stress and rewarming, cells were stained with JC-1, which was fluorescent dye indicator of mitochondrial membrane potentials, and were analyzed by flow cytometer.

Intracellular reducing power decreased in a temperature-dependent manner. Cells proliferation decreased under cold stress (4, 15, and 27℃) and the proliferation did not recovered by 24 hr rewarming; however, intracellular reducing power was sustained except for 4℃treatment.

Although intracellular reducing power decreased in a temperature-dependent manner, subsequent rewarming recovered that except for 4℃ treatment. The results indicated that low temperature (15℃<) itself was not critical damage for cells; however, cell metabolism was suppressed. At more severe low temperature (4℃), cells irreversibly lost part of reducing power which was needed for cellular metabolism. (COI: No)

1P-485

The role of BAG3 on the heat-induced cell death in human cancer cells

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[Purpose] Hyperthermia (HT) is a widely used physical treatment for various types of cancer. However, the cytoprotective effects caused, at least in part, by an increase in heat shock proteins (Hsps) in various cancers have rendered HT less effective. BAG3, a co-chaperone of Hsp70, is a cytoprotective protein that acts against various stresses, including heat stress. Here, we examined the role of BAG3 on the HT-induced cell death in cancer cells.

[Methods] HT were performed by immersing plastic vessels containing the cells in a water bath at 44°C for 90 min. Knock down (KD) of BAG3 was carried out by siRNA technology. Gene expression was analyzed using GeneChip microarrays and computational gene expression analysis tools.

[Results] Treatment of human oral squamous cell carcinoma HSC-3 cells with HT was significantly increased in apoptotic cells. The sensitivity to HT was markedly enhanced in BAG3-KD cells and the combination of BAG3-KD with inhibition of the JNK pathway further enhanced HT-induced apoptosis. Moreover, microarray analysis demonstrated two unique gene networks, designated as Pro-cell death and Anti-cell death, which were obtained from upregulated genes and were mainly associated with the biological functions of induction and the prevention of cell death, respectively.

[Conclusions] These findings indicate that BAG3 plays a protective role in cancer cell death induced by HT, and that the disruption of function of BAG3 may become an option in HT therapy. (COI: No)

1P-486

Lysosomal Proton Sponge Effect by a Cationic Gold Nanorod-Doxorubicin in Cancer Cells

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Despite the lysosomal proton sponge being considered to be a key process for stimulating the apoptosis of nanoparticle-based drug delivery, a clear relationship between the influx of Ca^{2+} and the proton sponge effect has still not been elucidated. Upon entering the lysosome, cationic nanoparticles activate the lysosomal proton pump leading to the retention of Cl^-, and ultimately causing lysosomal swelling. This increase in cytoplasmic Cl^- in turns triggers Ca^{2+} influx and induces cell apoptosis. Unfortunately, direct observation of released Cl^- arising from the rupture of swollen lysosomes, due to the presence of cationic nanoparticles, and the induction of cellular cytotoxicity associated with the proton sponge has not been clearly demonstrated. In this study, we demonstrated that the burst release of Cl^- stimulates the TRPM2 channels, and subsequently induces a massive Ca^{2+} influx, which independently increases apoptosis in cancer cells. Although the previous concept of elevated cancer apoptosis acting through the proton sponge effect is unclear, this study supports the concept that a massive Ca^{2+} influx mediated in response to a burst release of Cl^- significantly influenced cancer cell cytotoxicity. (COI: Properly Declared)

1P-487

Periodontitis elicits salivary gland atrophy via plasma TNF-α and infiltration of B-cells

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Low salivary secretion is well-known as a risk factor for periodontitis, but recent animal studies have suggested that periodontitis elicits low salivary secretion due to apoptotic salivary gland atrophy. In early studies, addition of B-cells or TNF-α has been reported to induce apoptosis. In this study, we examined involvements of the two factors with salivary gland atrophy in a rat periodontitis model. Under anesthesia, the unilateral second maxillary molar in Wistar male rats was tied with silk ligatures. In controls, the ligature was removed just after the ligation. After 4-weeks, the salivary glands and other organs were extracted and used for quantitative RT-PCR and immunofluorescence. The blood was collected to count blood cells and measure inflammatory cytokines in ELISA. In the periodontitis model, the salivary glands were smaller (atrophy) than those of control group. The lacrimal gland and lung were hypertrophy. TNF-α and lymphocyte in the blood were higher in periodontitis model. The mRNA level of TNF-α increased in the gingiva, but not in the salivary glands. The mRNA levels of the TNF-α receptors decreased in the parotid gland and did not change in the submandibular gland. The mRNA level of CD19, a B-cells marker, and CD19-immunoreactive cells were significantly increased in the salivary glands. These results suggest that periodontitis-induced apoptosis in the salivary gland is mediated by enhancement of plasma TNF-α and infiltration of B-cells. (COI: No)

1P-488

N-terminal region of apoptosis-inducing factor stabilizes formation of charge transfer complex

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Apoptosis-inducing factor (AIF) is considered to be a mitochondrial transmembrane protein. The proteolytic cleavage of the N-terminal region of mitochondrial-resident AIF (AIF_{mit}) is considered to be a critical step for nuclear translocation and induction of apoptosis. However, we found AIF_{mit} to bind to membrane via ionic bond, suggesting some novel role for the N-terminal region of AIF_{mit}. To elucidate such role, we investigated the differences in some properties between AIF_{mit} and the N-terminally cleaved soluble AIF (AIF_{sol}). Co-immunoprecipitation assay revealed that both AIF_{mit} and AIF_{sol} in a membrane fraction, but not in a soluble fraction, formed homodimer in a NADH-dependent manner. Under aerobic conditions, the addition of NADH to both AIF_{mit} and AIF_{sol} induced simultaneous decrease in the absorbance at 451 nm and increase in the absorbance at 750 nm, thus indicating similar ability to form a charge transfer $FADH_2$-NAD^+ complex. However, the estimated stoichiometry of AIF_{mit} and AIF_{sol} to NADH was approximately 1:1 and 1:3, respectively. Furthermore, the NADH oxidase activity of AIF_{sol} was significantly higher than that of AIF_{mit}. These findings indicate unstable form of charge transfer complex in AIF_{sol}. We conclude that the N-terminal region of AIF_{mit} plays a critical role for stabilizing the charge transfer complex and preventing electron transfer from reduced FAD to oxygen. (COI: No)

1P-490

Loss of GPx4 in vascular endothelial cells induces accumulation of lipid peroxide and cell death

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Oxidative stress is one of the risk factors for vascular endothelial cells (ECs) dysfunction. However, the importance of antioxidant enzymes in vascular ECs is not fully understood. In this study we attempt to elucidate the importance of Glutathione peroxidase 4 (GPx4), and the involvement of ferroptosis on cell death induced by GPx4 loss in human vascular ECs. In addition, we examined the effects of α-tocopherol (vitamin E) and brown rice on GPx4 loss condition. Cultured human umbilical vein ECs were transfected with siRNA that specifically knockdown GPx4 or scrambled control siRNA. GPx4 siRNA significantly increased the level of lipid oxidation, when compared with control siRNA. To examine the cytotoxicity, we assessed LDH activity. The LDH activity was significantly higher in GPx4 siRNA than control siRNA. On the other hand, vitamin E and extract of brown rice decreased the levels of lipid peroxidation, cytotoxicity, and delay of proliferation induced by GPx4 knockdown. Furthermore, ferrostatin-1, inhibitor of ferroptosis, also prevented cytotoxicity and delay of proliferation. However, caspase inhibitor did not rescue the cytotoxicity induced by GPx4 loss. These results showed that GPx4 was an essential antioxidant enzyme to maintain redox state and protected ECs from oxidative stress, and we found that GPx4 was a regulator of ferroptosis in ECs. Furthermore, vitamin E and brown rice can compensate for GPx4 loss by protecting cells against lipid peroxidation. (COI: No)

1P-491

Synergistic inhibition of Dinaciclib and Paclitaxel on breast cancer cell growth

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Paclitaxel is used as a chemotherapeutic drug for breast cancer for a long time, leading to the cell cycle arrested in G2/M phase. However, there are still many side effects after the chemotherapy. Dinaciclib is novel drug and it is a pan-inhibitor for cyclin-dependent kinase (CDK) and conducted the efficacy and safety with advanced breast cancer in phase II clinical trials. **Purpose:** Recently, paclitaxel combined with other drug to be a new strategy of therapy. Thus, the synergistic antitumor efficacy of dinaciclib combined with paclitaxel was examined in MCF-7 cells. **Method:** The cell viability was detected by MTT assay and based on the cell viability to calculate the combination index. The cell cycle and apoptosis related protein were assessed using Western Blot analyses. **Results:** On the basis of the combination index, 2 nM dinaciclib combined with 10 nM paclitaxel were used for treatment in cell biology experiments. When cells were treated for 24 and 48 hours, cell apoptosis was increased. In the protein level, the expression of p53 and p21 was up-regulating, and Bcl-2 was decreased after treatment for 24 and 48 hours; in addition, ultimately cleaved PARP was increased in time-dependent.**Conclusion:** Our data suggest there could be a synergistic inhibition effect of dinaciclib and paclitaxel in MCF-7 cells; moreover, p53 have a vital role in the combination treatment. Dinaciclib and paclitaxel combination might be a potential treatment for breast cancer in the future.

(COI: No)

1P-492

Bitter tastant and bacterial metabolite modulate glucagon-like peptide-1 secretion

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Glucagon-like peptide-1 (GLP-1) is one of incretin hormones secreted from enteroendocrine L cells. GLP-1 secretion is regulated by various luminal nutrients, bacterial metabolites, and hormones or neurotransmitters, and secreted GLP-1 enhances insulin secretion from pancreatic β cells. Although bitter tastant quinine and bacterial metabolite *S*-equol are known to cause weight loss and increase in insulin secretion, respectively, whether these effects are mediated by GLP-1 secretion remains unclear. We thus investigated the relationship between quinine, *S*-equol, and GLP-1 using mouse enteroendocrine L cell line GLUTag cells. Both quinine and *S*-equol induced an increase in intracellular Ca^{2+} levels, which are mediated by G_q signaling pathway downstream of putative G protein-coupled receptors for quinine and *S*-equol. Although both substances increased intracellular Ca^{2+} levels, both substances had no effect on GLP-1 secretion. Total internal reflection fluorescence microscopy and immunohistochemistry showed that GLP-1-containing vesicles remained unfused with the plasma membrane, and actin polymerization beneath the plasma membrane was enhanced upon application of quinine and *S*-equol. Interestingly, application of forskolin after quinine treatment restored GLP-1 exocytosis. These results suggest that quinine and *S*-equol do not promote GLP-1 secretion, and GLP-1 exocytosis is regulated by the complex signaling network including Ca^{2+}, cAMP, and actin cytoskeleton. (COI: No)

1P-493

Sequential phosphoinositide conversion is required for TGFβ–induced receptor endocytosis in ECs

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Receptor endocytosis is an important fundamental cellular process to receptor sensitization and desensitization. Phosphoinositide conversion regulates a diverse array of dynamic membrane events including endocytosis. However, it is not well understood which enzymes are involved in phosphoinositide conversion for receptor endocytosis. We found by siRNA–mediated knockdown that class II PI3K α isoform (PI3K-C2α), 5'-phosphatase synaptojanin1, and 4'-phosphatase INPP4B, but not PI3K-C2β, synaptojanin2 or INPP4A, were required for TGFβ-induced endocytosis of TGFβ receptor. TGFβ induced a rapid decrease in $PI(4,5)P_2$ at the plasma membrane (PM) with increases in PI(4)P and $PI(3,4)P_2$ in a manner dependent on TGFβ receptor kinase ALK5. Knockdown of synaptojanin1 abolished TGFβ-induced $PI(4,5)P_2$ decrease and PI(4)P increase while PI3K-C2α knockdown abolished $PI(3,4)P_2$ increase. Interestingly, PI3K-C2α knockdown also abolished TGFβ-induced $PI(4,5)P_2$ decrease, and TGFβ induced colocalization of synaptojanin1 and PI3K-C2α at the PM. These observations suggested the functional link of synaptojanin1 and PI3K-C2α. Finally, the phosphoinositide conversion was necessary for TGFβ–induced activation of Smad 2 and 3. These observations indicate that sequential phosphoinositide conversion mediated by synaptojanin1, PI3K-C2α, and INPP4B is essential for TGFβ receptor endocytosis and signaling. (COI: Properly Declared)

1P-494

The roles of p11 for the localization and heteromeric channel formation of TASK1 and TASK3 isoforms

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We have reported that adrenal medullary (AM) cells express TASK1 in the cell membrane. This study aimed to examine whether p11, an adaptor protein, plays a role for the subcellular localization and the heteromeric channel formation of TASK1 and TASK3 in rat adrenal cortical (AC) cells. Immunocytochemical analyses revealed that p11 was expressed in AC, but not AM cells, and that TASK1 channels were mainly located in the cytoplasm and in the cell membrane in AC and AM cells, respectively. Furthermore, GFP-TASK1 exogenously expressed in H295R cells, a human AC cell line endogenously expressing p11, were also located in the cytoplasm, whereas in PC12 cells expressing no p11, GFP-TASK1 and -TASK3 were mainly located in the cell membrane. Because p11 were reported to retain TASK1 in the ER by binding to its c-terminus, the lack of p11 expression in AM and PC12 cells may facilitate the trafficking of TASK1 to the cell membrane. This possibility was examined by exogenous expression of p11 in PC12 cells. When GFP-TASK1, GFP-TASK3, and p11 were simultaneously expressed in PC12 cells, GFP-TASK1and -TASK3 were mainly present in the cytoplasm, raising the possibility that the trafficking of TASK3 to the cell membrane is hindered by the formation of heteromeric TASK1/3 channels. These results suggest that the expression of p11 hinders trafficking of TASK1 from the ER to the cell membrane and consequently facilitate the formation of heteromeric TASK1/3 channels. (COI: No)

1P-495

Astrocytic spontaneous hormone exocytosis modulated by spontaneous cytosolic Ca^{2+} increase

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l Astrocytes, which comprise the largest population of glia, are now recognized as important participants in the maintenance of neural function by releasing or uptaking various signaling molecules called gliotransmitters. Brain-derived neurotrophic factor (BDNF) and neuropeptide Y (NPY) are essential neurotransmitters and released from astrocytes as well. However, the precise molecular mechanisms underlying the release of those peptide hormones from astrocytes are not fully understood. Here, we expressed green fluorescent protein Venus-fused BDNF and NPY (BDNF-Venus and NPY-Venus, respectively) in rat astrocytes to observe exocytotic dynamics of BDNF and NPY. Interestingly, total internal reflection fluorescence microscopy revealed that BDNF-Venus and NPY-Venus are released spontaneously from astrocytes. Additionally, we observed that intracellular Ca^{2+} concentration fluctuates spontaneously in astrocytes. Transient receptor potential A1 (TRPA1) channels are primarily expressed in astrocytes within the brain and reported to produce spontaneous Ca^{2+} signaling due to transmembrane fluxes. Blockage of TRPA1 channels reduced the number of Ca^{2+} transients and BDNF-Venus exocytosis, while activation of TRPA1 channels increased the number of Ca^{2+} transients and frequency of NPY-Venus exocytosis. Our results suggest that astrocytic spontaneous Ca^{2+} oscillations via TRPA1 channels may contribute to spontaneous release of gliotransmitters. (COI: No)

1P-496

Molecular mechanisms of deoxycholic acid induced glucagon-like peptide-1 secretion

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Glucagon-like peptide-1 (GLP-1) is secreted from enteroendocrine L cells and promotes insulin secretion from pancreatic β cells. GLP-1 secretion is triggered not only by the luminal nutrients but also by bacterial metabolites. For instance, deoxycholic acid (DCA), one of the secondary bile acids synthesized by intestinal microbiota, stimulates GLP-1 secretion. Although DCA is a ligand for G_s protein-coupled bile acid receptor, DCA induces both intercellular cAMP and Ca^{2+} to lead to GLP-1 secretion. In the present study, we investigated molecular mechanisms of how DCA elevated intercellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in enteroendocrine L cells. Application of an adenylyl cyclase inhibitor, dideoxyadenosine and G_q protein inhibitor, YM-254890 to mouse enteroendocrine L cell line GLUTag cells had little effects on the $[Ca^{2+}]_i$ increase by DCA. Moreover, removal of extracellular Ca^{2+} had little effect on $[Ca^{2+}]_i$ increase by DCA. Interestingly, depletion of endoplasmic reticulum Ca^{2+} by thapsigargin and application of ryanodine receptor blocker, dantrolene inhibited the $[Ca^{2+}]_i$ increase by DCA. These results suggested that DCA induces Ca^{2+} release the endoplasmic reticulum via ryanodine receptor. (COI: No)

1P-497

Effect of temperature on raft-dependent endocytosis during activation of T cells by concanavalin A

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(Background)
Temperature plays an important role in the immune response. Acclimatization occurs when there are changes in ambient temperature over a long period. We used the human leukemic Jurkat T cell line to study the effect of temperature on the immune system using concanavalin A (ConA), a plant-derived immunostimulant, as a trigger for T cell activation. Previously, we reported endocytic intracellular cluster formation during T cell activation by ConA with the aid of rafts and the cytoskeletons (actin and microtubules). Here, we investigated the effect of temperature on cluster formation (with the aid of three-dimensional images of the cells) and on the stability of rafts, actin, and microtubules.
(Results and Discussion)
When the temperature was changed between 23°C and 37°C (physiological temperature), clusters could be observed throughout this temperature range. Raft structure was stabilized at lower temperatures but destabilized at higher temperatures. Actin was stable when the temperature was higher than 27°C. When actin was depolymerized, clustering was not observed at 37°C but could be observed at 23°C. There were no changes in microtubules within this temperature range. Thus, raft clustering may be associated with raft stability at lower temperatures (<27°C) and with actin at higher temperatures (≥27°C). Hence, we provided insight into the associations between temperature, rafts, actin, and microtubules in the immune response. (COI: Properly Declared)

1P-498

Electrophysiological evidence for increased thrombopoiesis in the bone marrow in CRF rat model

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Uremic bleeding in chronic kidney disease (CKD) has been ascribable to decreased thrombopoiesis. However, serum thrombopoietin (TPO) levels are usually elevated in CKD patients, suggesting increased thrombopoiesis. The aim of this study was to determine the thrombopoietic activity in CKD. In the present study, male Sprague-Dawley rats that underwent 5/6 nephrectomy were used as the model of chronic renal failure (CRF). Age-matched sham-operated rats were used as controls. Single megakaryocytes were isolated from the rat bone marrow, and their size distribution was examined. Megakaryocyte membrane invaginations were monitored by confocal imaging of di-8-ANEPPS staining, and patch-clamp whole-cell recordings of membrane capacitance (Cm). TPO gene expression was assessed in various tissues. Circulating platelet counts and the number of large megakaryocytes in the bone marrow were significantly increased in CRF rats. Massive di-8-ANEPPS staining and increased membrane capacitance per cell surface area in large megakaryocytes demonstrated increased membrane invaginations. TPO transcription was decreased in the renal cortex but significantly increased in the liver and bone marrow of CRF rats. Increased thrombopoiesis in CKD was thought to be a reactive mechanism to platelet dysfunction. Increased TPO production from the liver and bone marrow compensated for the decreased production from damaged kidneys. (COI: No)

1P-499

The outer BRB in diabetic retina is regulated by interaction between microglia and RPE cells

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Outer blood-retinal barrier (BRB), mainly composed of retinal pigment epithelial (RPE) cells maintains the integrity of the retinal tissues. In this study, we aimed to investigate the mechanisms of the outer BRB disruption regarding the interaction between RPE and microglia. In mice with high-fat diet-induced obesity and streptozotocin-induced hyperglycemia, microglia accumulated on the RPE layer, as in those after intravitreal injection of interleukin (IL)-6. Although IL-6 did not directly affect the levels of zonula occludens (ZO)-1 and occludin in RPE cells, IL-6 increased *VEGFA* mRNA in RPE cells to recruit microglial cells. In microglial cells, IL-6 upregulated the mRNA levels of *MCP1*, *MIP1A*, and *MIP1B*, to amplify the recruitment of microglial cells. In this manner, IL-6 modulated RPE and microglial cells to attract microglial cells on RPE cells. Furthermore, IL-6-treated microglial cells produced and secreted tumor necrosis factor (TNF)-α, which activated NF-κB and decreased the levels of ZO-1 in RPE cells. As STAT3 inhibition reversed the effects of IL-6-treated microglial cells on the RPE monolayer *in vitro*, it reduced the recruitment of microglial cells and the production of TNF-α in RPE tissues in streptozotocin-treated mice. Taken together, IL-6-treated RPE and microglial cells amplified the recruitment of microglial cells and IL-6-treated microglial cells produced TNF-α to disrupt the outer BRB in diabetic retinopathy. (COI: No)

1P-500

Expression of Tyrosine Hydroxylase in CD4⁺ T Cells Alleviates Collagen-Induced Arthritis

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Purpose: Role of T cell-expressed tyrosine hydroxylase (TH) in rheumatoid arthritis (RA) is less clear. Herein, we aimed to show a contribution of TH expression by $CD4^+$ T cells to alleviation of Th17/Treg imbalance in collagen-induced arthritis (CIA). **Methods:** CIA was prepared by intradermal injection of collagen type II (CII) at tail base of DBA1/J mice. Expression of TH in the spleen and the ankle joints and percentages of TH-expressing Th17 and Treg cells in splenic $CD4^+$ T cells were determined. Overexpression and knockdown of TH gene in $CD4^+$ T cells were taken to evaluate effects of TH on Th17 and Treg cells in CIA. **Results:** TH expression was upregulated in both the inflamed tissues and the $CD4^+$ T cells of CIA mice. In splenic $CD4^+$ T cells, the cells expressing TH were increased during CIA. These cells that expressed more TH in CIA were mainly Th17 cells rather than Treg cells. TH gene overexpression in $CD4^+$ T cells from CIA mice reduced the Th17 cell activity, whereas TH gene knockdown enhanced it. In contrast, TH gene overexpression increased the Treg cell changes in $CD4^+$ T cells of CIA mice, while TH gene knockdown decreased it. **Conclusions:** These findings show that CIA induces TH expression in $CD4^+$ T cells, particularly in Th17 cells, and suggest that the increased TH expression during CIA represents an anti-inflammatory mechanism. (COI: No)

1P-501

Effects of 405 nm light by using light emitting diods on cultured HeLa cells

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We tested effects of 405 nm wavelength irradiation by using light emitting diods on cultured HeLa cells. Cells were plated in plastic dish and were maintained for 48 hours. The cells were irradiated with the light at 146 mW/cm^2. Reactive oxygen species (ROS) were monitored by fluorescent probe. Irradiation of 405 nm light didn't affect the viability of HeLa cells for 3 hr at least. First, we measured the intracellular ROS accumulation in the cells. The accumulation in the low glutathione (GSH) cells obtained by addition of 1-chloro 2,4 dinitrobenzene or buthionine sulfoximine (BSO) more rapidly increased compared with control cells. Then, we tested the effects of the light on low GSH cells obtained by preincubation with BSO for 24 hrs. The light irradiation for more than 1.5 hr induced cell death in the BSO loaded HeLa cells. Next, we tried to test the influences of the light irradiation on intracellular ROS scavengers (GSH and TRX) in the low GSH cells. The TRX was increased in both control and low GSH cells, but GSH was not changed in control cells and was decreased in low GSH cells. This result indicates that the intracellular GSH was mainly related to scavenging the ROS induced by the light, and TRX was not so closely related. These results suggest that the intracellular ROS induced by 405 nm light are containing singlet oxygen ($^1O_2$) and hydroxyradical (OH),and the intracellular glutathione plays an important role of the ROS (mainly $^1O_2$) for scavenging. (COI: No)

1P-502

Inhibitory effect of Corylifol C on RANKL-induced osteoclast differentiation and bone resorption

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Lately, the study of anti-resorptive agents from natural compounds has become a topic of interest. Corylifol C is a compound isolated from the seeds of *Psoralea corylifolia* that has been used as a traditional medicine in Asia. Corylifol C has previously been shown to have weak antioxidative effects; however, its effect on osteoclast differentiation and bone resorption remains unclear. In this study, we investigated the effects of Corylifol C on osteoclast differentiation and bone resorption. Corylifol C dose-dependently inhibited RANKL-induced osteoclast differentiation from 5uM. It is evaluated on bone marrow-derived monocytes by a TRAP staining and TRAP activity assay. Expression of RANKL-induced osteoclastogenesis-related marker genes including ACP5, MMP-9, DC-STAMP, Atp6v0d2, Ctsk, CLCN7 and NFATc1 was inhibited by Corylifol C treatment. Moreover, Corylifol C inhibits RANKL-induced bone resorption demonstrated by a bone resorption assay. The activation of c-Src by ROS are key steps in enhancing OC survival and differentiation. Corylifol C inhibited expression of c-Src protein and mRNA and decreased the generation of RANKL-mediated reactive oxygen species (ROS) in BMMs at 5uM. These findings suggest that Corylifol C inhibits osteoclast differentiation by inhibition of ROS and induces downregulation of c-Src in osteoclast. Our results revealed that Corylifol C could be a potential therapeutic agent of the treatment of bone-resorptive diseases. (COI: No)

1P-503

Sestrin 2 regulates osteoclast differentiation through interaction with p62 and TRAF6

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Sestrin 2(Sesn2), an autophagy inducer, interacts with p62 and Keap1 and prevents oxidative stress via Keap1-Nrf2 pathway. In addition, previous reports showed that p62 interacted with TRAF6 and leads to the activation of osteoclast differentiation. However, the role of Sesn2 in osteoclast differentiation is unknown. In the present work, we investigated the effects of Sesn2 in osteoclast differentiation with Sesn2 knock-out (Sesn2-/-) mice. The bone mass of Sesn2-/- mice increased more than that of wild type mice by μCT analysis. Also, the formation of multinuclear osteoclasts was inhibited in Sesn2-/- mice. Furthermore, NFATc1 and osteoclastogenesis - related gene expression were significantly diminished in Sesn2-/- mice during osteoclast differentiation. RANKL-induced TRAF6 downstream pathways were delayed in osteoclasts of Sesn2-/- mice. The interaction of p62 and TRAF6 also decreased in Sesn2-/- mice. These results suggest that Sesn2 regulates osteoclast differentiation via the interaction with p62 and TRAF6. (COI: No)

1P-504

A novel screening system to predict injured organs using cell-free DNA in serum

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Detecting organ injury (e.g. by toxins or chemicals) at earlier stage is important to prevent the adverse outcomes. For such purpose, we have developed a novel screening system using serum. This system is simple and minimally invasive, because we utilized serum to detect cell free circulating DNA (cfDNA), whose origin may be identifiable if they harbor tissue-specific epigenetic modification. Furthermore, our system may have a potential to detect fetal organ injury if cfDNA of fetal origin in maternal serum is identifable. First, we performed whole genome bisulfite sequence of several fetal organs on embryonic day 14.5 (E14.5) to identify the fetal organ-specific markers (specific un-methylated regions). The sequence data were compared with adult data that were obtained from Gene Expression Omnibus, and we selected several candidate regions for each organ. Next, to confirm whether these regions harbor tissue-specific epigenetic modification, we performed bisulfite amplicon sequence (BSAS) assay with genome DNA, derived from several fetal tissues. Finally, we produced organ-specific injury mouse model using chemical exposure (CCl4, doxorubicin, ethanol and nicotine). We could detect cfDNA harboring each organ-specific nature. The level of such organ-specific cfDNAs increased with the increase in chemicals causing organ-specific injury. These results indicate that our system may be useful to detect specific injured organs just by using serum. (COI: No)

1P-505

The stress-induced stress tolerance acquisition in ciliated protozoan *Paramecium caudatum*

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A unicellular organism, a ciliated protozoan *Paramecium* swims around by beating a thousand of cilia covering whole cell body. When being facing to toxic substances such as ethanol, they detach their cilia spontaneously possibly due to avoid damage by reducing a surface area (deciliation). Deciliated cells become unable to swim. In the present study, we found that cells acquired tolerance to a deciliatable concentration of ethanol by pretreatment with a lower undeciliatable concentration of ethanol. We investigated the effect of the pretreatment on the ethanol-induced deciliation by counting the number of undeciliated swimming cells. Findings of the study are: (1) the pretreated cells retained the acquired tolerance to ethanol for at least 24 h and lost it by 48 h; (2) the effect of pretreatment was attenuated by protein synthesis inhibitors; (3) the effect of pretreatment depended on the length and the strength of the pretreatment conditions; (4) the pretreatment conferred cells the tolerance to not only a deciliatable concentration of ethanol but a lethal concentration of ethanol and a high concentration of calcium which also induces deciliation; and (5) the cells acquired the ethanol tolerance during a starvation period. Judging from these results, we concluded that *Paramecium* cells acquire the stress tolerance while they are under mild stress conditions by synthesizing a certain protein(s). (COI: No)

1P-506

Calcium-dependent regulation of cortical actin filaments in mouse eggs

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In mammalian oocytes, actin filaments (F-actin) are densely localized in the cortex beneath the plasma membrane, and are involved in various events at fertilization, such as sperm incorporation, cortical granule exocytosis, and polar body emission. In the present study, we investigated the dynamics and the regulatory mechanism of F-actin organization in mouse eggs, in relation to repetitive transient rises in intracellular Ca^{2+} concentration, or Ca^{2+} oscillations, induced by sperm-borne egg-activating protein PLCζ. It was found that transient Ca^{2+} rises caused transient increases in the cortical F-actin except at the actin cap (AC), which is the thick layer of F-actin localized at the animal pole. At the AC, in contrast, F-actin transiently decreased upon first one or two Ca^{2+} rises. The AC was then thoroughly corrupted and F-actin was reorganized to emit the polar body. Transient increases in the cortical F-actin were suppressed by latrunculin A or cytochalasin D, inhibitors of actin polymerization, but not by CK-666 or SMIFH2, inhibitors of actin nucleation mediated by Arp2/3 and formins, respectively. The spatial pattern and the time course of Ca^{2+}-dependent transient decrease in F-actin at the AC closely resembled those observed in the egg treated with CK-666. These results suggested the cortical F-actin in mouse eggs is regulated by Ca^{2+} in different manners via distinct sets of actin-binding proteins at the AC and the other cortical regions. (COI: No)

1P-507

Target-gene disruption by CRISPR/xCas9 system in *Drosophila melanogaster*

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Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 9 system is an important genome editing tool, with customizable specificities. Genome editing mediated by this technology has been used to rapidly and efficiently modify endogenous genes in a wide variety of cell types and organisms. The specificity of CRISPR/Cas9-mediated DNA cleavage requires not only target sequences matching crRNA but also a protospacer adjacent motif(PAM) locating at downstream of target sequences. For the most commonly used Cas9 from *Streptococcus pyogenes* (*Sp*Cas9), the required PAM sequence is NGG. The NGG PAM requirement greatly limits the targeting scope of Cas9 applications. An new article published in Nature recently demonstrates that an expanded PAM *Sp*Cas9 variant (xCas9) can recognize a broad range of PAM sequences including NGT, NGA and NGC. Here we apply the CRISPR/xCas9 system to *Drosophila*, testing whether the evolved Cas9 variants with broad PAM compatibility can function efficiently in this model organism. As a result, on the NGG PAM sites tested, xCas9-3.7 showed comparable activity to *Sp*Cas9. For tested non-NGG PAM sites, xCas9-3.7 could produce DNA cleavage and indel-mediated disruption on the target site in *Drosophila melanogaster*, consistent with previous reports. Overall, these results establish that xCas9-3.7 nuclease can mediate target-gene disruption at non-NGG PAMs in Drosophila. (COI: No)

1P-508

Electrophysiological properties of inwardly rectifying K^+ channel in glioblastoma stem-like cells

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Introduction: Glioblastoma multiforme (GBM) is the most fatal malignant primary brain tumor. GBM contains functional subsets of cells called glioblastoma stem-like cells (GSCs), which are radio- and chemo-resistant and eventually lead to tumor recurrence. However, the molecular target for treatment of GSCs have not been extensively investigated. **Objectives:** The present study aimed to identify functional ion channels in GSCs. **Methods:** We established the stem-like cells from human GBM using three-dimensional cell culture. We measured whole-cell currents of the GSCs using patch-clamp techniques and analyzed cell growth of them with WST assay. **Results:** We found inwardly rectifying K^+ (Kir) currents in the GSCs. The Kir current was observed in 19 out of 25 cells. An inward conductance of the Kir currents was proportional to $[K^+]_o^{0.48}$. The selectivity sequence based on conductance ratios was K^+ (1.00) > Rb^+ (0.76) >> Cs^+ (0.10) > Na^+ (0.05). The Kir currents were blocked by extracellular Ba^{2+} and Cs^+ in a voltage- and a concentration-dependent manner. We found that a specific inhibitor of Kir decreased a growth rate of the GSCs. **Conclusion:** These results indicated that Kir channels contribute to cell growth of GSCs and have the potential to be therapeutic targets of GBM. (COI: No)

1P-509

Downregulating CXCR4 by miR-139 to restrain breast cancer stem cell-like phenotypes

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PURPOSE: High expression levels of the chemokine receptor CXCR4 correlate with chemotaxis, invasion and cancer stem cell (CSC)-like phenotypes. Recent studies of microRNA (miRNA) involvement in embryonic stem cell pluripotency highlighted the changing nature of the stemness characteristics of these small RNA molecules, but the physiologic role of miRNA in interfering with CXCR4 to restrain CSC-like cell property of breast cancer remains unknown.

METHODS: Identification of a stem cell-enriched side population was achieved with FACS and a sphere-forming assay. Wound healing, Boyden chamber assays and western blotting were used to study the contribution of miRNA to CSC-like properties. A delivery of lentivral-miRNA was used to examine tumor cell migration in nude mice xenotransplant model.

RESULTS: We identified the 3'-UTR sequences of CXCR4 mRNA as a target of miR-139. Increased miR-139 expression suppresses CSC-like cells with the mesenchymal traits. miR-139-overexpressing cells decreased the cell migration capacity via downregulation of the CXCR4/PI3K/p-Akt signaling. In addition, miR-139 inhibited the genesis of metastatic lung nodules as demonstrated in a lung cancer xenograft model in which nude mice were transplanted with MDA-MB-231 cells carring miR-139.

CONCLUSION: Our findings highlight the key role of the miR-139 targeting CXCR4 in decreasing Akt phosphorylation to interfere with mesenchymal stem cells morphogenesis and suppress migration and invasion of breast cancer. (COI: No)

1P-510

CHIP-mediated ubiquitination of Gal1 predicts prognosis of colorectal cancer

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Objective The precise roles of CHIP or Gal1 in colorectal cancer (CRC) are uncertain. Here, our study explored the relationship and clinical significance of CHIP or Gal1 in CRC. **Methods** Immunohistochemistry was performed to test the expression of CHIP or Gal1 on a CRC tissue microarray containing the tumor and corresponding normal tissues. The correlation of CHIP or Gal1 expression with clinicopathological parameters and survival was evaluated, respectively. The role of CHIP or Gal1 on antitumor was determined in vitro and in vivo. **Results** CHIP or Gal1 expression was significantly decreased or up-regulated in CRC compared with adjacent noncancerous tissues, respectively. Low tumoral CHIP or high Gal1 expression significantly correlated with clinicopathological characteristics in patients, as well as with shorter overall survival. Moreover, CHIP associated with Gal1 has a synergistic effect on the prediction of CRC prognosis. In vitro and vivo, high CHIP or low Gal1 expression inhibit CRC growth or metastasis. Futhermore, our results found that CHIP played an important role by regulating negatively Gal1. CHIP interacted with Gal1 and promoted its ubiquitination and degradation by proteasome, terminating Gal1 activity by immunocoprecipitation and ubiquitination experiments. **Conclusions** CHIP could inhibit CRC growth or metastasis through promoting Gal1 ubiquitination and degradation by proteasome. CHIP and Gal1 expressions are novel candidate prognostic markers in CRC. (COI: No)

1P-511

CD105 maintains the thermogenic program of beige adipocyte

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Beige adipocytes are thermogenic adipocytes distinct from classical brown adipocytes. Recent studies have revealed several key molecular regulators of beige adipocyte development. However, its molecular mechanism is not fully understood, and it's needed to clarify adequate markers of beige adipocyte precursors to elucidate the lineage of beige adipocytes.

CD105, also called endoglin, is a membrane protein composed of TGF-β receptor complex. It regulates TGF-β family signal transduction and vascular formation *in vivo*. Here, we show that CD105 maintains the thermogenic gene program of beige adipocytes by regulating Smad2 signaling. CD105 expression is high in beige adipocyte precursors of murine inguinal WAT and is essential for maintaining beige adipocyte precursors that are to differentiate into UCP1-expressing thermogenic beige adipocytes. *Cd105*-deficient beige adipocyte precursors showed decreased UCP1 production after adipocyte differentiation. *Cd105*$^{-/-}$ beige adipocyte precursors showed augmented Smad2 activation and decreased expression of thermogenic genes such as *Ucp1* and *Prdm16* after adipogenic differentiation. Smad2 signaling augmentation decreased the expression of thermogenic genes in beige adipocytes.

These data indicate the importance of CD105 in maintaining the thermogenic gene program of beige adipocyte by negatively regulating Smad2 signaling. (COI: No)

1P-512

Leucine and Caffeine induce mitochondrial biogenesis and downregulation of miRNAs in C2C12 myotubes

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We and other authors have demonstrated that small nutrients, such as Leucine (Leu) and Caffeine (CAFF), induce mitochondrial biogenesis through diverse mechanisms that converge in PGC1α activation. Recent evidence suggests that miR-494, -696 and -761 are involved in this process by negative modulation of PGC1α signaling. However, it remains unclear whether these miRNAs are regulated by nutrients stimuli. Therefore, our study was focused on the effect of Leu and CAFF on these miRNAs functions and how it affected mitochondrial biogenesis. **Methods:** After 5 days of differentiation of C2C12 myoblasts, myotubes were treated with Leu or CAFF (3mM) containing Dulbecco's modified Eagle's medium (DMEM) without serum and Leu for 24h. As a control was used serum and Leu-Free DMEM. After 24h of each treatment, cells were harvested and then, DNA, RNA, and whole protein fraction were isolated for immunoblotting and qPCR analyses. **Results:** Compared to control cells, mitochondrial DNA copy number increased significantly 24h after Leu addition, and especially in the CAFF treatment ($p<0.05$). Conversely, miR-494, -696 and -761 levels were downregulated in the Leu treated group, but in the CAFF treated group only miR-761 levels decreased. PGC1α protein level and phosphorylation rate of p70S6K improved in both treatment groups. **Conclusion:** These results suggested that supplementation of Leu or CAFF can induce mitochondrial biogenesis via modulating the expression of PGC1α-targeting miRNAs. (COI: No)

1P-513

Effects of supplementation of fatty acids on viability of B16F10 and neural stem cells

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Polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and arachidonic acid, are essential for the growth and functional development of the brain. Fetal calf serum (FCS), which is often added to culture media to enhance cell growth, contains several essential fatty acids, including DHA and arachidonic acid that are, not present in normal cell culture media. In the present study, we assessed the effects of oils containing essential fatty acids on cell viability. B16F10 mouse melanoma cells, were cultured in RPMI medium containing 10% FCS. Neural stem cells (NSCs) were collected from rat fetal forebrain embryonic day 14.5 and cultured as neurospheres. Oil was supplemented as an emulsion, followed by culture for 3 days. Cell viability was assessed by the MTS, a tetrazolium compound, method. Total lipids in the cells were extracted, and the composition of fatty acids was analyzed by gas chromatography. Supplementation with oil increased cell viability of B16F10 cells in the presence of 10% FCS. And supplementation with oil increased B16F10 cell viability in the absence of FCS. Fatty acid composition of cells was consistent with that of supplemented oil. In case of NSCs, DHA and arachidonic acid levels in the cells gradually decreased during passaging of cells. Supplementation with oil increased NSC viability. These data suggest that supplementation with oils is important for growth of B16F10 cells and NSCs. (COI: No)

1P-514

STAT6 promotes myoblast differentiation and fusion

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[Purpose] Skeletal muscle formation requires the myoblast fusion. It is known that interleukin-4 (IL-4) plays an important role in myoblast fusion to promote muscle formation. Previous studies have indicated that IL-4 activates Signal Transducers and Activator of Transcription 6 (STAT6) in other cell types. However, it is unknown whether STAT 6 is involved in the myoblast fusion. The aim of the present study was to clarify the role of STAT6 in myoblast fusion under cultured condition.
[Methods] Myoblasts were isolated from the lower limb of C57BL/6 mice (male, 8 weeks old). The myoblasts were transfected with short hairpin RNA (shRNA) for STAT6 or scrambled shRNA as a control and maintained in differentiation medium (DM) for 24, 48, and 72 h. The cells were fixed with 4% PFA, followed by stained immunohistochemically to examine the fusion index (percentage of nuclei inside the myotubes). The cells were also processed to determine the protein expression of differentiation and protein synthesis markers by Western blotting.
[Results] The fusion index was significantly increased in STAT6-knockdown cells compared with control cells in DM at 48 h and 72 h. We also found that the protein expression of differentiation and protein synthesis markers in STAT6-knockdown cells were significantly higher than the control cells in DM at 24, 48 and 72 h.
[Conclusions] These results suggest that STAT6 is implicated in myogenesis by regulating myoblast fusion. (COI: No)

1P-515

Analysis of Molecular and Cellular Roles of the GON domain in ER-to-Golgi transport

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ADAMTS9 is a metalloprotease that cleaves components of the extracellular matrix and is also implicated in intracellular protein transport. We found that the downregulation of human ADAMTS9 and its *C. elegans* orthologue GON-1 results in the inhibition of protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus. ADAMTS9 has a unique C-terminal domain called the "GON domain". The function of intracellular protein transport is dependent on the GON domain but independent of protease activity.
To investigate the role of ADAMTS9/GON-1 in the secretory pathway, we searched for genes whose depletion suppressed the *gon-1* phenotype in *C.elegans* and performed immunoprecipitation experiments in HEK293 cells. We found that the GON domain interacts with several suppressor gene products including a factor involved in the ER calcium homeostasis.
We investigated whether GON-1 is involved in calcium homeostasis. We found that calcium homeostasis is compromised by GON domain depletion. Furthermore, we found that the GON domain is involved in ubiquitination of Inositol trisphosphate receptor (IP3R). Now, we are investigating how the GON domain and the above-mentioned suppressor genes regulate IP3R. (COI: No)

1P-517

Relationships between exploration and anxiety in male Formosan wood mice (*Apodemus semotus*)

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The Formosan wood mice (*Apodemus semotus*) are the dominant rodent in Taiwan, and they live in the mountain areas of 1400~3700 meter-height. Past researches about wood mice were to focus on the ecological or reproductive behaviors in wild fields. Our recent study has found that male Formosan wood mice had higher searching locomotor activity in the laboratory environment as those in the wild fields. This study used the further behavioral tests, including the open field, light-dark box, and forced swimming tests, to examine the behavioral responses in male Formosan wood mice and compare with those responses in common laboratory mice, male C57BL/6 mice. Compared with C57BL/6 mice in the open field test, the total distance of movement significantly increased with male Formosan wood mice. In the light-dark box test, the latency to the light part was no difference between two mice. The Formosan wood mice showed higher duration in the light part compared with that of C57BL/6 mice. The Formosan wood mice showed lower duration in the immobility of forced swimming tests compared with that of C57BL/6 mice. Taken together, these results showed that male Formosan wood mice had higher activity, exploratory behaviors and more anxious responses, but did not have the depression-like behaviors. These behavioral responses might let Formosan wood mice have the surviving advantages in Taiwan mountains. (COI: No)

1P-518

Exploratory behaviors related to central dopaminergic activities in male Formosan wood mice

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Taiwan is a mountainous island with the steep Central Mountain Range along the longitudinal axis and the highest peak almost 4000 meters. The Formosan wood mice (*Apodemus semotus*) are the endemic wood mouse that generally resides in the highlands of Taiwan between 1400 and 3700 meters. Previous studies show that wood mice worldwide exhibit the differences of behavioural response compared with the common laboratory mice. Our recent studies have found that higher activity in the elevated plus maze test were highly correlated with central dopaminergic activities in male Formosan wood mice. This study used the further behavioral test, the open field test, to examine their behavioural responses compared with those in the common laboratory C57BL/6 mice. Compared with C57BL/6 mice in the open field test, the total distance of movement significantly increased with Formosan wood mice. The increased movements in the peripheral and center areas were observed in the Formosan wood mice compared with those of C57BL/6 mice. High locomotor and exploratory behaviours were highly correlated with central dopaminergic activities in male Formosan wood mice. Taken together, these results showed that male Formosan wood mice had higher activity and exploratory behaviours which might be related to the higher central dopaminergic activities. These behavioural responses might let Formosan wood mice have the surviving advantages in Taiwan mountains. (COI: No)

1P-519

Characterization of splicing variants of frog TRPA1 revealed divergence in their thermal property

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TRPA1 is known as a cold sensor in mammalian species, while it serves as a heat sensor in non-mammalian vertebrates. During the investigation of TRPA1 from several frog species, we found a novel splicing variant which has a single amino acid insertion near the N-terminus. In this splicing variant, a single valine residue was inserted in the 6th ankyrin repeat domain. We named the newly identified splicing variant as TRPA1(V+), and the TRPA1 splicing variant shared among all vertebrates as TRPA1(V-). TRPA1(V+) was shared among phylogenetically distantly related frog species. We then electrophysiologically characterized responses of heat stimulation of both variants from two frog species *Buergeria buergeri* and *Rana japonica*. In *R. japonica*, both variants responded to heat stimulation, although TRPA1(V+) showed higher temperature threshold for heat-evoked activation compared to that of TRPA1(V-). In contrast, TRPA1(-) from *B. buergeri* showed reduced thermal sensitivity instead *B. buergeri* TRPA1(V+) clearly responded to heat. In both species, both splicing variants were activated by an agonist cinnamaldehyde indicating that channel function is maintained even in *B. buergeri* TRPA1(V-). In conclusion, our results demonstrated the structural importance of the ankyrin repeat domain in modulating thermal sensitivity of TRPA1 in the course of evolution. (COI: No)

1P-520 (AP-7)

Fos expression in the hypothalamic nuclei after changes from hypergravity to normal gravity in mice

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It is important to determine the effects of change from microgravity to normal gravity on the central nervous system (CNS) after a long stay in the space. Fos expression is widely used as an anatomical/functional marker of the neuronal activation in the CNS. In the present study, Fos expression in the hypothalamic nuclei of mice with/without bilateral vestibular lesion (VL) were examined under normal gravity (1g) after exposure to centrifugation-induced hypergravity (2g) for 8 weeks, using immunohistochemistry. Mice were anesthetized deeply and perfused at 90 min after ending the exposure of hypergravity for 8 weeks. Control mice with/without VL were housed on normal condition. Immunohistochemistry for Fos revealed that Fos-immunoreactive (ir) cells were increased dramatically in the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA) after change from 2g to 1g. An increased number of Fos-ir cells in the PVN but not the LHA in VL mice were attenuated significantly in comparison with sham-operated mice. On the other hand, there was no change/difference in the neighbor nuclei such as the supraoptic nucleus and the arcuate nucleus in VL and sham-operated mice. As the PVN is known to be an important site to integrate the autonomic and neuroendocrine responses to the stress, these results suggest that acute response to returning from the hypergravity to normal gravity may cause autonomic/stress responses that are involved in the PVN via vestibular input. (COI: No)

1P-522

Impact of long-term stay in micro-gravity on vestibular function

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The vestibular organ consists of two components: the otolith organs, which perceive linear accelerations (gravity); and the semicircular canals, which perceive roll accelerations. The vestibular organ sends neural signal to the central nervous system, and regulates posture, eye movements, arterial pressure, and muscle-bone relationship, etc. However, it is known that the vestibular system is highly plastic, i.e., if subjects are exposed different gravitational environment, the function of the system is altered. To test this hypothesis, the otolith and canal functions were separately examined before and after the 6 months stay in the International Space Station (ISS), and correlate these functions to the body stability. Furthermore, we also test the hypothesis that noisy galvanic vestibular stimulation (nGVS) might ameliorate the impaired vestibular function. Otolith and canal functions were evaluated by vestibular evoked myogenic potential and by air-caloric test, respectively. Instantaneous fluctuations of the center of the body were determined using a gravicoder with or without nGVS. Astronauts were subjected to these tests before and after the stay in the ISS. The otolith function was impaired at 0-2 days after returning to the Earth and recovered after 2 weeks, but the canal function was preserved. The impaired otolith function was related to the body instability, which was ameliorated by nGVS. Thus, nGVS might be a countermeasure for spaceflight-induced body instability.

(COI: No)

1P-523

Effect of RBM3 on Glycolysis and Apoptosis in the Liver After Acute Cold Exposure

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Object

In this study, after acute cold exposure in mice who had fasted, some of molecular and signaling pathways responsible for the cold exposure response in the liver were investigated.

Materials and Methods

Male mice were randomly divided into control group (27±1°C) and acute cold exposure (4±1°C) for 2 h, 4 h, 6 h, and were fasted during the experiment. Liver tissue was collected after euthanasia. Change of *RBM3 mRNA*, Glycogen, ATP, AMP, liver glucolysis intermediates was measured. The expression of proteins in signaling pathway was detected by Western Blot.

Results

It was observed blood glucose remained stable during cold exposure for 0~4 h, and liver glycogen decomposition was unchanged during 2~6 h of cold exposure. FDP and PA showed a decrease after reaching the peak at 2 h after cold exposure with synchronous change ratio of ATP/AMP. In the liver, the mRNA level of RBM3 was significantly increased after 2 h cold exposure. The phosphorylation of AKT was found had a high level after exposure to the cold for 6 h. The phosphorylation of fructose-2, 6-bisphosphate and glycogen synthase kinase 3 beta was enhanced after exposure to the cold. In summary, the results suggested the liver possible mediated RBM3 expression via the HSP70/NF-κB pathway and enhanced cell survival by promoting the Bcl-2/Bax ratio, which RBM3 probable effects glucolysis via the AKT, GSK3β, and PFKFB2 signaling pathway during cold exposure. (COI: Properly Declared)

1P-524

Different adaptation of Chinese expeditioners during prolonged Antarctic and sub-Antarctic residence

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PURPOSE

Prolonged residence in Antarctica is characterized by exposure to isolated, confined, and extreme (ICE) environment. To understand the different patterns of psychophysiological responses provoked by environmental stress, we conducted a longitudinal assessment of mood and endocrine function in two groups of Chinese expeditioners who were deployed to sub-Antarctic (Great Wall Station, 62°S, $N = 12$) and Antarctic (Zhongshan Station, 66°S, $N = 16$) from December 2003 to 2005.

METHODS

Measures of mood, thyroid function, the levels of plasma catecholamine, and circulating interleukins were obtained at departure from China, mid-winter (Antarctica), end of winter (Antarctica), and return to China, respectively.

RESULTS

The Zhongshan Station crew experienced significant increase of negative mood and decrease in free thyroxine (FT4), norepinephrine (NE), and epinephrine (E) during the winter, increase in thyrotropin (TSH) and total triiodothyronine (TT3) when returning, whereas their counterparts at Great Wall Station only experienced increased TT3 after deployment. Compared with the Great Wall Station crew, the Zhongshan Station crew exhibited increased negative mood and severer endocrine dysfunction.

CONCLUSION

Chinese expeditioners who lived and worked at the Antarctic station and the sub-Antarctic station for over a year showed different change patterns in mood and endocrine hormones. (COI: No)

1P-525

Circadian Rhythm and Sleep during Prolonged Antarctic Residence at Chinese Zhongshan Station

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PURPOSE

Residence at Zhongshan Station for over 1 year exposes winter-over members to marked changes of light-dark cycle, in addition to geographic and social isolation. The aim of this study was to investigate the circadian rhythm and sleep phase of Chinese winter-over expeditioners at Zhongshan Station.

METHODS

This study was conducted on 17 healthy male participants before departure from Shanghai and during residence at Zhongshan Station for 1 year (before winter, mid-winter, and end of winter). Levels of aMT6s in 48h sequential urine samples were assessed, and the circadian rhythm was analyzed. Participants' sleep parameters were obtained from wrist actigraphy and sleep logs. MEQ and SPAQ were completed.

RESULTS:

The acrophase of aMT6s rhythm, sleep onset, sleep offset, and mid-sleep time were delayed significantly ($P < 0.05$) in Antarctica relative to departure values. The subjects had greater eveningness preference ($P < 0.05$) in mid-winter in Antarctica. The Global Seasonality Score and the prevalence of subsyndromal seasonal affective disorder increased ($P < 0.05$) during winter.

CONCLUSION:

Our results indicate that during polar nights Chinese expeditioners experienced the following problems: delayed circadian rhythm and sleep phase, later chronotype, and incidence of subsyndromal seasonal affective disorder. An appropriate combination of artificial bright light during dark winter months and a strict social schedule are recommended in a winter-over station in Antarctica. (COI: No)

1P-526

The sleep parameter and autonomic nervous response in menopausal women

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This study is to compare the change of sleep in the daily life in ten healthy women aged 45-55 years participated in this study on some nights each in the working day (WD) and holiday (HD).The subjects spent daily times as usual this experiment was carried out in each subject's home and measured the ECG using heart rate monitor (MyBeat) and sleep parameter using a Nemuri Scan mat (Paramount Bed Co. Ltd.) and the sleep diary. In the following morning, participants recorded the subjective perceptions of parameters using OSA questionnaire and sleep quality using VAS. Changes in autonomic function were estimated by the time domain for R-R intervals or the Lorenz plot method for 150min after sleep onset. The significant levels for the result were set at $P< .05$.

The total sleep time of HD tended to be higher than that of WD and the total time in bed of HD was significantly higher than that of WD. The mean sleep quality of VAS in the WD and HD was 58.7±21.4 and 59.6±24.7. The RR interval before sleep of HD was significantly higher than that of WD. However, the time course of R-R interval for 150 min after sleep onset followed the same progress on WD and HD. The correlation between the sleep quality and the total sleep time revealed the statistically positive significant in HD ($r=.554$, $p<.05$), but not in WD ($r=.041$, $p>.05$) . It is thought that we have the difference in the responsiveness of the sleep evaluation to sleep time between WD and HD in menopausal women. (COI: No)

1P-527

Time since injury and thermoregulatory responses in hyperthermic person with spinal cord injury

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Spinal cord injury persons suffer from thermoregulatory dysfunction. In general, skin vasodilatation and sweat responses in hyperthermia are absent in persons with cervical spinal cord injury (CSCI) and are not observed below the lesion in thoracic and lumbar spinal cord injuries (SCI). However, it remained unknown whether thermo-effector responses above the lesion in SCI were similar to able-body subjects (AB), and whether time since SCI modifies the responses. The present study conformed to the guidelines in the Declaration of Helsinki and were approved by the Review Board on Human Experiments, Wakayama Med. Univ. (#1091). Six CSCI (C5-7; injury year 1-22 yrs), 8 SCI (Th4-L1; 1-24 yrs) and 10 AB wore water-perfused suits circulating 33-°C water in a supine position. After 10-min baseline measurements, 36- and 50-°C water was perfused to upper and lower parts of the suits, respectively, until esophageal temperature raised by 1°C. The passive heat stress increased CVC and SR on the chest (C4; sensate) in AB and SCI, while not in CSCI. But the increase was attenuated in SCI compared with AB. Increases in CVC and SR on the lower abdomen (L1; insensate for CSCI and SCI) were absent in CSCI and SCI with a few exceptions. The higher SR on the chest related with the longer time since the SCI ($r=0.97$; $P<0.0001$). Thermo-effector responses in hyperthermia above the lesion are attenuated in SCI compared with AB, and these responses may be affected by time since the SCI. (COI: No)

1P-528

Neural network during cognitive tasks during whole body heat stress

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Hyperthermia-induced central fatigue impairs cognitive function as well as exercise performance. Using factional magnetic resonance imaging (*f*MRI), we evaluated the effect of hyperthermia on cognitive function. Twenty healthy individuals performed two visual cognitive tasks (Go/No-go task and Flanker task in a random order) before (i.e. normothermia) and during heat stress. External canal temperature during heat stress was increased by 1.1 °C from the normothermic condition. Reaction time of each task was shortened during heat stress, but the error rate was not changed. We observed broad activated brain regions, including the dorsolateral and ventrolateral prefrontal cortices, and motor-related areas such as supplementary motor area and premotor area during heat stress rather than during normothermia. These results suggest that heat stress increases the load of neural activity during performing cognitive tasks, relative to the normothermia. (COI: No)

1P-529

A study of ultradian rhythm expression with a mathematical model

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Sleep cycle and sleepiness appear in ultradian (U) rhythm. Sleep cycle is around 1.5 hours and sleepiness varies 1.5, 3-4, and 12 hours. U rhythm is considered to superimpose on circadian (C) rhythm. However, the mechanism oscillating several U rhythms is not fully understood. Therefore, we tried to explain the mechanism of oscillating several U rhythms using a mathematical model. As a precondition, it was assumed that an oscillator of U rhythm existed and generated several U rhythms by superimposing on C rhythm of clock gene expressions. In addition, 1.5-hour oscillator for U rhythm and 24-hour oscillator for C rhythm were assumed to have influences each other. U rhythm superimposed on C rhythm was calculated. As results, we reproduced and confirmed characteristics of U rhythm below. 1) Around 1.5, 3, 12, and 24-hour rhythms were expressed. Around 6, 8-hour rhythms were also produced. 2) To consider the temperature compensation of C rhythm, the effect of U rhythm on C rhythm should have a limitation depending on temperature. These results suggested that an oscillator of U rhythm might exist and generate several U rhythms by superimposing on C rhythm. (COI: No)

1P-530

Ultradian Calcium Rhythms in the PVN and SPZ in the Hypothalamus

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The suprachiasmatic nucleus (SCN), the master circadian clock in mammals, sends major output signals to the subparaventricular zone (SPZ) and further to the paraventricular nucleus (PVN), however, the neural mechanism of which is largely unknown. In this study, the intracellular calciumlevels were measured continuously in cultured hypothalamic slices containing the PVN, SPZ, and/or SCN. We detected ultradian calcium rhythms in both the SPZ-PVN and SCN regions with periods of 0.5 to 4.0 hours, the frequency of which depended on the local circadian rhythm in the SPZ-PVN region. The ultradian rhythms were synchronous in the entire SPZ-PVN region and a part of SCN. Since the ultradian rhythms were not detected in the SCN-only slice, the origin of ultradian rhythm is the SPZ-PVN region. In association with an ultradian bout, a rapid increase of intracellular calcium in a millisecond order was detected, the frequency of which determined the amplitude of an ultradian bout. Neurochemical interventions revealed that the glutamatergic mechanism is critical for generation and a tetrodotoxin-sensitive neural network for synchrony of the ultradian rhythm. The GABAergic system could have a role in refining the circadian output signals. The present study provides the first clue to unraveling the loci and network mechanisms of the ultradian rhythm. (COI: No)

1P-531

Thermosensors and neural circuit regulating temperature-dependent negative masking behavior in mice

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[Purpose] Masking is a direct behavioral response to environmental changes and plays an important role in the temporal distribution of activity. However, the mechanisms responsible for masking remain unclear. The purpose of this study is to identify the thermosensors and neural circuit regulating temperature-dependent negative masking behavior in mice. [Methods] We examined masking behaviors in all available thermosensitive transient receptor potential (TRP) channel–null mice (*Trpv1/3/4* and *Trpm2/8*). We also analyzed induction of *Fos*, a neuronal activation marker, in response to an acute warm temperature stimulus. Chemical lesion of one of the activated nuclei was performed as well. [Results] An ambient temperature rise decreased wheel-running activity, an example of negative masking, and this behavior was impaired in some TRP channel–null mice. *Fos* induction was observed in the thermoregulatory center, the preoptic area (POA), and the suprachiasmatic nucleus (SCN), which is the primary circadian pacemaker. We also observed *Fos* induction in several other nuclei, and partial chemical lesion of one of them slightly attenuated the masking behavior. [Conclusions] Our study revealed two thermosensors involved in temperature-dependent negative masking. Based on our findings using the neuronal activation marker *Fos* and chemical lesions in the brain, we propose a neural circuit that mediates this adaptive behavior. (COI: No)

1P-532

Real time recording of clock gene expression in multiple tissues of freely moving mice

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Clock genes are expressed in many tissues, both inside and outside the hypothalamic suprachiasmatic nucleus, the master clock controlling circadian rhythm. To understand the role of ubiquitous clock gene expression, we developed a *in vivo* recording technique of clock genes expression in freely moving mice over extended periods. In this system, quantification of gene expression in a steadily moving target was achieved by using photo-multiplier tube attached modified optical fiber and quantifies gene expression in peripheral tissues. Using this technique, we were able to measure circadian rhythms of clock gene expression over a prolonged period in the skin, liver and testis. Thus, our novel system successfully quantified clock gene expression in the body surface and deep areas in freely moving mice for a period sufficient to analyze circadian dynamics. (COI: No)

1P-533

The evaluation of activity and body temperature fluctuation in animal model of shift work

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We created an animal model of shift work by using mouse with feed restriction and exercise restriction by running wheel (RW). We used C57BL/6 mice and measured ambulatory activity, RW activity, body temperature and performed sleep monitoring with electroencephalogram (EEG) and electromyogram (EMG) recordings. The mouse was surgically implanted a radio transmitter into the peritoneal cavity for measuring ambulatory activity and body temperature, and the electrodes for measuring EEG and EMG under sevoflurane anesthesia. After the one week of recovery period, one week of baseline recording was started. After the baseline period, in shift work model group, the mouse was permitted to run on the RW and to eat the food during only light period and limited both of them in the dark period. In control group, we permitted them during the dark period only. Throughout the experiment, amount of ambulatory activity, RW activity, body temperature, EEG and EMG were recorded.

In shift work model group, peaks of ambulatory activity shifted from the dark period to the light period in the first day of the feeding and RW restriction. Body temperature decreased in the latter half of the dark period and increased in the first half of the light period. Non REM sleep increased in the dark period and arousal increased in the light period in the shift work model group. These results suggested that the biological changes in this mouse model might be equivalent to those of human in shift work condition. (COI: No)

1P-534

Optical imaging of circadian calcium rhythm in a solitary suprachiasmatic neuron

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In mammals, a master circadian clock is located in the hypothalamic suprachiasmatic nucleus (SCN) which is composed of multiple circadian oscillator cells. The SCN neurons are heterogeneous in not only cytochemical but also oscillatory properties. However, circadian properties of single neuron remain unknown. We examined circadian properties of solitary SCN neurons cultured on spatially isolated small wells, i.e., microislands. Culture dishes were prepared by opening a hole in the bottom of a 35 mm petri dish and attaching micropatterned MPC-polymer coated cover glass with silicon elastomer. SCN tissues were removed from newborn mice and dissociated into individual neurons with papain solution. These neurons were seeded on the micropattened-culture dish. The hSyn1-GCaMP6s was expressed in neurons using adeno-associated virus. We succeeded to demonstrate that spatially dissociated solitary SCN neurons exhibit circadian rhythms of intracellular calcium. Most of solitary single neurons, those without any physical contacts to other cells, showed sustained and stable circadian calcium rhythms. These results suggest that the circadian calcium rhythmicity of a solitary SCN neuron is cell autonomous. In the present methodology is suitable for examining physiological properties of single neurons. (COI: No)

1P-535

Chemical and thermal sensitivity of axolotl TRPA1

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As a nociceptive receptor, transient receptor potential ankyrin 1 (TRPA1) channel is expressed in sensory neurons of animals. TRPA1s from tetrapod vertebrates except rodents are activated by hot temperature with a relatively clear threshold. We recently examined characters of fish TRPA1s of zebrafish, medaka and pufferfish. The results suggested that a gradual heat-activation without clear threshold might be a common feature for TRPA1 of fish. To approach how noxious chemicals activate TRPA1 from tailed amphibians and which animal first acquires TRPA1 as a threshold detector instead of a gradual heat-sensor, here, we focused on TRPA1 from axolotls (*Ambystoma mexicanum*), which are members of order Urodela or tailed amphibians. Bade on the information on EST database of axolotl, we isolated a full-length cDNA of axolotl TRPA1 (axTRPA1). Sequencing analysis revealed that axTRPA1 cDNA encodes a protein which consists of 1120 amino acids. We studied the functional properties by two-electrode voltage clamp method using *Xenopus* oocytes. Allyl isothiocyanate, caffeine, methyl anthranilate and carvacrol activated axTRPA1 channels. Results indicated that axTRPA1 is heat-activated with the average threshold of 39.7 °C, suggesting that axTRPA1 already has acquired the functional property of land animals. (COI: No)

1P-536

Innate and acquired cold tolerant properties in hibernating Syrian hamsters (*Mesocricetus auratus*)

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Hibernators such as Syrian hamster (*Mesocricetus auratus*) keep their body temperature less than 10°C during hibernation. Despite very low body temperature, organs of hibernating animals are maintained in normal condition. Therefore, mechanisms to resist harmful cold temperature may operate during hibernation. The aim of this study was to clarify whether hamsters acquire the cold tolerant property specifically during hibernation or the cold tolerance is an innate property of hamsters. Hibernation-like hypothermia was forcibly induced by anesthesia and cooling, and the hypothermic hamsters were compared with naturally hibernating hamsters. Histological analysis with hematoxylin-eosin staining showed that there is no apparent injury in all organs examined. In contrast, in the artificially-induced hypothermic hamsters, tissue injury was manifested in the heart. In support of the histological data, elevation of cardiac enzyme activity was evident in the blood biochemical test. These results indicate that the heart of hamster acquires cold tolerance specifically during hibernation. When artificial hypothermia was induced in non-hibernators, rats and mice, tissue injury in the heart was more severe than that of hamsters. This suggests that the heart of the hamster possesses cold tolerance innately. Taken together, the present study demonstrates that the heart of the hamster has both innate and hibernation-specific cold tolerant properties to avoid cold injury during hibernation. (COI: Properly Declared)

1P-537

Effect of blue light blocking glass on melatonin secretion and sleep quality in humans

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Light has the strong effect on circadian rhythm, and especially blue light at night has a negative effect on melatonin secretion and sleep quality. Melatonin is a hormone secreted by the pineal gland during sleep at night. Recently, smart phone is popularly used in whole day even including night, resulting in the increased rate of insomnia and short of sleep. In the present study, we investigated that the effect of blue light blocking glass on melatonin secretion and sleep quality in humans. Seven men participated in this study for overnight from 18:00 h to 8:00 h of next day. Each subject underwent two conditions with and without wearing blue light blocking glass on a different day. Subjects sat in front of 5000 lux fluorescent and blue light lumps until sleep at 0:00 h in a climatic chamber set at 26°C and 50% relative humidity. Saliva samples for melatonin were collected at 20:00 h, 23:30 h, 6:00 h and 8:00 h. The melatonin concentrations were not significantly different between two groups. Total percentage of slow-wave sleep and the sleep latency to stage 3 were not significantly different with both conditions. Our results suggested that blue blocking glass have individual effects under strong light by 5000 lux.
(COI: Properly Declared)

1P-538

Cell autonomous cold resistance of a mammalian hibernator, Syrian hamster

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Most mammals maintain core body temperature (T_b) at around 37ºC, and deep hypothermia leads to cardiac arrest, or tissue injury. However, small mammalian hibernators can endure extreme hypothermia (their T_b drops to below 10ºC) during hibernation (HIB). To address mechanisms for the resistance to hypothermic stress, we first examined organ function during HIB in a mammalian hibernator, Syrian hamster. We found that hamsters did not exhibit obvious hepatic or renal dysfunction during HIB. Our RNA-seq analysis revealed that 42 genes were differentially expressed between normothermic state and hypothermic state during HIB both in liver and kidney. Some of these genes exhibited distinct responses to hibernation and artificial hypothermia induced by anesthesia and cooling. To examine whether cell autonomous cold response was different between non-HIB and HIB, we subjected primary hepatocytes to cold stress at 4ºC. Almost all the hepatocytes maintained their membrane integrity even after 5 days of cold culture both in non-HIB and HIB hamsters. In addition, hepatocytes from HIB hamsters tended to survive longer than non-HIB when they were subjected to prolonged cold culture for about 2 weeks, or to rewarming to 37ºC after cold culture. These data indicate that hamster hepatocytes have inherent cell autonomous cold resistance, which is further enhanced during HIB. (COI: No)

1P-539

Alternative splicing of cold-inducible RNA-binding protein mRNA in hypothermic animals

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Although low temperature exhibits protective effects on cell functions, it paradoxically damages organ functions. Interestingly, hibernators can survive even under severe hypothermic conditions less than 10°C during hibernation. To investigate the mechanism of tolerance to hypothermia, we focused on cold-inducible RNA-binding protein (CIRP) and examined its gene expression.We revealed that CIRP mRNA is constitutively expressed in various organs of non-hibernating euthermic hamsters with three alternative splicing variants with RT-PCR method. The short form contains open reading frame for full-length CIRP. On the other hand, the long form had an insert containing a stop codon, coding a C-terminal deletion isoform of CIRP. In contrast, only the short product was amplified in the hibernating animals. Next, we induced hypothermia in hamsters by cooling after anesthesia or injection of an adenosine A1 receptor agonist. When body temperature was rapidly decreased, the hibernation-specific splicing was not reproduced. In contrast, slow decreasing of body temperature caused unification of CIRP mRNA variants with either induction methods of hypothermia. These results suggest that decrease in body temperature, which is comparable with that observed in hibernators, can reproduce the hibernation-specific splicing pattern. This alternative splicing mechanism might permit rapid expression of function of CIRP only by switching the splicing factors during entering hibernation. (COI: No)

1P-540

Weighted gene co-expression network analysis in chronic kidney disease and hemodialysis patients

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Chronic kidney disease (CKD) is a general term for heterogeneous disorders affecting the structure and function of the kidney. Most of patients with End-stage renal disease (ESRD) which is the terminal stage of CKD chose to be treated with dialysis to increase their lifespan in developed country. In CKD or hemodialysis patients, genes involved in immune system, oxidative stress and mitochondrial dysregulation were revealed by microarray analysis, however, several points remain unknown. In this study, Microarray data of GSE15072 was downloaded from the Gene Expression Omnibus (GEO) database and we examined the microarray data set of healthy controls, CKD patients, and hemodialysis (HD) patients using weighted gene co-expression network analysis (WGCNA) and identified co-expression modules.

Turquoise module genes were most significantly enriched in GO terms related to ion channel activity. In this module, genes involved in neurological and mental disorder or cardiovascular diseases were also contained. Brown module genes were most significantly enriched in GO terms related to immune response. In this module, genes involved in the survival of memory CD4 positive T cells were identified in addition to genes of the immune system known as differentially expressed in HD patients. These findings are expected to contribute to the prevention of complications such as cardiovascular events and infectious diseases in CKD and HD patients. (COI: No)

1P-541

Reflected conduction caused by subcellular sodium channel redistributions

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Brugada syndrome (BrS) is characterized by the higher incidence of sudden cardiac death due to ventricular tachycardia/fibrillation. It is believed that such fatal arrhythmias are elicited by the closely coupled premature ventricular contractions via the reflected conduction (or phase-2 reentry). Although a loss of function mutation in cardiac sodium (Na) channels has been linked to BrS, the relation between the loss of function of Na channels and the arrhythmogenic mechanism remains unclear. Here, we conducted computer simulations of AP propagation in a human ventricular myofiber model where myocytes were electrically coupled with both gap junction and intercellular cleft conductor, and investigated the relation between subcellular Na channel distributions and the development of reflected conduction. We found that the myofiber model with specific subcellular Na channel distribution (Na channels were preferentially localized in the intercalated disks and Na channels along the lateral side of each myocyte were markedly reduced) resulted in early repolarization followed by reflected conduction. Subcellular Na channel redistribution might be responsible for lethal arrhythmia in BrS. (COI: No)

1P-542

Simulation study on the nitrogen homeostasis disturbed by defect of glutamine synthase in liver

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Since ammonia is toxic for the central nervous system, its blood concentration should be maintained at low level. Human excrete most of nitrogen as urea in urine, and urea synthesis exclusively occurs in liver. Ammonia is an inescapable metabolic intermediate during urea synthesis from various nitrogen compounds in hepatocytes. Most of ammonia is produced in hepatocytes and many of it is converted into urea and the rest is converted into reusable glutamine.

The liver is super-parallel metabolic filter with hundreds of thousands hepatic lobules. Blood flows into a hepatic lobule from fine branches of hepatic artery and portal vein and goes out from central vein. While periportal (PP) blood abundantly contains external molecules absorbed in the gastrointestinal tract, perivenous (PV) blood carries almost adjusted substances. Therefore, metabolic heterogeneity inevitably arises between PP and PV. Moreover, it is known that many enzymes heterogeneously express between PP and PV. For nitrogen metabolism, activity of urea cycle is dominant in PP and glutamine synthase (GS) activity is confined in PV. Recently, it is shown that hepatic GS deficient transgenic mice exhibit hyperammonemia.

In this study, we made a systematic nitrogen homeostasis model which incorporated metabolic heterogeneity in liver. This model successfully represented hyperammonemia caused by GS defect, and we explored causal relationships found in systematic shift of nitrogen homeostasis in the GS knockout mouse. (COI: No)

1P-543

Cortical cerebral blood flow response induced by manual acupuncture of the auricular region in rats

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We aimed to determine whether acupuncture to the auricular or abdominal region increases cortical regional cerebral blood flow (rCBF). The rCBF was measured using laser speckle contrast imaging in urethane-anesthetized rats. Acupuncture stimulation was performed manually at the auricular concha or abdomen. The former's stimulation significantly increased the rCBF of the bilateral cerebral cortex in the frontal, parietal, and occipital lobes without altering the systemic arterial pressure. In contrast, abdominal stimulation affected neither rCBF nor systemic arterial pressure. The increase in the rCBF was completely abolished by the severance of the somatic nerves that innervated the auricular region, comprising the trigeminal nerve, facial nerve, auricular branch of the vagal nerve, glossopharyngeal nerve, and great auricular nerve. The increase in the rCBF was not influenced by i.v. administration of naloxone, suggesting that endogenous opioids are not involved in the present response. We conclude that the application of acupuncture to the auricular region induces naloxone independent increase of the rCBF without increasing arterial pressure. (COI: No)

1P-544

Influence of press tack needle acupuncture on the secretion of orexin

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Background: Press tack needle (PTN) is a very short acupuncture needle with an adhesive plaster. Orexin, a neuropeptide secreted mainly from the hypothalamus, is involved in the regulation of stress responses. In this study, the influences of PTN treatment on the secretion of orexin were investigated.

Methods and Results: Male Wistar rats were used for the study.

1) Differences in the influence on secretion of orexin A according to the length of the needle were investigated. PTNs (sham: without needle) of lengths 0.3 mm, 0.6 mm, and 1.2 mm were inserted into acupoint GV20 in intact rats; 24 h later, plasma levels of orexin A were measured. The decrease in plasma levels varied according to the lengths.

2) The influence on psychological (social isolation) stress model of the rats was investigated. After isolated stress loading for 8 days, an aggressive behavior and secretions of orexin A into plasma and in the hypothalamus increased. However, these increases were inhibited following PTN (1.2 mm) treatment at GV20.

3) Involvement of orexin in causing increased aggressive behavior was investigated. After isolated stress loading similar to experiment 2, orexin 1 or 2 receptor antagonists were injected. The administration of orexin 2 receptor antagonist restrained this behavior.

Conclusion: These results suggest that PTN treatment at GV20 inhibits the secretion of orexin A and has an anti-stress effect. The inhibitory effect of orexin A may be related to the anti-stress effect. (COI: No)

1P-546

Family history of hypertension has an effect on blood pressure response with fragrance inhalation

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[Purpose] Fragrance inhalation of essential oils used in aromatherapy can affect blood pressure (BP) and autonomic nervous system in humans. Considerably wide individual variation has been observed in the response, but the background factors behind the wide individual variation remain unknown. Previous studies have reported that resting circulatory variables and baroreflex sensitivity, and also the response of BP and muscle sympathetic nerve activity with various stimuli are different between subjects with and without family history of hypertension (FH). We tested the hypothesis that the response of BP with fragrance inhalation of grapefruit essential oil (GF) was different between subjects with (FH+) and without FH (FH-). [Methods] Eighteen healthy subjects participated in this study (FH+; n=8, FH-; n=10). While subjects in the supine position, they breathed blank air through a face mask for 10 min as baseline (BL). Then, they inhaled air with the fragrance of GF from Douglas bags for 10 min. Beat-by-beat heart rate (HR) and BP were recorded continuously.[Results] At 6 to 10 min of BL, HR but not BP was significantly ($P < 0.05$) higher in FH+ group than FH- group. During GF inhalation, diastolic BP in FH- group increased significantly from BL whereas it remained unchanged in FH+ group.[Conclusions] The FH is a background factor behind the individual variation observed in the response of BP with fragrance inhalation of grapefruit essential oil. (COI: No)

1P-547

Physiological effects in CNS and the autonomic nervous system by drinking jasmine tea

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To investigate physiological effects after by jasmine tea, we measured the contingent negative variation, CNV of EEG. The autonomic nervous activities were analyzed by measuring heart rate and heart rate variability. Psychological conditions were monitored by describing two psychological tests, the multiple mood scale (MMS) and the General Arousal Checklist (GACL). Subjects were young 6 students for jasmine tea and 7 for coffee. Area of the late CNV in Fz was increased after drinking jasmine tea and in other positions areas were decreased, suggesting that jasmine tea might induce relaxation. On the other hand, after drinking coffee areas of the late CNV in all positions were increased, suggesting awakening effect. Reaction time for pushing button to target signal of CNV after drinking jasmine tea did not changed but after drinking coffee the time was reduced in almost 8% comparing to the control. These results suggested that drinking coffee stimulated responsibility of nervous function from sensory to motor system but no effect by jasmine tea. HF in heart rate variability was increased after drinking jasmine tea and coffee, suggesting that tea and coffee induced relaxation and stimulated parasympathetic activity. Liveliness of mind in MMS was decreased after jasmine and startle of mind in MMS was increased after drinking coffee. Difference in GACL was observed. (COI: No)

1P-548

Contribution of oxytocin to the anti-stress effect of Kampo medicine *Kamikihito*

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Purpose: *Kamikihito* (KKT) is a Kampo medicine (Japanese traditional herbal medicine) that is administered to patients with psychological symptoms such as anxiety, depression and sleeplessness. Oxytocin (OT), a hypothalamic nonapeptide, is linked to increased levels of social interaction, well-being and anti-stress effects. We investigated whether KKT has anti-stress effects and the contribution of OT to the mechanism.

Method and Results: Male Wistar rats were allocated into control, acute stress (Stress), and KKT (300 mg/kg/day po)-treated acute stress (KKT+Stress) groups. Rats in the Stress and KKT+Stress groups were exposed to a 90-min restraint stress procedure involving novel physical stress. This model is also used as a model of irritable bowel syndrome. Stress induces defecation; thus, the fecal pellets were quantitated. The fecal weight in the KKT+Stress group was significantly lower than that in the Stress group. OT secretions of cerebrospinal fluid were collected by microdialysis and analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS). In the Stress and KKT+Stress groups, the OT levels were increased during stress loading. At 30 and 60 min after stress loading, the levels in the Stress group were decreased; however, those in the KKT+Stress group remained higher than before stress loading.

Conclusion: These results suggested that KKT has anti-stress activities and that increased OT secretion may be a mechanism underlying this phenomenon. (COI: No)

1P-549

Asymmetric Dimethylarginine and Endothelin B Receptor Modulation in *Piper Sarmentosum* Treated Rats

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Hypertension is a major modifiable risk factor for cardiovascular diseases that leads to morbidity and mortality worldwide.Less than half of hypertensive patients managed to achieve blood pressure (BP) goal with current treatments.*Piper sarmentosum* (PS) have been widely used traditionally and proven for its antihypertensive and antioxidant effects. This study aims to evaluate the antihypertensive potential of PS aqueous extract (PSAE) and investigate on how it modulates nitric oxide (NO), asymmetric dimethylarginine (ADMA) and arginine level as well as endothelin B receptor (ETBR) expression in spontaneous hypertensive rats (SHR). PS leaves were extracted with distilled water and freeze-dried. Its antioxidant capacity was examined using free radical scavenging and ferric reducing ability test. SHRs were divided into four groups (n=6); C (negative control), K (PSAE 500mg/kg), P (3 mg/kg perindopril) and M (PSAE 500 mg/kg + 1.5 mg/kg perindopril). Treatments were given via oral gavage for 28 consecutive days. The BP and heart rate were determined using the non-invasive blood pressure monitoring tail cuff technique and recorded weekly. SHRs' blood was sampled to determine the NO, ADMA and arginine levels. The ETBR expression in SHRs' kidney arterioles were determined by immunohistochemistry. PSAE shows good antioxidant activities, reduces ADMA thus increasing the serum NO level. The arginine level is maintained, and it upregulates ETBR hence ameliorating the BP of SHRs. (COI: No)

1P-550

Theobromine increases plasma cholesterol levels by increasing ABCA1 protein

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Theobromine is a methylxanthine, which is found abundantly in cacao. Although the structure of theobromine is similar to that of caffeine, its physiological effects are different. Theobromine increases HDL cholesterol levels in humans; however, the detailed mechanisms are not well known. In this study, we assessed the mechanisms through which theobromine increases cholesterol levels in rats. Forty-eight male Wistar rats were assigned to two groups. One group was fed a standard rat chow (control diet) and the other group was fed the control diet with 0.05% theobromine added. Every 10 days, 6 rats from each group were sacrificed under anesthesia, and the blood, liver, and muscle tissues were collected. Theobromine did not affect the body and liver weights of the rats. Plasma cholesterol levels at 30 and 40 days were significantly higher in the theobromine group than in the control group. Detailed lipoprotein analysis revealed that HDL and LDL cholesterol levels at 30 days in the theobromine group were significantly higher than those in the control group. The mRNA levels of ABCA1 were significantly higher in the theobromine group. Furthermore, western blot analysis revealed theobromine significantly increased the ABCA1 protein levels in the liver at 10 and 40 days. These results indicate that theobromine enhances cholesterol transport by increasing ABCA1 levels. As theobromine is a PDE inhibitor, increased ABCA1 levels may lead to elevation of cAMP levels. (COI: No)

1P-551

Nonequivalent effect of CO_2-water bath on muscle fatigue caused by isotonic- and isometric-exercise

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With CO_2-hot-springs bath, the blood flow is increased in the local skin and skeletal muscle underwater, even beneath a neutral water temperature. Alteration of the blood flow may influence the muscle fatigue during exercise through the supply of metabolic substrate and the removal of the metabolic products. To investigate whether CO_2-water bath influenced the muscle fatigue, the subjects were immersed their forearm into artificial CO_2-water containing comparable amount of CO_2 with natural CO_2-hot spring (CO_2>1g/L) in between intermittent exercise. Two groups of 6 (IT: isotonic exercise group) and 9 (IM: isometric exercise group) subjects participated in the experiment. To induced muscle fatigue, IT repeated bending and stretching a middle finger with loading weight, and IM repeated continuous holding a hand dynamometer at 35% of maximum grip strength as long as possible. During 10-min rest in between two sets of repetitive exercise, the subjects put the forearm into arm-bath apparatus filled with general tap water or CO_2-water of a same temperature (33 °C) under room temperature of 24 °C. In IM, decrease in holding time showed fatigue, but any difference of fatigue state was not observed between two waters for forearm bath. In IT, CO_2-water bath showed significant facilitation of recovery from muscle fatigue compared to tap-water bath. The effect of CO_2-water bath on the fatigue might be depending on the way of fatigue induction under the present experimental conditions. (COI: No)

1P-552

Change in the foot pressure distribution to dental occlusion adjustment by micro tapping with paper

Masanori Takemura[1]; Akio Kawamura[2]; Kenichi Ichihashi[1]; Mitsuharu Kaya[3]; Junzo Tsujita[4] ([1]*Ichihashi Clinic, Japan*; [2]*Kawamura Dental Clinic*; [3]*Hyogo University of Health Science*; [4]*Institute of Health & Sports Medical Science*)

It was reported that a dental malocclusion influences a posture balance in the previous study. Kawamura has developed the method to dental occlusion adjustment temporarily by a micro tapping using kitchen paper instead of a sprint. We're experiencing a change of muscle tonus and/or posture by the micro tapping clinically. The purpose of this study is to clarify the change by the micro tapping in the foot pressure distribution of a standing position. Thirty orthopedic patient with a dental malocclusion participated in an investigation. The foot pressure distribution and posturography with eye open and eye close were measured before and after the micro tapping using kitchen paper for temporarily dental occlusion adjustment. The measurements of before and after the micro tapping were compared by paired t-test. And it was compared by 2-factor (low and high Romberg ratio group) ANOVA. The center of pressure of each left and right foot and the load balance in calculated by the foot pressure distribution didn't change before and after the tapping. The trajectory length (mm/s) in center of pressure was decreased significantly to 5.7±1.98 at after tapping from 6.9±3.18 at before tapping. The trajectory area (mm^2/s) was decreased significantly to 3.0±.32 at after from 4.4±.74 at before for high Romberg group. Alteration of the foot pressure distribution occurred by the micro tapping. This is regarded by whether activation of labyrinthine function or reduction of muscle tonus. (COI: No)

1P-553

Analysis of Ultrasound Changes in Vastus Lateralis Muscle following Transcutaneous Vacume Treatment

Junzo Tsujita[1]; Tomonari Shibutani[2,6]; Hiroshi Ueno[3]; Yoichiro Yamashita[4]; Arijit Banerjee[5]; Mitsuharu Kaya[6]; Masanori Takemura[7]; Kenichi Ichihashi[7] (*[1]Institute of Health and Sports Medical Science, Japan*; *[2]MJ Company, Japan*; *[3]JCRAFT, Japan*; *[4]Osaka Electro-Communication University, Japan*; *[5]Amgsaki-city Bord of Education, Japan*; *[6]Hyogo University of Health Sciences, Japan*; *[7]Ichihashi Clinic, Japan*)

Background: We report that transcutaneous vacuum treatment improves ROM and decreases pain. However, no study has reported whether transcutaneous vacuum improves the gliding function and flexibility of muscle and fascia. Objective: This study aimed to determine the effects of transcutaneous vacuum treatment on fascial gliding of the vastus lateralis muscle. **Methods**:Transcutaneous vacuum treatment (vacuum and rolling [approximately 0.5Hz]) was applied to both left and right vastus lateralis muscle. Deep fascial motion during treatment of five subjects and during passive movement of knee joint from 0 to 45 degree in seven subjects were measured by B-mode ultrasound. Data were statistically evaluated using two-way ANOVA. **Results**:Deep fascial motions during treatment were the superficial layer; 5.6±2.35 mm and the deep layer; 6.3 ± 2.19 mm. Ratio (%) for the motion during the knee joint passive flexion from 0 to 45 degree were 16.9 (the superficial layer), and 14.6 (the deep layer). Motion of the deep fascia before treatment was 26.8 ± 7.57 mm and after treatment was 32.2 ± 7.39 mm. Ttreatment effect was significant, and then motion after was longer than motion before as a result of ANOVA. C**onclusion**: the transcutaneous vacuum provided mild myofascial stimulation without requiring articular movement. Transcutaneous vacuum treatment was a more effective therapeutic method for improving gliding function and flexibility of muscle and fascia. (COI: Properly Declared)

1P-554

Changes of HRV and resting-state amygdala functional connectivity after SKY practicing

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SKY is comprised a unique breathing technique sudarshan kirya. Daily practicing of SKY increases well-being and relief of emotional disorders such as anxiety. Especially, SKY practicing improves the vagal tone as reflected by heart rate variability (HRV) analysis. Amygdala is a key hub of emotional response and affects the cardiac autonomic tone. However, whether the improvement of HRV by SKY is modulated by the functional connectivity of amygdala in the brain remains unknown. We recruited 25 healthy participants who had learned SKY for resting-state magnetic resonance imaging (rs-fMRI) and photoplethysmographic (PPG) HRV analysis. EPI and HRV data were collected simultaneously for each participant immediately before and after SKY practicing and watching video. Results showed that SKY practicing but not video watching significantly increased RMSSD. FC of right amygdala and right insula/parahippocampol cortex was positively correlated with increased RMSSD after SKY practice. Similarly, FC of left amygdala and right amygdala/parahippocampol cortex was also positively correlated with increased RMSSD. However, these correlations were not found after watching video. These results demonstrated that SKY practicing but not watching video significantly increased RMSSD and the improvement of HRV by SKY practicing is correlated with the functional connectivity of bilateral amygdala with important forebrain regions involved emotional regulation. (COI: No)

Poster Presentations

Day 3

（March 30, 13:30-14:20/14:20-15:10）

2P-001～2P-016	Skeletal muscle & locomotion (2)
2P-017～2P-042	Exercise (2)
2P-043～2P-081	Circulation & Respiration: Cardiac Physiology (2)
2P-082～2P-086	Circulation & Respiration: Lung Physiology (2)
2P-087～2P-108	Circulation & Respiration: Vascular Physiology (2)
2P-110～2P-161	Endocrine, Reproduction & Development (2)
2P-162～2P-187	Neuroscience: Neural development and repair
2P-188～2P-200	Neuroscience: Synapse & neural cellular communication (2)
2P-201～2P-222	Neuroscience: Neuron-glia interactions/functions of glia
2P-223～2P-248	Neuroscience: Imaging of brain
2P-249～2P-274	Neuroscience: Learning, memory & neuronal plasticity (2)
2P-276～2P-298	Neuroscience: Neurologic and psychiatric diseases (2)
2P-299～2P-325	Neuroscience: Somatosensory & Pain (2)
2P-326～2P-342	Neuroscience: Autonomic physiology (2)
2P-343～2P-373	Neuroscience: Others (2)
2P-374～2P-382	Epithelial Transport, Secretion & Absorption: Epithelium (2)
2P-383～2P-394	Epithelial Transport, Secretion & Absorption: G-I tract (2)
2P-395～2P-394	Epithelial Transport, Secretion & Absorption: Renal Physiology (2)
2P-395～2P-431	Molecular & Cellular Biology: Channels & Transporters (2)
2P-432～2P-498	Molecular & Cellular Biology: Cellular Physiology (2)
2P-499～2P-518	Adaptation, Environment & Evolution (2)
2P-519～2P-529	Genomics & Biodiversity
2P-530～2P-539	Education
2P-540～2P-552	Alternative Medicine (2)

2P-001

Application of CGRP upregulates MyHC I mRNA through cAMP-dependent manner in C2C12 cells

Yoshiaki Mori[1]; Junko Yamaji[2] (*[1]Department of Rehabilitation Sciences, Kansai University of Welfare Sciences, Japan*; *[2]Department of Nutrition Sciences, Kansai University of Welfare Sciences, Japan*)

Our previous study using differentiated C2C12 cells indicated that myosin heavy chain type I (MyHC I), myosin heavy chain type II_b (MyHC II_b), and interleukin-6 (IL-6) mRNA expression levels were significantly increased by the application of calcineurin (CN) activators, such as chlorogenic acid. In this study, we examined the effects of calcitonin gene-related peptide (CGRP) on these mRNA levels in C2C12 cells. Effects of CGRP have been studied in two skeletal muscle cell lines, L6 and C2C12 cells, and these cell lines appear to express CGRP receptors coupled to adenylyl cyclase activity. However, the effects of CGRP on expression of MyHC mRNA have not been reported in these cells. C2C12 cells were induced to differentiate to myotubes by medium exchange to D-MEM containing 2%FBS. The cells were incubated in D-MEM containing 2%FBS with chemical compounds at the beginning of differentiation and removed after 24hr, and were maintained in differentiation medium for 3 days. MyHC I, MyHC II_b, and IL-6 mRNA expression levels were measured by the real-time PCR method. MyHC I mRNA levels were significantly increased by the administration of CGRP, although MyHC II_b and IL-6 mRNA levels were not affected by it. Additionally, the effects of forskolin on these mRNA levels were almost identical to that of CGRP. These results suggest that the effects of CGRP on upregulation of MyHC I mRNA levels do not depend on the CN-mediated mechanisms, but on the cAMP-mediated mechanisms in C2C12 cells. (COI: No)

2P-002

Essential role of calcineurin but not cAMP in mRNA expression of MyHC II and IL-6 in murine myocytes

Junko Yamaji[1]; Yoshiaki Mori[2] (*[1]Dept. of Nutrition sciences, Kansai University of Welfare Sciences, Japan*; *[2]Dept. of Rehabilitation sciences, Kansai University of Welfare Sciences, Japan*)

Forskolin is a diterpenoid from Coleus forskohlii, activating adenylate cyclase and increasing intracellular cAMP levels. CGRP is a neuropeptide released from motor neuron endings and also activates cAMP-dependent pathway. However, contribution of the cAMP-dependent pathway in mRNA expression of MyHc Ⅱ in skeletal muscle remains elusive.

Our recent study demonstrated that calcineurin activation enhanced IL-6 mRNA and that IL-6 production by the calcineurin activation in myocytes might increase MyHC $Ⅱ_b$ mRNA.

In this study, we exmamined that the effects of CGRP and forskolin on expression levels of MyHC $Ⅱ_b$ and IL-6 mRNAs in C2C12 cells. C2C12 cells were cultured by medium containing with or without agent at the beginning of differentiation. The mRNA expression levels were measured by quantitative RT-PCR method using Taqman probes.

Then our results are as follow: (1) The MyHC $Ⅱ_b$ mRNA level was significantly upregulated by IL-6 induced by calcineurin activators and was significantly attenuated by calcineurin inhibitor. (2) Both MyHC $Ⅱ_b$ and IL-6 mRNA levels were not affected by medium supplemented with CGRP. (3) The mRNA expression levels of MyHC $Ⅱ_b$ and IL-6 was also not affected by medium supplemented with forskolin.

These results suggested the production of IL-6 induced by calcineurin activation increases MyHC $Ⅱ_b$ mRNA but that CGRP-cAMP pathway is not participated in mRNA expression of IL-6 and MyHC $Ⅱ_b$ in C2C12 cells. (COI: No)

2P-003

Differential Scanning Calorimeter reveals interaction between water and myoproteins

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MRI reflects not only water content, but also water states in biological tissue. Details of tissue water state are not clarified yet, but interaction between water and macromolecules is generally considered to restrict molecular motional freedom similarly to the freezing of water. As in the case of melting of ice, differential scanning calorimeter (DSC) showed the restriction as extra heat absorption with temperature. With DSC, skinned fibers of sartorius muscle from *Rana Catesbeiana* at rigor condition showed extra heat absorption at -24, -21, 0, 46, 65℃. The peak at 46℃ and 65℃ would represent denaturation of myosin and actin, respectively, because selective removal of myosin or actin diminished the corresponding peaks, and the temperature values are close to those reported for the denaturation of corresponding proteins from rabbit psoas (Dergez et al.). The denaturation and removal of myosin and actin filaments differentially affected the peaks at -24, -21℃. The peak at -24℃ was affected mainly by actin filaments, and the peak at -21℃ was affected by both of myosin and actin filaments. Integrated heat absorption in the range from -80 to +20℃ was affected by actin filaments. These results suggested actin and myosin independently and cooperatively restrict surrounding water, and the over all heat capacity depends mainly on actin filaments. (COI: No)

2P-004

Microscopic heat pulses induce activation of cardiac thin filaments in the *in vitro* motility assay

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During excitation-contraction coupling of the heart, sarcomeres are activated via thin filament structural changes, i.e., from the "off" state to the "on" state, in response to a release of Ca^{2+} from the sarcoplasmic reticulum. Here we investigated the effects of rapid heating by Infra-red (IR) laser irradiation on the sliding of thin filaments reconstituted with human α-tropomyosin and bovine ventricular troponin in the *in vitro* motility assay. Temperature was varied from 25 to ~46˚C within 2 s. In the absence of $Ca^{2+}$and in the presence of ATP at 25˚C, F-actin moved at 4.9±0.1 μm/s, although reconstituted thin filaments did not move. Then, IR laser irradiation elicited movements of reconstituted thin filaments, with a sliding velocity of 5.0±1.0 and 8.6±0.5 μm/s at ~34 and ~38˚C, respectively, which increased to 14.5±0.6 μm/s at ~41˚C, with a temperature coefficient (Q_{10}) of 5.5. The sliding velocity of F-actin was increased with increasing temperature, which was faster than reconstituted thin filaments between 25 and ~41˚C, with a Q_{10} of 2.4. The heating-induced acceleration of thin filament sliding was likewise observed in the presence of $Ca^{2+}$and ATP; however, the temperature dependence was >2-fold less pronounced. These findings suggest that in mammals, the "on-off" equilibrium of the cardiac thin filament state is partially shifted toward the "on" state in diastole at the body temperature, enabling rapid and efficient myocardial dynamics in systole. (COI: No)

2P-005

Withdrawn

2P-006

Thalamocortical Axon Activity in Motor Cortex Exhibits Layer-Specific Dynamics during Motor Learning

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In motor circuits, subcortical and cortical structures are interconnected in real time. The thalamus is the hub through which neural signals are transmitted from the basal ganglia and cerebellum to the neocortex. However, thalamocortical axonal activity during motor learning remains largely undescribed. We conducted two-photon calcium imaging of thalamocortical axonal activity in the motor cortex of mice learning a self-initiated lever-pull task. Layer 1 (L1) axons came to exhibit activity at lever-pull initiation and termination, while layer 3 (L3) axons did so at lever-pull initiation. L1 population activity had a sequence structure related to both lever-pull duration and reproducibility. Stimulation of the substantia nigra pars reticulata activated more L1 than L3 axons, whereas deep cerebellar nuclei (DCN) stimulation did the opposite. Lesions to either the dorsal striatum or the DCN impaired motor learning and disrupted temporal dynamics in both layers. Thus, layer-specific thalamocortical signals evolve with the progression of learning, which requires both the basal ganglia and cerebellar activities. (COI: Properly Declared)

2P-007

Leg muscle activity during postural control under optokinetic stimulation in healthy subjects

Junya Komagata[1,2]; Atsushi Sugiura[1]; Hiroshi Takamura[2]; Yujiro Masu[2]; Toshihiro Kitama[1] (*[1]Center for Life Science Research, University of Yamanashi, Japan*; *[2]Department of Physical Therapy, Health Science University, Japan*)

Stroke patients often show a gait asymmetry due to their loss in muscle strength and perceptual deficits on the affected side. The weight-bearing and the augmentation of the muscle strength would be critical for the rehabilitation of the patients. The present study examined the effect of OKS on postural stability and electromyographic (EMG) activity in the legs of normal subjects. We asked 5 healthy students (aged 20-22 years old) wearing a head-mounted display device (HMD) to stand upright quietly on a stabilometric platform. For the OKS, a pattern of random dots in a virtual 3D spherical space was moved continuously at 40 °/s in rightward horizontal (HOKS) and clockwise torsional (TOKS) directions. Postural stability was evaluated by measuring the body's center of pressure position (CoP), total length of sway path (SP), and sway area (SA). EMG activity was recorded from the bellies of the tibialis anterior (TA) and gastrocnemius (GC). During each OKS direction, CoP tended to shift rightward and the SP and SA values were higher than in the static condition. Analysis of the EMG activity showed (1) higher right TA activity during HOKS and TOKS and right GC activity during TOKS than in the static condition; and (2) significant correlation between the increase in right GC activity and each SP and CoP slope value. TOKS and HOKS induced significant changes in body balance and EMG activity, suggesting the OKS via HMD could be applied in stroke rehabilitation. (COI: No)

2P-008

Effects of neonatal dopamine depletion on behavioral responses to anxiogenic tasks in adult rats

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Dopamine (DA) neurons in the midbrain is involved in several brain functions, including motor control, and anxiety. Although rats with neonatal DA depletion exhibit motor hyperactivity, characterization of their behavior under anxiogenic conditions is lacking. In the present study, we investigated behavioral responses to anxiogenic tasks in adult rats with neonatal DA depletion. The open field (OF), and elevated plus maze (EPM) tests were performed under low and bright light conditions to obtain detailed behavioral characterizations. The ameliorative effects of pretreatment with methamphetamine MAP (4 mg/kg) and ATX (1.2 mg/kg/day) on abnormal behaviors induced by neonatal DA depletion were also assessed. Rats that underwent 6-hydroxydopamine treatment 4 d after birth showed significant increases in motor activity and decreases in anxiety-related behaviors in OF tests under both conditions and in EPM tests under bright condition. Rats with neonatal DA depletion did not show normal behavioral responsiveness to changes in the intensity of anxiogenic stimuli. Pretreatment with MAP and ATX ameliorated motor hyperactivity but not abnormal anxiety-related behaviors. These results suggest that the dopaminergic system plays a crucial role in the development of neural networks involved in locomotor activity and anxiety-related behavior, and that the mechanisms underlying abnormal anxiolytic responses partially differ from those underlying motor hyperactivity. (COI: No)

2P-009

Primary motor cortex single cell activity during quadrupedal vs. bipedal gait in Japanese macaques

Marc A Maier[1]; Katsumi Nakajima[2,3]; Kazunori Morita[2,3]; Akira Murata[2]; Masahiko Inase[2] (*[1]FR[3636], CNRS / Université Paris Descartes, Sorbonne Paris Cité, France* ; *[2]Department of Physiology, Kindai University, Faculty of Medicine, Japan* ; *[3]Department of Physiology, School of Medicine, Iwate Medical University, Japan*)

The role of distributed neural structures in bipedal locomotion in humans and non-human primates is still debated. It remains unclear whether the functional roles attributed to the motor cortex, deduced from the cat model, also holds for non-human primates. We recorded the activity of 109 cells from hindlimb/trunk regions of the primary motor cortex (M1) in 2 *Macaca fuscata* during treadmill locomotion (~1.0 m/s), as well as EMG activity.Each cell was recorded during quadrupedal (QP) and bipedal (BP) gait. Experimental procedures were approved by the Animal Care and Use Committee of Kindai University and performed in accordance with the Guidelines for Proper Conduct of Animal Experiments of the Science Council of Japan. Most M1 cells modulated their activity phasically or phasic/tonically for both QP and BP gait. During BP (vs. QP) gait, M1 population activity showed higher mean discharge frequency during the step cycle (25.4±19.7 vs. 21.2±18.7Hz) and higher peak frequency (78.8±57.0 vs. 59.5±42.7Hz). Peak activity occurred during BP stance in 63% of cells, compared to 52% for QP. Among 26 cells tested, 11 showed a significant correlation between their cycle-modulated firing rate and the simultaneous EMG activity of at least one hindlimb muscle. These results suggest that partly overlapping populations of monkey M1 cells are implicated in BP vs. QP gait execution and that M1 is significantly involved in generating final outputs, probably via spinal interneuronal circuits. (COI: No)

2P-010

Features of fine motor skills in 5-year-old children with developmental coordination disorders

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Poor fine motor skill is one of the most common problems experienced by children with developmental coordination disorder (DCD). This study aimed to elucidate difficulties in fine motor performance and the factors associated with problems in children with DCD. Thirty nine Japanese children aged five years, 18 with DCD and 21 typically developing (TD) were examined. A web camera was positioned above children's hands and recorded during the Movement Assessment Battery for Children 2 (M-ABC2) posting coins task. Then average speed, acceleration and total trajectory length of children's hand movement were calculated. We also carried out the grip strength test and the finger to nose test. Differences in all scores between the DCD and TD groups were analyzed. In addition, correlation analyses were used between scores on the M-ABC2 posting coins task, video tracking data, and other factors. The average speed was significantly slower, and the total trajectory length was longer in the DCD group. They also scored worse on average grip strength and the correct answer rate of the finger to nose test. A significant correlation was found between scores on the M-ABC2 posting coins task and average grip strength. These results suggest that children with DCD exhibit slowness and extra movement in fine motor performance, weaker grip strength, and problems in the sense of position and movement. Moreover, overshoot dysmetria appears to point to some degree of dysfunction in the brain. (COI: No)

2P-011

Serotonin-induced synchronization to both respiratory rhythm and body movement in the pons

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Respiratory rhythm is belonging to the medullary rhythm generator, and body movement is the spinal rhythm generator. Parabrachial Nucleus (PB) in the dorsolateral pons conjunctive system of medulla respiratory activity and spinal motor activity. It is known that serotonin strongly modulates the rhythm generator like respiratory or body movement. However, the relationship between respiratory rhythm and body movement at the PB modulated by serotonin has not been well investigated. In this study, we analyzed the relationship between respiratory rhythm and body movement using neonatal rat medulla-spinal cord preparation with/without pons; and we also examined the effect of serotonin on the relationship between respiratory rhythm and body movement in PB. In the case of the pons-medulla-spinal cord preparation, the respiratory rhythm was synchronized to body movement when serotonin was applied. On the other hand, even if serotonin was applied, the relationship between respiratory rhythm and body movement was not synchronized without pons. 5-HT1A blocker abolished this relationship. Also, we examined the distribution of optical signals in the pons triggered by body movement. We found the optical signals which were induced by body movement in the dorsal pons. These results suggested that serotonin caused the synchronization between respiratory rhythm and body movement through the pons, and 5-HT1A receptor in the pons related this synchronization. (COI: No)

2P-012

Neuronal tuning to speed and acceleration of locomotion in mouse cerebellar cortex

Koji Ikezoe; Kazuo Kitamura (*Faculty of Medicine, University of Yamanashi, Japan*)

Proper adjustment of locomotor activity is necessary for animals to travel efficiently and cooperate with others. Speed and acceleration of locomotion are important clues to keep or change the speed as animal intends. Cerebellum is involved in control of locomotion. However, whether and how speed and acceleration are represented in neuronal activities in cerebellar cortex is poorly understood. Here, we sought to characterize speed and acceleration tunings of cerebellar neurons. We recorded activities of multiple single-units using silicon probes from lobule IV/V and VI of the cerebellum in a head-fixed mouse walking on a treadmill in a virtual reality environment. The treadmill comprised a circular disk and constrained a mouse to walk nearly straight forward. Water-restricted mice were trained to walk for three seconds without stopping to obtain a water reward. Most of units changed their firing rate during locomotion. Preferred speed of individual units was uniformly distributed over all range of speeds. On the other hand, the distribution of preferred acceleration was biased to be positive. A joint tuning for speed and acceleration was well-fitted with a linear sum of tunings for individual parameters. From these results, we suggest that individual neurons in the cerebellum encode information of locomotion speed and acceleration, and thus these neurons and their downstream target can contribute to regulate locomotion speed properly. (COI: No)

2P-013

Characteristics of eye movements of 5-year-old children with developmental coordination disorder

Manabu Saito[1,2,4]; Shuhei Koeda[3]; Misaki Mikami[3]; Taiahiro Aoki[5]; Kazutaka Yoshida[1]; Yui Sakamoto[1]; Junko Yamada[3]; Kenji Tsuchiya[6]; Taiichi Katayama[7]; Kazuhiko Nakamura[1,2]
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According to a meta-analysis, the pathological state of Developmental coordination disorder (DCD) is mainly described by Internal Modeling Deficit (IMD) hypothesis, which consists of different effector systems, and one of them is eye movement control. The purpose of this study is to investigate the characteristics of eye movement of 5-year-olds.
The subjects were 64 children of 5-year-olds who participated community-based Health Check-up. There were 30 DCDs, 14 with other diagnoses (ODs), and 20 without diagnosis (NDs). Eye movements were measured using a new eye tracking device 'Gazefinder'. The tasks were Fixation, Pursuit, and Saccade. The total time of task is 106 sec. Gaze points were plotted every 0.02 sec. We analyzed the consequence between the score of Movement Assessment Battery for Children 2 (MABC-2) and Gaze fixation rate (FR), Average distance between the center of the object and the gazing point (AD), Longest gaze fixation time (LFT), Number of saccades (NS) and Search time (ST) for objects in each task. In addition, the same contents were compared between three groups.
AD and LFT in Fixation tasks, LFT, FR in Pursuit tasks and FR in Saccade tasks were significantly related with and MABC-2 scores. FR, LFT in Fixation tasks, of DCDs and several FRs, NS and AD in Pursuit tasks and Saccade tasks of DCDs were significantly different compared to the NDs.
This study showed that some elements of eye movements are related to coordinated body movements. (COI: No)

2P-014

Postural adjustments associated with transition from quadrupedal to bipedal locomotion in monkeys

Takashi Suzuki[1]; You Komagiri[1]; Kazunori Morita[1]; Akira Murata[2]; Masahiko Inase[2]; Katsumi Nakajima[1] ([1]*Dept. Physiol, Iwate Med. Univ., Japan*; [2]*Dept. Physiol, Facult Med., Kindai Univ., Japan*)

Neural mechanisms underlying postural transition during whole body movements have been poorly understood. As the fundamental step, we kinematically analyzed behavioral processes of the transition from quadrupedal to bipedal locomotion on a treadmill in two Japanese monkeys. In the sagittal plane, during quadrupedal locomotion, the body axis angle was maintained at about 5° from the horizontal plane. The postural transition was initiated by swift forward movement during the swing phase of either hindlimb. At around the touchdown of this foot, the trunk started to rise. It reached near vertical (about 70°) within a single step (i.e., a pair of stance and swing phases, 0.51~0.60 s). We defined the hindlimb to trigger the trunk righting up as the support limb. In the frontal plane, the head and hip positions fluctuated mediolaterally, less correlated with the step cycle during quadrupedal locomotion. Just before the transition, the head and hip positions were shifted toward ipsilateral side of the support limb. Conversely, these positions were moved to the contralateral side during the stance phase and then to the ipsilateral side during the subsequent swing phase of the transition period. Such left and right trunk tilts, each once in a step, led to cyclical trunk sway during stable bipedal locomotion. The results suggest that two kinds of postural adjustments occur before and during the postural transition, and they successfully secure dynamic stability during locomotion. (COI: No)

2P-015

Distinctive compositions of nicotinic acetylcholine receptors in slow and fast muscles

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Nicotinic acetylcholine receptors (AChRs) expressed in the neuromuscular junction are pentamers, composed of α, β, δ, and ε (or γ) subunits. Recent studies suggested that the subunit compositions of AChRs in zebrafish slow muscles lack ε/γ subunit. To investigate the distinctive compositions and functions of AChRs in slow and fast muscles further, we generated a double knockout zebrafish line that lacked both ε and γ subunits (ε/γ-DKO). Unexpectedly, instead of converting fast muscle synapses to slow muscle-type, fast muscles failed to express pentamers lacking ε or γ and completely lacked synaptic transmission in the NMJ. We took advantage of this mutant, whose synapses in fast muscles are specifically silenced with minimal impact on cell fate and metabolism. By analyzing locomotion and synapses in this mutant, we investigated the functional contribution of fast and slow muscles in juveniles. We also analyzed adult ε-KO zebrafish whose synapses in fast muscles are silenced. In the juvenile stage, mutants completely lacked the characteristic escape response upon touch: a large bending of the trunk (c-bend) followed by the robust forward propulsion. Unexpectedly, adult mutants swimming with silenced fast muscles showed robust forward propulsion upon stimuli. These data revealed that fast muscles underlie C-bend and forward propulsion in juveniles, while forward propulsion in adults can be executed sufficiently by slow muscles. (COI: No)

2P-016

The effects of sensory and cognitive functions on motor coordination in 5-years old children

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For motor coordination, the Forward model of motor control that predicts and controls the next movement is involved. The pathological state of Developmental coordination disorder (DCD) is mainly described by Internal Modeling Deficit (IMD) hypothesis, which consists of different effector systems. This research aimed to clarify sensory function and cognitive function affecting motor coordination in 5-year-old children.
To evaluate outcome of motor coordination, Movement Assessment Battery for Children 2nd Edition (MABC-2) was conducted for 94 children aged 5 years in health checkup in 2016. To evaluate sensory function, the Japanese sensory profile (SP), for cognitive function evaluation, Wechsler Intelligence Scale for Children 4th edition (WISC-Ⅳ), and for both function, Japanese Version of Miller Assessment for Preschoolers Short version (S-JMAP) were implemented.
The results of multiple regression analysis revealed that visual cognition, oculo-motor coordination, and sense of position and motion affect the control of fine motor and gross motor. These are main factors of deficiency of Internal Modeling Deficit (IMD) and have been confirmed to be important factors constituting motor coordination. In addition, attention and memory, durability, and muscle tone also contribute to motor coordination and may be other factors affecting the internal model. (COI: No)

2P-017

Habitual physical exercise attenuates classical brown adipose tissue mass in interscapular region

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[Purpose] It has been widely accepted that brown adipocyte can expend the energy as heat. Thus, establishing the new therapy via function of brown adipocyte would be expected to prevent obesity and its-related disorders. However, there has been well unknown the effect of habitual physical exercise on formation of brown adipocytes in interscapular region. The purpose of the current study was to investigate the morphological alteration in brown adipose, and to elucidate the molecular mechanism of this phenomenon.
[Methods] The animals (Wistar rats) were randomly divided into 2 groups: a control group (CG) and a habitual exercised group (EG). The EG rats were subjected to running on a treadmill set at a 5-degree incline 5 days per week for 10 weeks. In interscapular, brown adipose tissue and muscle, which is connected with brown adipose tissue, were removed and used for each analysis via sample preparation.
[Results] The mass of brown adipose tissue were significantly decreased in EG compared with CG. Under this condition, levels of complex in PRDM16/PPARgamma and/or PRDM16/EHMT1, stimulation factors for browning of myf-5 positive skeletal muscle, were significantly reduced in EG. In addition, expression levels of TLE3 proteins, which inhibit action of PRDM16 by competing for its interaction with PPARgamma, were significantly augmented by EG compare with CG.
[Conclusions] EG has inhibitory effect on browning of myf-5 positive skeletal muscles in interscapular region. (COI: No)

2P-018

Changes in Atf3 and Ankrd2 following denervation induced skeletal muscle atrophy

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Purpose: In order to investigate the mechanisms of muscle atrophy, we analyzed the expressions of Atf3 (activated transcription factor 3, which can promote regeneration of peripheral neurons) and Ankrd2 (ankyrin repeated domain 2, which exhibits elevated expression of following skeletal muscle denervation) mRNAs in a sciatic nerve transected denervation (DN). These changes were compared with the ubiquitin ligase (Atrogin-1, MURF1) and their up-regulator Myogenin. Method: Male Fischer-344 rats were used in this study. The right sciatic nerve was tied and transected as DN model. A left hind-limbs were analyzed as contra-lateral controls, and all data were expressed as the ratio of the contra-lateral side. Gene expression (Ankrd2, Atf3, Myogenin, Atrogin-1 and MURF1) was analyzed by real time qPCR before and after 6 hours, 1, 4, and 10 days of operation. Analysis was performed on the slow type SOL and fast type PLA muscle. Results: Degrease ratio in muscle mass was SOL50%, PLA50% at 10day. Significant decrease of the expression ratio in Atf3 was observed in after 6h, and Ankrd2 was observed at 1day. However, two ubiquitin ligase and Myogenin consistently elevated after 1 and 4 days. These results showed that expression of Atf3 likely precede to the expressions of ubiquitin ligase and Myogenin, and Ankrd2 showed similar changes of two ubiquitin ligases.
Conclusion: Results clearly indicated that Atf3 and Ankrd2 is possible novel markers for the skeletal muscle atrophy. (COI: No)

2P-019

Understanding Cardiac Hypertrophy Process After Training with Different Intensity In Wistar Rats

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Introduction : The mechanism for cardiac hypertrophy process that would be a benefit for improvement of cardiovascular endurance needed to be investigated throughly. Specific intensity of training may play a role for homeostasis process in cardiac during training.

Objective : to examine the effect of different intensity of treadmill training on cardiac hypertrophy process and autophagy gene expression in male Wistar rats.

Methods : 20 male Wistar rats were assigned to four groups : sedentary control, low (10 m/minute), moderate (20 m/minute) and high (30 m/minute) intensity of treadmill training, 30 minutes a day, 5 times a week, for 8 weeks. Heart weight/weight ratio were measured after the experiments. Left ventricle myocardium were taken for microscopic analysis with HE staining and autophagy gene expression (p62, LC3) with reverse transcriptase PCR.

Results : We observed significant differences of heart weight/weight ratio between control and moderate and high intensity training. This is supported by microscopic result in which cardiac hypertrophy were found in moderate and high intensity, with focal fibrosis in high intensity. Interestingly, autophagy marker altered by different intensity of training, specifically stimulated by mild and moderate intensity, and inhibited by high intensity.

Conclusion : Training with different intensity creates different cardiac hypertrophy process based on heart weight/weight ratio, microscopic examination and autophagy gene expression. (COI: No)

2P-020

Alteration of Autophagy Gene Expression by Different Intensity of Exercise in Skeletal Muscles

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Exercise-induced skeletal muscle adaptation requires both addition and clearance of cellular components. Autophagy is a catabolic process that is responsible for the degradation of cellular components. However, the alteration of autophagy by different intensity of exercise in skeletal muscle is still unknown. Therefore, in the present study, we investigate whether mild, moderate, and high-intensity exercise have different impacts on autophagy gene expression in gastrocnemius and soleus muscles of Wistar rats. 20 male Wistar rats were assigned to four groups : sedentary control, mild-intensity (10 m/minute), moderate-intensity (20 m/minute), and high-intensity treadmill exercise (30 m/minute) for 30 minutes/day, 5 times/week. After 8 weeks of exercise program, mRNA from gastrocnemius and soleus muscles were evaluated for expression of autophagy genes with Reverse Transcriptase PCR. The results showed that expression of autophagy gene expression (LC3 and p62) were decreased in mild and moderate-intensity exercise of gastrocnemius and soleus muscles, but LC3 was decreased, while p62 increased in high-intensity of gastrocnemius and soleus muscles of Wistar rats. This study shows that mild and moderate-intensity exercise stimulate autophagy gene expression in gastrocnemius and soleus muscles, but high-intensity exercise inhibits autophagy gene expression in gastrocnemius and soleus muscles of Wistar rats. (COI: No)

2P-021(Y-01)

Effect of Swimming Exercise to Cardiac PGC-1α and HIF-1α Gene Expression in Mice

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Hypoxia caused by exercise condition may alter PGC-1α and HIF-1α as expression in cardiac cells. Both factors are transcriptional factor which play a role in conserving energy during metabolic process. However, there is less information about long term moderate intensity exercise such as swimming effects on PGC-1α and HIF-1α expression in cardiac cells.

Aim of this study is to observe the effect of force swimming exercise to the expression PGC-1α and HIF-1α gene expression in cardiac muscle.

On this study, male BALBc mice were divided into two groups (n=10): Control (C) and Swimming (ST). ST group was subjected to moderate intensity swimming for 4 weeks, 30 minutes/day for 5 days. Cardiacs of animal model were preserved for cardiac morphology study using HE staining, PGC-1α and HIF-1α mRNA expression study.

We observed that cardiac PGC-1α expression in ST was significantly increased 1.5 folds compared with control group (0.64 vs 0.44; CI = 95%, -0.378- 0.019 ; p = 0.035), but expression of HIF-1α was significantly decreased 0.4 folds lower than control group (0.35 vs 0.52; CI=95%, 0.27- 0.31; p=0,025)

Taken together, ST group increased the expression of PGC-1α but decreased the expression of HIF-1α in mice cardiac muscle in responses of chronic hypoxia condition.

Keywords: Cardiac, swimming, HIF-1α, PGC-1α, hypoxia, moderate intensity (COI: No)

2P-022

Influence exercise intensity moderate (walking) delay changes of physiology aging for elderly

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Sedentary elderly lifestyle increase free radical, leads to mitochondrial dysfunction and telomere shortening progressively. Exercise Intensity Moderate *(EIM)* repairs structure of mitochondria and telomere length, combine with VO_{2max} level it can caused delay aging process through telomerase elevation activity. Aims, was to investigate improvement of cell function and cardiorespiratory by increasing telomerase level and VO_{2max} influence of *EIM* 12 weeks for sedentary elderly women. **Method:** The research was quasi experimental. Statistical sample included 73 sedentary elder women aged 65.86 ± 5.21 years were divided in two groups: 36 subject experimental group (EG) and 37 subject control group (CG). The exercise program is *EIM*, 3 times a week for 12 weeks, 50–85% HR_{max} during 30 minutes. The variable was examined at 0,6 and 12 weeks. Telomerase measured by ELISA kit and predicted VO_{2max} by 6 min walk test. Data processed by Friedman test with post hoc Wilcoxon and spearman test. **Results :** Telomerase and predicted VO_{2max} were significantly increased about 39.37% and 25.08% ($p<0.05$) on EG Our data showed a significant correlation between telomerase level and predicted VO_{2max} at 12 weeks (r = -0,214, p=0,024). **Conclusion**: 12 weeks *EIM* showed that biomolecular activity through increased of telomerase level are significantly related with VO_{2max} can improved structure and function cells that can delay aging process.

Keyword: aging, *EIM*, telomerase, VO_{2max} (COI: No)

2P-023

Drastic changes in arterial pressure during high intensity of treadmill exercise in rats

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[Purpose] A single bout of exercise induces cardiovascular responses. Although the patterns of heart rate (HR) changes at different intensities are well established, the details of intensity-dependent arterial pressure (AP) responses, especially changing patterns of AP at high intensity of physical activities remain unknown. Therefore, the present study investigated to identify the details of AP responses at different intensities of running exercise in rats.

[Methods] To measure AP and HR, the catheter of radio transmitter was implanted into the rat's abdominal aorta. After 5-7 days, the rats were subjected to forced running for 60 min (≦20m/min) for treadmill habituation. After this, incremental exercise test was conducted until exhaustion. Exercise intensity were started from at 10m/min and the speed was increased per 3 min by 2m/min every 3min.

[Results] HR almost progressively increased depending on exercise intensity as well as previous study, although the response was relatively mild after the exercise intensity reached to the high level. On the other hand, AP showed a moderate increase during the low and middle intensity of exercise, followed by a marked increase at high-intensity exercise levels.

[Conclusion] This study showed that, not like HR responses, AP exhibits a drastic increase at a high intensity of exercise just before reaching exhaustion. This specific response in AP may be important to exert the highest performance before exhaustion. (COI: No)

2P-024

Differential improvement of performance by motor imagery of human ankle dorsal and plantar flexion

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Whether the effects of motor imagery on motor performance depend on the strength of central control of the agonist muscles remains unclear. By a 4-weeks training of motor imagery (3 sessions per week, totally 12 sessions), we examined the differences in the motor performance before and after the ankle dorsal flexion imagery (DI group, N=10), plantar flexion imagery (PI group, N=10), and without imagery training (control group, N=9). The motor performance included the output force during maximum voluntary contraction (MVC), electromyogram (EMG) of tibialis anterior and triceps surae muscles, and simple reaction time (SRT) of ankle dorsal and plantar flexion. After the imagery training, the subjective vividness of motor imagery became relatively higher only in the DI group. Interestingly, the output force and EMG activity of the agonist muscle during MVC were larger in the DI group by the imagery training, while those in the PI group showed minimum changes as well as in the control group. The SRT showed no changes before and after imagery training in either group. These findings suggest that the effects of motor imagery on the performance depend on the property of agonist muscle with differential strength of the central command. (COI: No)

2P-025

The long-term exercise doesn't affect blood humoral immunity

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[Introduction] There is no study considering effect of the long-term exercise on humoral immunity by a measuring T helper 2 (Th2) and immunoglobulin. In this study, we investigated the effect of the long-term exercise on blood humoral immunity.
[Subjects and Methods] Ten healthy men belongs to rugby football club participated in this study (age: 19.2 ± 0.2 years, height: 172.6 ± 2.0 cm, weight: 86.9 ± 3.3 kg). Subjects carried out 25-days rugby football camp. Venous blood samples were collected from the subjects pre and post 25-days training camp. Th2 levels and immunoglobulin (Ig) levels (IgG, IgM) were measured using the collected blood samples.
[Results] The Th2 levels (pre: 2.07 ± 0.2 %, post: 2.04 ± 0.2 %), the IgG levels (pre: 1179.8 ± 56.2 mg/dL, post: 1167.2 ± 61.2 mg/dL), and the IgM levels (pre: 136.0 ± 18.0 mg/dL, post: 139.1 ± 17.4 mg/dL) didn't change after camp.
[Conclusions] Th2 levels, IgG levels, and IgM levels didn't change after the long-term exercise. This study revealed that the long-term exercise doesn't affect blood humoral immunity. (COI: No)

2P-026

Seasonal effect on resting energy expenditure is age and percent body fat dependent

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The seasonal variation of resting energy expenditure (REE) is still under debate. This study aims to examine the seasonal changes in REE and its relevant factors among Korean adults.
867 healthy volunteers (385 men and 482 women) aged 20-69 years enrolled in four seasons were divided into subgroups of age intervals, body mass index (BMI), and percent of body fat (PBF) quartiles. REE, body composition, glucose metabolism, thyroid hormones, and catecholamines have been strictly measured.
Seasonal factor contributed to the calculation of REE independently to anthropometric indices with the additional variation reduced from 6 to 2% among younger and older persons. Adjusted REE in the winter was 5.4-13.9%, 7.8-14.3%, and 8.6-11.9% higher than that in the summer in every age, BMI, and PBF subgroups, respectively. T3 and log-transformed norepinephrine (NE_{log}) were higher whereas log-transformed epinephrine (EPI_{log}) was lower in the winter compared to that in the summer. The magnitude of winter-summer difference in REE and T3 and of summer-winter difference in EPI_{log} reduced 3 folds between the lowest and highest intervals of age and PBF, whereas the difference in NE_{log} was constant across age and PBF intervals. No such change in analyses related to BMI intervals.
In summary, the season was an independent predictor of REE and its effect was attenuated by the increment of age and PBF, but not BMI. This work was funded by the NRF (2015M3A9B6028310). (COI: No)

2P-027

Exercise Prevents Hypertension by Modulating Sleep-Related Cardiovascular Autonomic Function in SHRs

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Purpose: Autonomic dysfunction and sleep problems have previously been shown to be associated with hypertension to be independent predictors of cardiovascular diseases. Various studies have indicated that exercise is an important factor in prevention and treatment of hypertension. However, the antihypertensive mechanism is multiple and not yet fully understood. This study aimed to clarify the underlying physiological mechanisms of exercise affecting hypertension.
Methods: Male spontaneously hypertensive rats (SHRs) were used in this study. They were divided into two groups, the wheel-exercised group and the sedentary control group. Polysomnographic recordings were recorded simultaneously for 24 hours once a week over 11 weeks in rats. Exercise training period was 8 weeks and started at 12 weeks old.
Results: Compared with sedentary SHRs, exercised SHRs showed a significant suppression of the age-related elevation in blood pressure (BP), in which the reduction of BP was correlated with the elevation of parasympathetic activity and baroreflex sensitivity and the reduction of sympathetic activity mainly during quiet sleep. Moreover, exercise increased paradoxical sleep time and theta power, and attenuated the flattering of circadian rhythm.
Conclusions: Wheel exercise can modulate sleep-related cardiovascular dysfunction and flattering of circadian rhythm, and in turn prevent the progression of hypertension, which may further reduce the incidence of cardiovascular diseases. (COI: Properly Declared)

2P-028

Does sport discipline at a young age influence the incidence of hypertension? -J-Fit+study-

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Purpose Exercise training such as endurance and resistance exercise influences cardiovascular function. However, it is unclear whether the sport discipline at a young age associated with cardiovascular disease in later life. We aimed to clarify the effect of competitive sport discipline at a young age on the development of hypertension in later life.
Methods Study 1: A total of 219 young male competitive athletes (19.7±1.3 yr) were divided into 3 groups (endurance: n=32, power: n=45, combined: n=142) and assessed blood pressure (BP) and carotid-femoral pulse wave velocity (cfPWV).
Study 2: A total of 1,233 former male competitive athletes (56.0±8.9 yr), who participated the follow-up examination, were categorized by their sport discipline at college years (endurance: n=119, power: n=385, combined: n=729) and compared their medical history of hypertension.
Results Study 1: BP and cfPWV were significantly different between groups (all $P<0.05$); the values were the lowest in endurance athletes and the highest in power athletes.
Study 2: During the follow-up periods (38±10 years), multivariable adjusted hazard ratios were 1.00 (reference), 1.30 (95% CI: 0.91-1.93) and 1.63 (1.12-2.44) endurance, combined and power alumni, respectively (P= 0.005 for trend).
Conclusions These results suggest that sport discipline was associated with current BP and arterial stiffness in young athletes and sport discipline at a young age might influence the incidence of hypertension in later years. (COI: No)

2P-029

Regular exercise suppresses obesity-associated HCC development

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Recent studies suggest that regular exercise can help prevent cancer and inhibit tumor growth, however its molecular mechanism has not been fully understood. We have previously revealed that HFD (high fat diet)-induced obesity promoted hepatocellular carcinoma (HCC) development in mice treated with DMBA (7,12-dimethylbenz(a) anthracene), a chemical carcinogen. Moreover, these obesity-associated HCC revealed a marked accumulation of lipid in the area of HCC and the increased level of blood deoxycholic acid (DCA), which could cause liver injury. Therefore, we speculated that regular exercise might contribute to suppressing HCC development by HFD-induced metabolic alterations. Thus, we tried to investigate whether regular treadmill running could reduce lipid accumulation in the liver and blood DCA level, and possibly HCC development in HFD-induced obese mice treated with DMBA. Indeed, the mice with exercise showed reduced accumulation of lipid in the liver and the decreased level of blood DCA compared with the mice without exercise. Furthermore, interestingly, exercise attenuated HCC development in mice and suppressed senescence-associated secretory phenotype (SASP), an inflammatory phenotype in the hepatic stellate cells provoked by DCA in the tumor area. Taken together, we suggest that a regular exercise such as running can prevent fatty liver and liver cholestasis, and thus obesity-associated HCC development by improving lipid and bile acid metabolism. (COI: No)

2P-030

Lower urinary tract symptoms are associated with reduced peak aerobic capacity in old people

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[Purpose] The prevalence of lower urinary tract symptoms (LUTS) increases with age and 78% of Japanese >40 years old have LUTS that include difficulty of urination, frequent urination, and urinary incontinence. In the present study, we examined whether LUTS were associated with reduced peak aerobic capacity (VO_{2peak}) in middle-aged and older people.
[Methods] Participants (n=441, 71.8±7.1 (SD) yr) in the Matsumoto Health Promotion Program answered validated questionnaires assessing LUTS with the International Prostate Symptom Score (IPSS) and the Overactive Bladder Symptom Score (OABSS). VO_{2peak} was measured by graded walking test. Subjects who did not return questionnaires, lacked the VO_{2peak} measurement, or had pain during urination were excluded from analyses. Finally, 91 men and 216 women were analysed.
[Results] Men had higher (worsened) IPSS and OABSS than women (both, $P<0.001$). In simple regression analysis, both IPSS and OABSS were positively correlated with age while inversely correlated with VO_{2peak} (all, $P<0.01$). Multiple regression analyses indicated that after adjustment for physical characteristics and other possible covariates, male sex and lower VO_{2peak} were the major determinants of higher IPSS ($P<0.041$) while advanced age and male sex were the major determinants of higher OABSS ($P<0.037$).
[Conclusions] Both higher IPSS and OABSS were associated with reduced VO_{2peak} in middle-aged and older people. Moreover, VO_{2peak} was an independent factor affecting IPSS. (COI: No)

2P-031

Assessment of thermal load during exercise in junior high school students using wearable sensors

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[Background] Young students sometimes become victims of heat stress during sports activities. However, the physiology and etiology remain unclear yet. [Aims] The aim of the present study was to assess thermal load of junior high school students during sports activities on fields with wearable sensor devices for heart rate(HR) and acceleration(ACC). The measurements were performed for 4 months from early summer to fall periods to evaluate the influence of thermal load(i.e. factors of environment and personal habituation). [Method] Twenty-three male junior high school students(age of 13 - 15) participated in the study. The practiced and played soccer game or baseball on a field as school activity, during which HR, ACC and temperature in the clothes were monitored with the all-in-one wearable sensor(myBeat, Union Tool Co.). The schedule of exercise intensity, duration, rest, and drinking was not controlled. We also assessed wet bulb globe temperature(WBGT) on the field as environmental variables. Ratings of fatigue and thirst sensations, and body weight were measured before and after the activity. [Result] Linear correlation was observed between HR and ACC. Moreover, the relationship was changed with length of the exercise and WBGT. [Conclusion] Wearable sensors for HR and ACC were easiest way to obtain biosignals on fields even in young students. Moreover, continuous and repeated measures of the signals could be an useful way to evaluate thermal loads of sports activity. (COI: Properly Declared)

2P-032(Y-02)

Respiratory Muscle Training (RMT), Aerobic Fitness and Performance in Sri Lankan Rowers

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Introduction: RMT is known to improve respiratory functions and performance in sportsmen. Proper training is important to increase the aerobic fitness of rowers. **Objective:** To investigate the effect of RMT on aerobic fitness and rowing performance in Sri Lankan rowers. **Methods:** A case controlled randomized study was conducted in 20 male rowers (test group n = 11; control group n= 9) aged 20-35 years during the competitive period. At the beginning of the study, performance was assessed in all rowers using the 2000m & 5000m rowing ergometer while Monark cycle ergometer 828E was used to determine VO_2max according to YMCA protocol. Subsequently, rowers in the test group were prescribed a RMT program while the control group was prescribed a general exercise program for non- respiratory muscles for a 12 week period. **Results:** The mean age of the rowers was 33± 1.4 years. The mean ergometer time trial for test and control groups were 2000m (6.3± 0.2, 7.2± 0.2 minutes) and 5000m (18.2±0.7, 19.5± 0.3 minutes) respectively. There was a significant improvement in rowing ergometer performance in the RMT group (2.1%) compared to control group (0.8%) ($p<0.01$). The mean aerobic fitness for test and control groups were (41.3± 5.2) and (38.7± 5.2) respectively. But, there was no significant difference in VO_2max in the RMT group compared to control group ($p>0.05$). **Conclusions:** The 12-week RMT program increased rowing performance although no improvement in aerobic fitness was observed. (COI: No)

2P-033

The expression and distribution of mitsugumin53 in skeletal muscle after lengthening contraction

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Delayed onset muscle soreness (DOMS) is commonly experienced after unaccustomed exercise, especially after activities that involve lengthening contraction (LC), and characterized by tenderness and movement related pain, a kind of mechanical hyperalgesia. After the recovery from DOMS led by the first bout of exercise, mechanical hyperalgesia is reduced after the subsequent bout compared with that after the first bout. To understand the mechanisms for this adaptive response, called repeated bout effect, we have focused on several membrane proteins that have functions in membrane stabilization or maintenance of sarcolemma. We previously reported that mitsugumin 53 (MG53), a peripheral membrane protein involved in membrane repair, was significantly upregulated at both protein and mRNA levels in a DOMS model of rat, when the animals were recovered from DOMS 5 days after the exercise. In the present study, we immunohistochemically examined the distribution of MG53 in the rat muscle after exercise. MG53 was weakly observed in the muscle fibers and blood vessels in the control muscle without exercise. On the other hand, in the exercised muscle, significant increase of MG53-staining was observed in blood vessels. The results proposed that the increased expression of MG53 in the muscle blood vessels after the first exercise possibly raise the ability to repair muscle cell membranes, and thus, might contribute to the repeated bout effect on DOMS. (COI: No)

2P-034

Neuroendocrine response to long-term exercise

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[Introduction] It is reported that the exercise has an influence on the neuroendocrine system. However, the report that examined neuroendocrine response to long-term exercise does not exist. Therefore, this study compared the neuroendocrine response to the long-term exercise with the short-term exercise.
[Methods] The subjects of the short-term exercise group were 10 university students who were exercised routinely. They carried out running as far as possible within 12 min. The subjects of the long-term exercise group were 10 university students who were members of rugby football clubs. They participated in the 25-day rugby football camp. Blood samples were collected from the subjects before and after each exercise.
[Results] Adrenaline and noradrenaline, ACTH, cortisol were significantly higher after exercise than before exercise in the short-term exercise group ($p < 0.01$) . On the other hand, Adrenaline and ACTH, cortisol showed no significant difference in the long-term exercise group. But only noradrenaline was significantly increased after exercise ($p < 0.05$) .
[Conclusion] After the short-term exercise, each sympathetic system and endocrine system aggravated it. However, after the long-term exercise, Noradrenaline increased by hyperactivity of the sympathetic system. (COI: No)

2P-035

The relationship of body mass index and aerobic capacity in primary school students in Jakarta

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There is not much known about the relationship between body composition and aerobic capacity in primary school students in Indonesia. Baseline data on body composition and aerobic capacity may provide valuable information for future studies, and also for public health policy makers regarding physical activity lifestyle and health related physical fitness in children.
Purpose: To evaluate the relationship of body mass index and aerobic capacity in a sample of primary school students in Jakarta, Indonesia.
Methods: Two hundred thirty nine primary school students age 9 - 15 years old were participated. Weight and height were measured; body mass index (BMI) was calculated. Aerobic capacity was measured using the multi-stage 20 m shuttle run test. Participants were grouped by gender and BMI category. Means were compared between groups using independent t-test for height and Mann Whitney U test for the rest of the parameters. Relationship between BMI and VO_2max as aerobic capacity parameter was evaluated with Spearman correlation within each gender.
Results: There were significant differences between gender on height, shuttle run test, and VO_2max. There were no significant differences of VO_2max between underweight, normoweight, overweight and obese students. There was negative weak correlation between BMI and VO_2max on both groups (female r_s=0.382, $p<0.01$; male r_s=0.375, $p<0.01$).
Conclusion: Lower BMI correlates with better aerobic capacity in primary school students in Jakarta. (COI: No)

2P-036

The analgesic effect of voluntary running in a rat model of persistent inflammatory pain

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Background: Clinical and animal studies suggest that physical exercise may help improve the pain condition. However, forced exercise is said to complicate the correct interpretation of the effect of physical exercise on pain, as it involves varying degrees of stress. The aim of this study was to investigate the analgesic effect and the mechanisms of voluntary running in an animal model of persistent inflammatory pain.
Methods: Wistar rats were divided into control, non-running following formalin injection (NOR), and voluntary running following formalin injection (VR) groups. Formalin (1%, 50 µl) was injected into the hindpaw. Rats in the VR group were given free access to running wheels. The formalin-induced sensitization was tested with the von frey test at 0, 1, 6, and 11 days after the injection. In addition, the expression of activated microglia in the spinal cord dorsal horn was analyzed by immunofluorescent staining on day 6.
Results: In the NOR group, the paw withdrawal times significantly increased from 1 to 11 days, and the expression of activated microglia in the spinal cord dorsal horn was significantly increased compared with the control group; however, voluntary running significantly inhibited these changes.
Conclusion: These results suggest that starting physical exercise early may promote the recovery of inflammatory pain, and inhibiting the activation of microglia was involved in this phenomenon. (COI: No)

2P-037

A Randomised Controlled Trial Evaluating Effect of Walking Advice on Improving Depressive Symptoms

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Background & aims: Evidence of the efficacy of walking advice for treating depressive symptoms is scarce.
Methods: This is a pilot randomized controlled trial to test short-term effects and feasibility of walking advice on relieving depressive symptoms. A total of 30 adolescents with Hamilton Rating Scale for Depression (HAMD) score 8-18 (mean age of 20.8 years old) were randomly assigned to either receiving walking advice group (n=15) or no advice control group (n=15). During 4-week intervention period, subjects were suggested to walk an additional 2000 steps daily or follow their normal daily walking steps. They were evaluated with Hospital Anxiety and Depression Scale, Sheehan Disability Scale, Pittsburgh Sleep Quality Index, HAMD, and salivary cortisol level.
Results: After 4-week intervention period, the attrition rate for advice group was 33.3%. The advice group and control group showed an increase of walking steps of 2053 (SD = 2750) and 868 (SD = 2883), respectively (mean difference = -255.02; d = 0.42, P = 0.28). The HADS-anxiety score was lower in advice group compared to control group (mean difference = -2.46; d = 0.97, P = 0.015). There was no significant between-group difference in HAMD score, other secondary outcomes, and cortisol level.
Conclusions: Our findings showed that a simple advice of walking extra steps is feasible and may increase step count in college students. Subjects receiving walking advice showed a decrease in self-report depressive symptoms. (COI: No)

2P-038

Acute effects of mechanical compression in hypoxia on arterial stiffness

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Purpose: This study aimed to examine the effects of mechanical compression in hypoxia on arterial stiffness. **Methods:** Eight healthy male adults participated in experiments of four different protocols (*i.e.*, rest in normoxia (NR), rest in hypoxia (HR), mechanical compression in normoxia (NMC), and mechanical compression in hypoxia (HMC)) in random order on separate days. Throughout a 40-min measurement, the subjects breathed normoxic (20.9%O_2) or hypoxic (15.3 – 15.5%O_2) gas via a facemask connected to the oxygen generator. Also, in NMC and HMC, a 20-minmechanical compression was conducted in a lower limb in the latter 20-min of the measurement. **Results:** During mechanical compression, no significant difference was observed in the heart rate between NES and HES. Interestingly, CAVI, which is an index of arterial stiffness, significantly reduced, and the reduction in CAVI was significantly greater in HCO than that in NCO. **Conclusion:** These findings suggest that mechanical compression in hypoxia can induce a similar or even greater reduction in arterial stiffness compared with those in normoxia. (COI: No)

2P-039(Y-03)

Factors affecting oxygen pulse in a healthy Thai population

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An oxygen pulse (O_2 pulse) is the amount of oxygen consumed per heartbeat. The O_2 pulse has been demonstrated to be a powerful predictor of mortality in patients with cardiopulmonary disease. However, this interpretation is often inaccurate due to interfering factors, factors which vary in different populations. The aim of this study is to evaluate the relationship between O_2 pulse and many factors in healthy Thais. Two hundred healthy Thai subjects aged 20 to 69 years old were recruited. All subjects underwent CPET using a treadmill and an incremental protocol until symptom limitation. Ethical approval was obtained from the KKUEC (HE561451). A comparison between age-matched males and females showed significantly higher O_2 pulse values in males (12.4±1.9 vs. 8.5±1.0 ml/beat). There was a positive correlation between O_2 pulse values and weight (r = 0.6639, p<0.001), height (r = 0.6401, p<0.001) and BMI (r = 0.2945, p<0.001). There were also negative correlations between O_2 pulse values and age (r = -0.1866, p<0.01) and genders (r = -0.7881, p<0.001). Moreover, O_2 pulse values in healthy adults were positively correlated with pulmonary function as assessed by %predicted FEV_1 (r = 0.3376, p<0.001), FEV_1/FVC (r = 0.4348, p<0.001) and MVV (r = 0.4392, p<0.001). The present study suggests that O_2 pulse values in healthy adults depend on the individual's weight, height, BMI, age and gender. Furthermore, a low O_2 pulse may reflect decreasing pulmonary function. (COI: No)

2P-040

Circulatory dynamics and autonomic nervous activities between sprinters and distance runners

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The autonomic nervous system (ANS) has an important role in physical performance. However, ANS activity between sprinters (SPR) and distance runners (DR) has been poorly explored. Not much research has been conducted to determine the differences between SPR and DR. We aimed to investigate the influences of the ANS and circulatory dynamics on the different types of exercise training. Male 14 SPR and 17 DR aged 18–25 years (21.4±0.4years) were recruited from the sports club. We measured BP and heart rate variability (HRV) at rest and during the postural change from the supine to the sitting position. Spectral analysis of HRV was performed to evaluate low-frequency (LF) , high-frequency (HF) , and LF/HF ratio to observe the changes in cardiac sympathetic and parasympathetic nerve activities. Significant differences were observed between the 2 groups with regard to body composition. The SPR had significantly higher HRV and BP in all the positions than the DR. During the postural change from the supine to the sitting position, LF/HF ratio was significantly higher in the DR than in the SPR. The changes in the SBP of the SPR and DR after postural change to the sitting position were significant, while those during postural change were significantly reduced in the DR group. We observed different responses between the 2 groups, suggesting significant differences in ANS activity and circulatory dynamics between the SPR and DR. (COI: No)

2P-041

Exercise habit is correlated to lower fall risks among elderly people living in urban areas

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In the present superaged society that has limited financial resources for efficient health care for the elderly population, fall prevention is an urgent issue. This study examined the relationship between exercise habit and fall risks among elderly people living in an urban area. A total of 1,014 elderly people (65–89 years) living in Osaka City were enrolled in this study and assigned to 5 different age groups. The motor abilities of the participants were evaluated by the Functional Reach Test (FRT) and the Timed Up and Go (TUG) test, with the cutoff value for fall risk set at 25 cm and 13.5 s, respectively. Exercise habit (≤ 1 h, 1–2 h, or ≥ 2 h /W), history of falls, and anxiety regarding falls were also assessed. The results of the FRT and TUG showed deterioration in values with aging. History of falls and anxiety regarding falls were also more frequent in higher age groups (all $p < .05$). Prevalence of exercise habits was similar among the age groups. In the age groups ≥ 75 years, exercise habit was correlated with a decrease in fall risks, based on the FRT results (all $p < .05$). The mean FRT value of the participants with the greatest exercise habit was equivalent to that of 10-year younger generations with the lowest exercise habit. Exercise habit was correlated with decreased anxiety regarding falls ($p = .003$), but not with the history of falls. Our results suggest that exercise should be promoted among elderly people living in urban areas for fall prevention. (COI: No)

2P-042

Asymmetry of plantar flexor muscle but not Achilles tendon in high jumpers

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Purpose: Athletes have sporting-related specific morphological characteristics. High jumpers perform asymmetrical movements during the takeoff phase between jump and non-jump legs. In particular, muscle and tendon in jump leg is exposed to high mechanical stress in archiving superior takeoff. Thus, it is hypothesized that high jumpers would exhibit asymmetrical hypertrophy of muscle and tendon in jump leg compared with non-jump leg. To test this hypothesis, we examined the asymmetry of ankle plantar flexors (i.e., planter flexor muscle and Achilles tendon) in high jumpers. **Methods:** Fifteen well-trained high jumpers participated in this study. Volumes of plantar flexor muscle and Achilles tendon in the high jumpers were measured using magnetic resonance imaging. **Results:** The plantar flexor muscle volume was significantly greater in jump leg than in non-jump leg. Among plantar flexor muscle, volume of the soleus, but not gastrocnemius medialis and lateralis, was significantly greater in jump leg than in non-jump leg. In contrast, Achilles tendon volume did not differ significantly between jump and non-jump legs. **Conclusions:** The present findings demonstrated that high jumpers have exhibited asymmetry of plantar flexor muscle but not Achilles tendon in ankle plantar flexors. Thus, we suggest that high mechanical stress such as that during the takeoff phase in high jumpers may be not necessary for Achilles tendon hypertrophy. (COI: No)

2P-043

nNOS regulation of myocyte contraction and $[Ca^{2+}]_i$ handling with fatty acid supplementation

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We examined nNOS regulation of left ventricular (LV) myocyte contraction with fatty acid in sham and angiotensin II (Ang II)-induced hypertensive (HTN) rats. Our results showed that palmitic acid (PA, 100 μM) increased the amplitudes of sarcomere shortening and intracellular ATP in sham but not in HTN despite oxygen consumption rate (OCR) was increased by PA in both groups. PA increased nNOS-derived NO only in HTN. Inhibition of nNOS with S-methyl-L-thiocitrulline (SMTC) prevented PA-induced OCR and restored PA potentiation of myocyte contraction in HTN. Mechanistically, PA increased intracellular Ca^{2+} transient ($[Ca^{2+}]_i$) without changing Ca^{2+} influx via L-type Ca^{2+} channel (I-LTCC) and reduced myofilament Ca^{2+} sensitivity in sham. nNOS inhibition increased $[Ca^{2+}]_i$, I-LTCC and reduced myofilament Ca^{2+} sensitivity prior to PA supplementation; as such, normalized PA increment of $[Ca^{2+}]_i$. In HTN, PA reduced I-LTCC without affecting $[Ca^{2+}]_i$ or myofilament Ca^{2+} sensitivity. However, PA increased I-LTCC, $[Ca^{2+}]_i$ and reduced myofilament Ca^{2+}sensitivity following nNOS inhibition. Myocardial FA oxidation (18F-fluoro-6-thia-heptadecanoic acid, 18F-FTHA) was comparable between groups, but nNOS inhibition increased it only in HTN. Collectively, PA increases myocyte contraction through stimulating $[Ca^{2+}]_i$ and mitochondrial activity in healthy hearts. PA-dependent cardiac inotropy was limited by nNOS in HTN, predominantly due to its modulatory effect on $[Ca^{2+}]_i$ handling. (COI: No)

2P-044

A novel superforated-patch technique revealed the Ca^{2+}-triggered arrhythmogenesis from the T-tubules

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In hearts, aberrant intracellular Ca^{2+} dynamics potentially trigger ventricular arrhythmias, via the development of EAD and/or DAD. For studying the mechanisms underlying Ca^{2+}-triggered arrhythmogenesis, the perforated-patch recording technique is desirable because it conserves the intracellular Ca^{2+} dynamics; however, the conventional techniques are hard to do and time-consuming. Here, I have developed a novel "superforated patch" recording technique, which provides a series resistance below 20 megohms within 5 min after the formation of giga-seal. To do this, nystatin was solubilized to water by titrating with alkaline (KOH) solution, and was dispersed using Pluronic F-127. In "superforated-patch" clamped mouse ventricular cells, lowering the extracellular K^+ level induced Ca^{2+}-triggered arrhythmogenic activities Preceding the low-K^+ induced arrhythmogenic activities, the membrane potential fluctuated at an amplitude and frequency of ~10 mV and ~1 s. This membrane fluctuation had a latency of ~10 s after the development of low-K^+ hyperpolarization, which is attributed to the diffusion of low-K^+ solution into the T-tubule. I conclude that the Ca^{2+}-induced arrhythmogenic activity develops on the T-tubule membrane, which then generates the fluctuation of the plasma membrane potential via the decremental conduction along the T-tubule. The membrane potential waveform of the T-tubule membrane might be totally different from that of the surface plasma membrane. (COI: No)

2P-045

Propagation of repolarization induced in a cell array of human ventricular cell models

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In the early stage of cardiac electrophysiology, it was found that the action potential plateau could be interrupted by applying a short hyperpolarizing current pulse. Since this response occurred only when the membrane was hyperpolarized beyond a certain threshold level, a possibility of propagation of repolarization might be involved when the action potential durationwere different within a multicellular preparation such as the Purkinje fiber. However, it was difficult to demonstrate the propagation of repolarization directly in experimental studies. This theoretical study examined the hypothesis in a one-dimensional array of 600 human ventricular myocyte (HuVEC) models. The whole HuVEC models in the array were continuously depolarized through conduction of action potential after blocking the inactivation of late Na^+ current. Then, the cell model at the left end of the array was repolarized by applying a short current pulse. The repolarization propagated in the rightward direction through the gap-junction current, which was evoked by the removal of Mg^{2+}-block of I_{K1}. This mechanism is self-regenerative due to progressive increase in the I_{K1} conductance during repolarization beyond ~ -50 mV. (COI: No)

2P-046

Screening for novel RyR2 inhibitor by ER Ca^{2+} monitoring

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Type 2 ryanodine receptor (RyR2) is a Ca2+ release channel on the ER Ca2+ store and plays a central role in the EC coupling in the heart. Mutations in RyR2 are known to cause arrhythmic diseases such as catecholaminergic polymorphic ventricular tachycardia (CPVT). Because spontaneous Ca2+ release from the ER, which induces triggered activity, is likely to occur in cardiac cells in patients with CPVT, drugs that suppress RyR2 are expected to have antiarrhythmic effects. We have recently developed an efficient high-throughput screening platform for RyR1 inhibitors using ER Ca2+ monitoring in HEK293 cells expressing mutant RyR1. In this study, we aimed to search for RyR2 inhibitors by ER Ca2+ monitoring and to examine their effects on noncardiac and cardiac cells. HEK293 cells stably expressing RyR2 and R-CEPIA1er were generated and the R-CEPIA1er signal was measured in a 96-well plate by a FlexStation III spectrophotometer. By screening of approximately 1,600 of well-characterized compounds, we identified four compounds that increased the R-CEPIA1er signal above the set reference value. All four hit compounds decreased frequency of spontaneous Ca2+ oscillations in HEK 293 cells expressing RyR2 and correspondingly reduced the Ca2+-dependent [3H]ryanodine binding. Three out of four compounds suppressed Ca2+ waves in cardiac cell-line HL1 cells without suppressing action potential induced Ca2+ transients. These compounds are promising candidates for novel anti-arrhythmic drugs. (COI: No)

2P-047

Molecular architecture of catecholamine-induced arrhythmogenicity in rat pulmonary vein

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Pulmonary veins (PVs) are the major origin of atrial fibrillation. Recently, we revealed that IP_3R_2 in rat PV cardiomyocytes cooperates with Na^+-Ca^{2+} exchanger (NCX) on T-tubule to provoke the ectopic automaticity in response to norepinephrine (NE), and identified a unique Cl^- current that facilitate the automaticity. Here, we further characterized molecular architecture of arrhythmogenic properties in rat PV. Microarray, RT-PCR and immunohistochemistry uncover that one of Ca^{2+}-stimulable adenylyl cyclase regionally expressed in supraventricular area including PV. Immunocytochemistry of cardiomyocytes detected the enriched expression of the AC along T-tubule of PV myocytes, while atrial myocytes hardly displayed T-tubules. HEK293 cells exhibited sustained Ca^{2+} oscillation in response to UTP under isoproterenol pre-application. By contrast, gene-knockout of our interest lost the ability to keep the Ca^{2+} oscillation sustained in the same conditions. NE-induce automaticity in PV cardiomyocytes was reversibly arrested by AC inhibitor. In addition, mass spectrometry identified a CLCN2 interacting protein. The β-subunit was subcloned from rat PV and introduced into PC12 cell. With co-expression of the β-subunit, CLCN2 current exhibited a unique voltage-dependency. These findings suggest that specialized expression of NCX, IP_3R_2, and the AC along T-tubule potentiates the arrhythmogenicity of rat PV, and that rat heart possessed a unique β subunit of the Cl^- channel. (COI: No)

2P-048

High throughout screening of RyR2 inhibitors as candidates for novel antiarrhythmic drugs

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Type 2 ryanodine receptor (RyR2) is a Ca^{2+} release channel on the endoplasmic reticulum (ER) and plays a central role in excitation-contraction coupling in the heart. When the RyR2 activity is enhanced by miss-sense mutations and/or excessive phosphorylation, spontaneous Ca^{2+} release occurs from the ER, which often results in fatal ventricular arrhythmia. Therefore, specific inhibitors of RyR2 are expected to have an antiarrhythmic effect on the arrhythmia related to abnormal RyR2, but currently specific drugs have not been reported. Recently, we have developed a procedure to screen ryanodine receptor inhibitors by ER Ca^{2+} monitoring (Murayama et al., Mol Pharmacol, 94: 722-730, 2018). Using the procedure, we have reported three promising compounds from 1535 compounds in a library of well-characterized drugs(See Poster by Tamura et al.). The purpose of this study is to find more RyR2 inhibitors by screening libraries containing a larger number of non-characterized compounds. HEK293 cells expressing RyR2 and R-CEPIA1er, a genetically encoded ER Ca^{2+} indicator, were generated and ER Ca^{2+} was monitored with FlexStation3 fluorometer. We further identified eight compounds that inhibited RyR2 at 10μM or less. These compounds are also good candidates for antiarrhythmic drugs. Further characterization of these compounds may help to establish the more effective RyR2 inhibitors and antiarrhythmic drugs. (COI: No)

2P-049

Anti-arrhythmic force of leak current enhancement in manufactured atrial fibrillation of rat

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The origin of atrial fibrillation (AF) is known to be an ectopic excitability in pulmonary veins (PVs). We have investigated the trigger of AF, and discovered the several molecular causes underlying the abnormal excitation. However, inhibitors targeting these molecules were apart from clinical use due to low selectivity, week potency or potential side-effects. Here, we screened the effects of the potential drug seeds *in vivo* using manufactured AF model, then studied the molecular mechanisms of the pharmacological effect. To manufacture the AF model, bipolar pacing catheter was penetrated into rat right atrium (RA), and rapid pacing inside RA for 24 h was demonstrated. The criteria of atrial arrhythmia was defined as 0.1 or greater in coefficient variance of RR intervals. By the rapid pacing, variability of RR interval increased gradually, and reached the criteria within 10 h. In the model, serum cytokines were up-regulated, while L-type Ca^{2+} channel was down-regulated in atrium. During the modeled atrial arrhythmia, leak current activator was applied by intravenous injection. AF was reproductively defibrillated in the procedure. By patch-clamp method, leak current was observed in isolated PV, atrium and ventricular myocytes, whose amplitudes were magnified by the activator. Electrical excitabilities of these myocytes were suppressed by the activator. These results suggest that the leak current activator potentially defibrillate the AF by suppressing ectopic excitability. (COI: No)

2P-050

Interventricular difference in calcium sensitivity with lower expression of calcium binding proteins

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Left ventricle (LV) and right ventricle (RV) have distinctive structural and functional characteristics as well as heterogeneous physiological properties. Consistent with the less mechanical afterload, the RV has a thinner free wall than the LV, and the movement of its contraction is geometrically different. Despite these definite differences, the studies of basic excitation-contraction coupling and calcium homeostasis of RV has been less studied than in LV. To establish the interventricular difference, we evaluated the basic electrophysiological and calcium-contractile properties of myocyte with or without β-adrenergic stimulation. Analyses of contraction and Ca^{2+}signaling and action potential duration (APD) in isolated RV myocytes showed more prominent APD prolongation with less significant changes in sarcomere shortening and calcium transient, implying less efficient E-C coupling RV myocytes. Comparing with LV, RV myocytes showed round peak, slower early relaxation, and faster late relaxation, suggesting the difference of calcium sensitivity between two ventricles. To investigate the difference, we examined the expression level of calcium-binding proteins that regulate myofilament activities. Results showed that the calcium binding proteins such as troponin I were lower in RV. Taken together, our results suggest that calcium binding proteins of RV was differ from that of LV, which is a clue to explain the different physiological properties of the RV. (COI: No)

2P-051(Y-04)

Mitochondrial fusion promoter attenuates left ventricular dysfunction in pre-diabetic rats

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Purpose: Obese-insulin resistance impairs cardiac mitochondrial dynamics by decreasing the process of mitochondrial fusion which is associated with mitochondrial dysfunction and fragmentation. These alterations were led to myocardial apoptosis and cardiac dysfunction. We investigated the effects of a mitochondrial fusion promoter (M1) on cardiac function in high-fat diet (HFD) induced obese-insulin resistant rats. We hypothesized that M1 improves left ventricular (LV) function by improving mitochondrial function.

Methods: Fifteen male Wistar rats were randomly assigned into HFD fed group and normal diet (NDV) fed group. After 12 weeks of specific feeding, HFD rats received either saline solution (HFDV) or M1 (HFM1; 2 mg/kg, i.p.) for 14 days. The LV function and cardiac mitochondrial function were determined.

Results: HFD rats developed obese-insulin resistance indicated by increasing plasma insulin and HOMA index. They also had LV dysfunction indicated by reduced LV ejection fraction (%LVEF). M1 treatment improved %LVEF compared to HFDV group. M1-treated rats also had improved cardiac mitochondrial function as indicated by decreased mitochondrial ROS level, mitochondrial depolarization and swelling.

Conclusion: M1 exerts cardioprotection by attenuating LV dysfunction in pre-diabetic rats via improved cardiac mitochondrial function. (COI: No)

2P-053

The use of fetal heart rate variability to identify evolving brain injury after asphyxia

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[**Purpose**]

Exposure to hypoxia-ischaemia (HI)

before birth is a major cause of brain injury in preterm fetuses. However, currently we lack reliable biomarkers to identify fetuses at risk and phase of hypoxic-ischaemic brain injury in antepartum. The aim of this study was to identify whether fetal heart rate variability (FHRV) could distinguish the timing and severity of the HI brain injury.

[**Methods**]

Singleton fetal sheep at 0.7ga were randomly assigned to either sham control (n=12), 25 min of complete umbilical cord occlusion (UCO) (n=17) or 15 min (n=10). Fetal heart rate (FHR) and FHRV were assessed before 24 h until 72 h after HI in two phases: latent and secondary phases.

[**Results**]

During the latent phase (first 6h post UCO) FHRV was biphasic with initial suppression followed by elevation in both groups. The elevation of FHRV is prominent in 25 min group. The 25min group was tachycardiac, whereas the 15 group showed mild reduction of FHR in the early latent phase. In the secondary phase (6-72h), 25 min group showed bradycardia and FHRV was profoundly suppressed. FHRV and FHR normalized in 15 min group.

[**Conclusion**]

Our study uniquely showed phasic pattern of FHRV after HI in preterm fetal sheep. A pattern of increased FHRV accompanying tachycardia in the early latent phase could be the injurious sign of HI injury. These findings suggest that analysis of FHRV after HI may be able to help determine the timing and severity of fetal HI. (COI: No)

2P-054

Generation mechanism of transient EAD in a mathematical ventricular model

Yuichiro Ito; Hiroyuki Kitajima; Toru Yazawa (*Department of Engineering and Design, Kagawa University, Japan*)

Electrical activity occurs in the cell membrane of cardiomyocytes. This electrical activity formes the action potential and the action potential generates pumping of the heart. An abnormality in the action potential turns into an arrhythmia, which may cause sudden death. Studies of arrhythmias using mathematical models are important to reduce the risk of sudden death. In this study, we investigated mechanism of early afterdepolarization (EAD) using a mathematical model. We obtain that EAD occurred transiently during convergence process of sodium ion concentration. (COI: No)

2P-055

Alternans in a Mathematical Crustacean Cardiac Model

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Alternans is a beat-to-beat alternation in the action potential duration for a cardiac cell and may cause sudden cardiac death. Thus, studies of alternans using mathematical models are important to reduce the risk of sudden death. In this study, we investigate a mathematical model of the crustacean heart. Because the crustacean cardiac pacemaker cells consist of a small number of neurons (four small cells (SC) and five large cells (LC), which drive muscle cells (MC)). Moreover, the network structure of the heart and the central nervous system, and the types of synapses between the SCs and LCs, the LCs and MCs, were clarified through an experiment on American lobsters. In this paper, we studied all combinations of the parameters (conductances of all ionic currents) and determined that a key parameter of generating alternans is GKCa (conductance of calcium-dependentpotassiumcurrent). By decreasing the value of GKCa for both the SC and LC, we can reproduce the firing patterns of an experiment on a hermit club. (COI: No)

2P-056

Dynamical mechanisms of phase-2 early afterdepolarizations in human ventricular myocyte models

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Early afterdepolarizations (EADs) cause lethal ventricular arrhythmias in long QT syndromes (LQTS). The aim of this study was to elucidate the mechanisms of EAD generation in LQTS by the slow-fast decomposition analysis based on bifurcation theory. We have developed LQTS type 1 (LQT1) and 2 (LQT2) model cells from the mathematical models of human ventricular myocytes (Kurata et al, 2005; ten Tusscher-Panfilov, 2006; O'Hara et al, 2011), assuming the inhibition of the delayed-rectifier K^+ channel currents (slow component I_{Ks} for LQT1 and rapid component I_{Kr} for LQT2). Roles of ionic currents in EAD generation and how to control EAD formation were investigated by the slow-fast decomposition analysis to construct bifurcation diagrams for a fast subsystem as functions of slow variables. Slow activation gating variable of I_{Ks} or slow inactivation gating variable of the L-type Ca^{2+} channel current I_{CaL} were identified as slow variables during EAD generation. Bifurcation diagrams for fast subsystems showed stable equilibrium points (EPs) at depolarized potentials and EP destabilization via Hopf bifurcations with increasing the slow variables. EADs can be regarded as transient oscillations of the full system trajectories around the stable and unstable EPs in the vicinity of Hopf bifurcation points during slow changes of the slow variables. Inhibition of I_{CaL} to modulate bifurcations, as well as acceleration of I_{Ks} activation, was effective in eliminating EADs. (COI: No)

2P-057

Mechanisms of L-type Ca^{2+} channel blockers to produce EAD in drug-induced arrhythmia

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Drug-induced arrhythmia is a great concern in drug development and had been predicted by checking if candidate drugs block the rapidly activating delayed rectifier K^+ current (I_{Kr}) and prolong action potential duration. However, this approach excluded potentially useful drug candidates that may not induce arrhythmia. The inaccuracy of the method for arrhythmia risk prediction can be explained by the different occurrences of early afterdepolarization (EAD) even under the same prolonged action potential duration. In the present study, we examined the possibility that various properties of L-type Ca^{2+} channel block in I_{Kr} blockers account for the different EAD occurrences under the same prolonged action potential duration. We adopted O'Hara Rudy model, the *de facto* standard Human ventricular model, and simulated contributions of various properties of L-type Ca^{2+} channel blockers. We found that the different EAD occurrences can be explained by the drug effects on voltage dependence in L-type Ca^{2+} channel. These results suggest that the risk in drug-induced arrhythmia should be predicted not only by checking block of I_{Kr} and prolongation of action potential duration but also by checking drug effects on the voltage dependence in L-type Ca^{2+} channel. (COI: No)

2P-058(Y-05)

Crossbridge thermodynamics in right heart failure

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Right-ventricular (RV) failure is an event consequent to RV hypertrophy secondary to pulmonary arterial hypertension. In this pathology, the contractile force produced by failing RV tissue samples is unexpectedly not different from that produced by healthy samples. This finding has led to the hypothesis that crossbridge thermodynamics is unaffected in RV failure. To test this hypothesis, we characterised crossbridge thermodynamics in health and in RV failure by performing experiments on isolated rat RV trabeculae using our calorimeter. Our experiments involved three interventions: (i) partition of total measured muscle energy expenditure to reveal the crossbridge heat, (ii) perturbing muscle length sinusoidally in order to interrogate crossbridge dynamic stiffness, and (iii) subjecting muscles to a range of force-length work-loop contractions to assess crossbridge energy efficiency. We found that none of these energetic characteristics of crossbridges differed between the control and failing groups. In addition, none of these indices was dependent on the extent of RV hypertrophy. Thus, we conclude that crossbridge thermodynamics in RV failure is indeed preserved. One implication of our findings is that treatment strategies for RV failure should therefore differ from those that are currently used to target left-ventricular failure where crossbridge thermodynamics is impaired. (COI: No)

2P-059(Y-06)

LysoPC plays a crucial role in cholesterol-induced nonobese MS cardiomyopathy

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Purpose: Many studies have reported the pathogenic mechanisms of cardiomyopathy in obese metabolic syndrome (MS); however, it is remain unknown in nonobese MS. Therefore, cholesterol (CHOL)-induced nonobese MS animal models were used to investigate this issue.
Methods: Control (C), high CHOL (HC), and HC with 10% fructose in drinking water (HCF) diets were fed to SD rats for 12 weeks.
Results: HC and HCF diets had no effect on body weight but caused high blood pressure, hypercholesterolemia, and hypoinsulinemia. Moreover, the HCF-fed rats exhibited insulin resistance with low plasma HDL level. Echocardiography illustrated that CHOL can decreased interventricular septum and left ventricular posterior wall thickness, and increased left ventricular internal diameter. The sympathetic and renin–angiotensin system and myocardial adrenergic signaling were activated by CHOL, and subsequently resulted in cardiac overwork. Simultaneously, the decreased ejection fraction attributed to the impaired ventricular *end-systolic elastance* and preload recruitable stroke work. CHOL-induced myocardial damage via lysoPC was demonstrated by lipidomics analysis. Furthermore, CHOL can directly triggered cytosolic PLA2 RNA expression in H9C2 cardiac myoblast cells, revealing the novel pathogenic mechanism of nonobese cardiomyopathy.
Conclusions: We suggest that blockade of lysoPC expression provide a novel therapeutic strategy for treating of nonobese MS cardiomyopathy. (COI: No)

2P-060

Successful establishment of a murine model of cardiac reverse remodeling

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Adverse cardiac remodeling is the hallmark of the pathology of heart failure, which remains the leading cause of death in the world. Transverse aortic constriction (TAC) model has been widely used as a murine model of adverse cardiac remodeling, contributing to identifying the molecular processes during its development. However, many patients who have established heart failure and adverse cardiac remodeling requires its reversal (reverse remodeling), the underlying molecular process of which is still poorly understood. We, therefore, aimed to establish a murine model of cardiac reverse remodeling by releasing the constriction after TAC. We evaluated the recovery of left ventricular mass (LVM) and left ventricular ejection fraction (LVEF) two weeks after TAC release. When we released the constriction two weeks after TAC, both LVM and LVEF almost normalized, indicating complete reverse remodeling. Surprisingly, when we released the aortic constriction eight weeks after TAC, LVM and LVEF only recovered partially, suggesting incomplete reverse remodeling. These results indicate that the duration of pressure overload affects the potential of the heart to successfully undergo reverse remodeling. This reverse TAC model should be useful to identify the molecular processes of cardiac reverse remodeling and molecular targets which facilitate reverse remodeling. We are currently studying the roles of the ubiquitin-proteasome system in cardiac reverse remodeling using this model. (COI: No)

2P-062

Forced expression of DFCP1 attenuates cardiac fibroblasts activation via promoting autophagic flux

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Purpose: Double FYVE domain-containing protein (1DFCP1) is a key mediator of omegasome-derived isolation membrane, the platform of autophagosomes formation. However, its role in cardiac hypertrophy is poorly understood. We aimed to test the hypothesis that DFCP1 regulates cardiac fibroblasts activation via affecting autophagic flux and ER stress signaling.
Methods: Rat models of transverse aortic constriction (TAC) and primary cardiac fibroblasts treated with TGF-β1 were used in this study.
Results: In TAC rats and cardiac fibroblasts treated with TGF-β1, DFCP1 expression was decreased, accompanied by the damage of autophagic flux and activation of PERK/ATF4 and IRE1α/XBP-1s branches of ER stress. Gene silence of DFCP1 by siRNA aggravated the damaged autophagic flux and activated PERK/ATF4 branch. Overexpression of DFCP1 by adenovirus alleviated the impaired autophagic flux and relieved the activation of PERK/ATF4 signaling. Furthermore, autophagy inducer rapamycin and ER stress inhibitor 4-PBA attenuated the siDFCP1-induced activation of cardiac fibroblasts by restoring autophagic flux and inhibiting ER stress; and overexpression of DFCP1 attenuated this pathological process induced by autophagy inhibitor 3-MA or ER stress agonist thapsigargin.
Conclusions: DFCP1 played an important role in activation of cardiac fibroblasts via restoring autophagic flux and inhibiting ER stress signaling. (COI: Properly Declared)

2P-063

Chronic isoproterenol stimulation induced different cardiac disorders in *Tric*-deficient mice

Daiju Yamazaki (*Division of Pharmacology, National Institute of Health Sciences, Japan*)

Trimeric intracellular cation (TRIC) channel subtypes, namely TRIC-A and TRIC-B, are intracellular monovalent cation channels to mediate counter-ion movements facilitating physiological Ca^{2+} release from internal stores. *Tric-a*$^{-/-}$ and *Tric-a*$^{-/-}$*b*$^{+/-}$ mice normally grow and reproduce under standard rearing condition, and no abnormalities were observed in heart. To investigate the function of TRIC channel in heart, we conducted chronic isoproterenol (Iso) stimulation and analyzed cardiac disorders in *Tric-a*$^{-/-}$ and *Tric-a*$^{-/-}$*b*$^{+/-}$ mice. High-dose Iso (60 mg/kg/day) administration for 14 days reduced survival rate to 50% in *Tric-a*$^{-/-}$ mice. In contrast, all of *Tric-a*$^{-/-}$*b*$^{+/-}$ mice died within 7 days by high-dose Iso stimulation. In *Tric-a*$^{-/-}$ mice, mitochondrial dysfunction-mediated vacuolization was induced by continuous stimulation of Iso for 24 hours. Moreover, serum cardiac troponin T and evans blue-permeabilized cardiomyocytes were significantly increased in Iso-treated *Tric-a*$^{-/-}$ mice after 1 and 3 days. Finally, significantly cardiac fibrosis but not hypertrophy was induced by Iso stimulation in *Tric-a*$^{-/-}$. Because of no survivors in high-dose Iso-treated *Tric-a*$^{-/-}$*b*$^{+/-}$ mice, low-dose Iso (20 mg/kg/day) was applied. We found that *Tric-a*$^{-/-}$*b*$^{+/-}$ heart showed significantly hypertrophy by low-dose Iso stimulation. These results suggested the existence of different function between TRIC-A and TRIC-B in heart. (COI: No)

2P-064

SDH deficiency induced metabolic switch and dilated cardiomyopathy

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Fatty acid oxidation (FAO) is a primary energy source meeting the energy requirements of the hearts. Cardiomyopathy is characterized by alterations in myocardial metabolism with elevated glucose use and reduced FAO. However, the reasons of this substrate shift are poorly understood. Here we investigated the mechanism underlying the metabolic switch of dilated cardiomyopathy in succinate dehydrogenase (SDH)-deficient mice. Mitochondria are principal sites of metabolism in eukaryotes. The tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) play central roles in mitochondrial metabolism. Mitochondrial Complex II (CII), also known as succinate dehydrogenase (SDH), makes a functional link between these two essential processes. Mice with cardiomyocyte-specific ablation of Sdhb or Sdhc develop severe DCM progressing to heart failure and early-aged death, which is similar to the dilated cardiomyopathic symptoms of patients harboring mutations in SDH. Loss of SDH in cardiomyocytes resulted in mitochondrial dysfunction and down-regulation of multiple key genes promoting FAO, whereas glucose metabolism-associated genes were up-regulated. SDH-deficient mice displayed severe metabolic disorders and heart failure. Thus, these findings provide new insights in treating cardiomyopathy and other heart diseases. (COI: No)

2P-065

Chronic response of renal and lumbar sympathetic nerve activity to myocardial infarction in rats

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Dysfunction of autonomic nervous activity, especially activation of sympathetic nerve activity (SNA), has long been considered a deleterious factor influencing mortality of acute myocardial infarction (MI). However, detailed changes on SNA following MI remains unclear. In this study, we directly and continuously monitored renal sympathetic nerve activity (RSNA) and lumbar sympathetic nerve activity (LSNA) before and after MI induced by ligation of the left anterior descending artery (LAD). Wistar rats were subjected to either LAD ligation (MI group; $n = 13$) or sham surgery ($n = 11$). The rats had a telemetry transmitter and bipolar electrodes attached for continuous measurement of mean arterial pressure (MAP), heart rate (HR), RSNA, and LSNA. After 4 days of control measurements, measurements were temporarily interrupted and the rats operated by LAD ligation or sham surgery via left thoracotomy with inhalation anesthesia. Rats in the MI group were divided into two subgroups: those that survived for 24 days (MI-Survivor) and those that died within 3 days after LAD ligation (MI-within 3Days). In the MI-within 3Days group, LSNA increased abruptly, while RSNA did not change significantly relative to pre-MI values on Day 1. In the MI-Survivor group, RSNA increased gradually from Day 9, and reached a statistically significant level by 27% ± 14% on Day 24. Altogether, this study shows that LSNA and RSNA respond differently to MI over time, and in a region-specific manner after MI. (COI: No)

2P-066(Y-07)

Inhibition of p16^{INK4a} protects against myocardial ischemia/reperfusion injury

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Aim: The apoptosis of cardiomyocytes is an essential pathological process in myocardial ischemia/reperfusion (I/R) injury. We aimed to investigate the role of p16^{INK4a} in regulating apoptosis in cardiomyocytes.
Methods: The expression and functional role of p16^{INK4a} was examined in oxygen glucose deprivation/reperfusion (OGD/R)-induced cardiomyocyte apoptosis model and murine model of acute myocardial I/R injury.
Results: The expression of p16^{INK4a} was significantly increased in the heart from acute I/R injury mouse model and OGD/R-induced cardiomyocyte apoptosis model. Transfection with p16-siRNA reduced OGD/R-induced cardiomyocyte apoptosis *in vitro*; myocardial specific p16^{INK4a} KO mice displayed reduced infarct size after I/R injury *in vivo*, indicating that inhibiting p16^{INK4a} might suppress cardiomyocyte apoptosis thus reducing myocardial I/R injury. The Akt/mTOR/P70S6K phosphorylation level was decreased in OGD/R-induced cardiomyocyte apoptosis model, which was recovered by transfection with p16-siRNA. Furthermore, inhibition of Akt and mTOR could abolish the effect of p16-siRNA in reducing cardiomyocyte apoptosis, indicating that the Akt/mTOR signaling pathway was involved in the regulatory effect of p16^{INK4a} in cardiomyocyte apoptosis.
Conclusion: Inhibition of p16^{INK4a} can inhibit cardiomyocyte apoptosis thus reducing myocardial I/R injury through activation of the Akt/mTOR signaling. (COI: No)

2P-067

The cytotoxic effect of 2-APB in H9c2 cells

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Aim 2-aminoethoxydiphenyl borate (2-APB) was broadly known and used as IP_3 receptor, stored operative calcium (SOC) channels and non-selective TRPs channels blocker in many studies. Recent studies indicated that 2-APB possessed a protective role in ischemia-reperfusion (I/R) injury through inhibition of reactive oxygen species (ROS) and/or Ca^{2+} elevation in the reperfusion period of I/R in different organs, such as liver and kidney. As 2-APB is limited by its non-selective effects on cellular Ca^{2+} homeostasis. Our aim was to determine the cytotoxicity of 2-APB in H9c2 cells and whether the 2-APB-induced cell death was related to cellular Ca^{2+} homeostasis.
Method The cell viability of H9c2 cells was determined by MTT assay.
Result In our study, we found that 2-APB could protect against H_2O_2 induced-cell death; however, 2-APB treated alone was found a cytotoxic effect in H9c2 cells in a dose-dependent manner. Our data also indicated that the cytotoxic effect of 2-APB was ceased in the environment without Na^+ and Ca^{2+}, suggested that the cytotoxicity of 2-APB was relative to Na^+ and Ca^{2+} signaling pathways.
Conclusion In conclusion, in spite of the protective effect of 2-APB in organs and cells against ischemia reperfusion injury, our data suggested that 2-APB might also serve cytotoxicity in H9c2 cells through Na^+ and Ca^{2+} signaling pathways, and the application of 2-APB in ischemia-reperfusion injuries of different organs, should be concerned in further clinical studies. (COI: No)

2P-068

Protective Effect of Intermittent Hypoxia Against Oxidative Stress Injury in Rat Cardiomyocytes

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The reactive oxygen species (ROS) contribute to oxidative stress which lead to diseases such as ischemic heart disease. Recently, increasing evidence indicated that short-term intermittent hypoxia (IH) could yield cardioprotection similar to ischemia preconditioning, but detail of the mechanisms remain unclear. The aim of this study was to determine whether IH exposure enhances antioxidant capacity that contributes to cardioprotection against oxidative stress injury in cardiomyocytes. Primary rat neonatal cardiomyocytes were cultured with an oscillating O_2 concentration between 20% and 5% every 30 min for IH. DCFDA, Fura-2 and Rhod-2 were performed to analyze the ROS, cytosol and mitochondrial Ca^{2+}. RT-PCR were performed to detect the antioxidant enzymes. Our results show that IH-induce slightly increased of $O_2^{-\cdot}$. In addition, IH protects cardiomyocytes against H_2O_2-induce cell death. Also, H_2O_2-induce Ca^{2+} imbalance and mitochondrial membrane potential depolarization were attenuated by IH. Treatment with blocks to eliminate the ROS produced by IH can abolished the protective effects of IH on the Ca^{2+} homeostasis and mitochondrial membrane potential. Furthermore, IH treatment up-regulated the expression of Cu/ZnSOD and MnSOD. Our findings suggest that IH protects the cardiomyocytes against H_2O_2-induce oxidative stress and cell death through maintenance of Ca^{2+} homeostasis, mitochondrial membrane potential and up-regulation of antioxidant enzymes. (COI: No)

2P-070

The cardiac end-systolic pressure-volume (force-length) relation is contraction-mode dependent

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We present a new perspective on a century-old uncertainty regarding the contraction mode-dependency of the cardiac end-systolic pressure-volume (force-length) relation. The uncertainty arose when studies queried the pressure-volume diagram published by Otto Frank in the late 19th Century. In the diagram, Frank presented end-systolic pressure-volume relations that were dependent on the modes of contraction: one for isovolumic contractions that located above that for afterloaded ejecting contractions. Although his results were confirmed by many subsequent investigators, some studies found only a single relation that was independent of the mode of contraction.

We reconciled these findings by developing a modelling framework for the cardiac force-length relation. The model was parameterised using experimental data from rat cardiac trabeculae undergoing isometric and work-loop (shortening) contractions at 37 °C (approved by Animal Ethics Committee of The University of Auckland; R1341). Within the framework, we explored the cardiac force-length relation under wide ranges of both preloads and afterloads.

Our data show that the isometric relation is located above a family of work-loop relations. We found that under low preload and/or high afterload conditions, the isometric relation is indistinguishable from the work-loop relations. These two conditions explain the commonly-observed apparent single relation, thereby reconciling the discrepant findings in the literature. (COI: No)

2P-071

Glycolytic pathway is activated in rat embryonic heart just after the beginning of the heartbeat

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Backgrounds: The heartbeat in rat embryo begins at embryonic day 10.0 (E10.0). Although ATP demand during this phase of development is increased to initiate and maintain the heartbeat, it remains unclear how energy metabolism is changed just after the beginning of the heartbeat.

Methods and Results: The embryos at E10.0 in Wistar rats were divided into two groups by the hearts without (pre-) or with (post-) heartbeat. Total RNA was extracted from the embryonic hearts and microarray analysis was performed. Pathway analysis for up-regulated genes in post-heartbeat group compared with pre-heartbeat group revealed that the pathway of glycolysis ranked next to the best matched pathway of muscle contraction. The pathway of glycogenolysis was also found as a significant enriched pathway, whereas pathways of TCA cycle and oxidative phosphorylation were not found. In isolated embryonic myocytes, baseline oxygen consumption rate (OCR) assessed by extracellular flux analyzer was extremely low in both groups, while extracellular acidification rate was detectable in both groups and tended to be high in post-heartbeat group. Interestingly, increased response in OCR by adding mitochondrial uncoupler FCCP was observed in post-heartbeat group but not in pre-heartbeat group, suggesting increased substrate availability in post-heartbeat group.

Conclusions: The findings indicate that glycolytic pathway is predominantly activated just after the initiation of the heartbeat in rat embryonic heart. (COI: No)

2P-072

Rapid heating induces high-frequency sarcomeric oscillations in living rat neonatal cardiomyocytes

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It is well established that an increase in body temperature, albeit by a slight magnitude, results in dramatic changes in the function of various organs, coupled with altered cellular homeostasis. In the present study, we investigated the effects of infra-red laser irradiation on sarcomere dynamics in living rat neonatal cardiomyocytes, by taking advantage of sarcomere length nanometry (Shintani et al., *J Gen Physiol* 2014). We found that a rapid increase in temperature to 38–42°C induced $[Ca^{2+}]_i$-independent high-frequency (~5–10 Hz) sarcomeric auto-oscillations (Hyperthermal Sarcomeric Oscillations; HSOs). In myocytes with intact sarcoplasmic reticular function, HSOs coexisted with $[Ca^{2+}]_i$-dependent spontaneous beating in the same sarcomeres, with markedly varying frequencies (~10 and ~1 Hz for the former and latter, respectively). We simulated HSOs with UT-Heart, a multi-scale, multi-physics heart simulation model. Our simulation predicts that there is a "reverse stroke" during the power stroke of myosin, which is vital for HSOs, as well as for rapid myocardial relaxation for subsequent ventricular filling. Based on these findings, we will discuss the physiological significance and the molecular mechanisms of HSOs. (COI: Properly Declared)

2P-073

Roles of Epac1 in the regulation of contractility in cardiac muscle

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To elucidate the contribution of exchange protein activated by cAMP 1 (Epac1), a PKA-independent cAMP effector, to cardiac myofilament response to β-adrenergic receptor (β-AR) stimulation, we examined the contractility as well as the phosphorylation status in skinned (demembranated) myocardium prepared from transgenic mice overexpressing Epac1 in the heart (Epac1TG). Ca^{2+} sensitivities of force and ATPase activity as well as tension cost (ATPase activity/force) were significantly increased in cardiac myofilaments from Epac1TG compared to non-transgenic mice (NTG). In addition, phosphorylation level of myosin regulatory light chain (RLC) was significantly greater in Epac1TG than that in NTG without any changes in the phosphorylation of TnI or MyBP-C. We also observed that pharmacological activation of Epac with 8-CPT-AM, an Epac-specific but not isoform-selective cAMP analogue, increased Ca^{2+} sensitivities of force and ATPase activity as well as phosphorylation levels of RLC and myosin phosphatase target subunit (MYPT) in skinned myocardium, but its increase was blunted by the addition of a phospholipase C (PLC) inhibitor (U73122) or a protein kinase C (PKC) inhibitor (Bisindolylmaleimide I). These results suggest that Epac1 activation by β-AR stimulation promotes RLC phosphorylation and subsequent increases in Ca^{2+} sensitivity and tension cost in cardiac myofilaments through PLC/PKC/MYPT signaling pathways, independently of PKA. (COI: No)

2P-074

In vivo nano-analysis of the dynamics of individual sarcomeres in the beating mouse heart

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PURPOSE: We aimed to clarify the underling mechanisms by which sarcomere contraction and relaxation are organized in the living mouse heart. METHODS: We developed a high-speed (100 fps), high-resolution (20 nm) spinning disc confocal-imaging system for the beating heart in anesthetized mice (e.g., *J Gen Physiol* 2016; *Prog Biophys Mol Biol* 2017). In the present study, we systematically analyzed sarcomere dynamics in a single myofibril consisting of ~30 sarcomeres in a ventricular myocyte, simultaneous with hemodynamic parameters (i.e., electrocardiogram and left ventricular pressure). RESULTS: First, the average sarcomere length (SL) values were 1.88±0.29 and 1.66±0.19 μm, respectively, in diastole and systole; however, the individual SL values varied markedly during the cardiac cycle. Second, the correlation (R) between the dynamics of an individual sarcomere and that of a whole myofibril varied markedly, i.e., from -0.2 to 0.8. Third, sarcomeres along a myofibril alternately contributed to myocardial dynamics, and the contribution ratio was constantly ~65% in sequential heartbeats. CONCLUSIONS: Sarcomeres behave distinctly under the physiologic condition, suggesting that mechanical interactions between sarcomeres along a myofibril organize myocardial dynamics. Likewise, the alternate contribution of sarcomeres to myocardial dynamics may underlie the heart's partial activation and protect sarcomeres from being disorganized via repeated contractions. (COI: No)

2P-075

Role of pannexin hemichannel on stretch-induced mitochondrial hyperpolarization in cardiomyocytes

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The mitochondrial respiratory chain transfers protons from the matrix to the intermembrane space to keep mitochondrial transmembrane potential ($\Delta\psi_m$), which provides the driving force for proton influx through ATP synthase to synthesize ATP. We have previously reported that myocardial stretch enhances respiratory chain to hyperpolarize mitochondrial membrane potential, however, the underlying mechanisms are not clear. In the present study, we investigated the involvement of ATP release via pannexin hemichannel in stretch-induced mitochondrial hyperpolarization. Isolated mouse ventricular myocytes were loaded with a $\Delta\psi_m$ indicator, Tetramethylrhodamine ethylamine (TMRE) and a mitochondrial marker, MitoTracker (MT). The cells were exposed to 5-8 % axial stretch using computer-controlled piezo-manipulated carbon fibers, attached to both cell ends, to assess stretch-induced changes in $\Delta\psi_m$ by confocal microscopy. The $\Delta\psi_m$ was estimated by the TMRE signal, which was normalized to the MT signal in the confocal plane (TMRE/MT)The stretch significantly hyperpolarized $\Delta\psi_m$, while treatment with hemichannel inhibitors, carbenoxolone, abolished the response. The present results suggest that myocardial stretch enhances respiratory chain secondarily following stretch-induced ATP release via pannexin hemichannel. (COI: No)

2P-076

Comparison of cardiomyocyte kinetics of rat left ventricle and turtle ventricle

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During the evolution of vertebrates, the heart has undergone marked anatomical and functional changes to adapt to different environments and modes of living. However, changes in the heart at the cellular level have remained unclear. We observed myocardial tissue sections of ventricles of various vertebrates, such as cartilaginous fishes (elephant shark, red stingray), amphibian (African clawed frog), reptile (common slider) and ave (Japanese quail) and mammals (gray shrew opossum, rat, and mouse). Interestingly, cell cross-sectional area perpendicular to the longitudinal axis of cardiomyocytes was significantly larger in mammals than in nonmammals, regardless of the size of the bodies and hearts. To analyze changes in cardiomyocyte function, cardiomyocytes were isolated from left ventricles of mammalian rat and ventricles of reptile turtles. Rat cells were enlarged and its sarcomere length was short, compared with turtle cells. While maximum cell contraction of turtle cells was significantly higher than that of rat, both the rates of contraction and relaxation were much greater in rats than in turtles. To evaluate passive extensibility, isolated cells were stretched in the longitudinal axis of cardiomyocytes using a laboratory-made tensile tester. Larger loads were required to stretch rat cells for the same length as compared with turtle cells. These results suggested that cardiomyocytes have evolved to restrict the range of active/passive deformation while quickly pulsating. (COI: No)

2P-077

Hydrogen Sulfide Exerts Cardioprotection in Sepsis by Inhibiting Endoplasmic Reticulum Stress

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To investigate the role of Hydrogen sulphide (H_2S) in the development of Sepsis-induced myocardial dysfunction (SIMD),ten SIMD patients and ten healthy controls were enrolled. The plasma levels of cardiac troponin I (cTnI), creatine kinase (CK), tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were significantly higher in the SIMD group than in the control group.The left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) were significantly lower in the SIMD patients than the controls. Furthermore, the plasma H_2S levels were lower in the SIMD patients, and the H_2S level was positively correlated with LVEF and LVFS. Subsequently, we established a mouse model of SIMD by lipopolysaccharide (LPS) administration and used the H_2S donor NaHS to clarify the role of H_2S in the pathogenesis of SIMD. LPS-treated mice exhibited decreased LVEF and LVFS and increased myocardial damage as well as elevated levels of TNF-α, IL-1β, cTnI and CK. Additionally, the protein levels of toll-like receptor (TLR)-4 and endoplasmic reticulum stress (ERS) markers were overexpressed in the SIMD mice; these changes were more severe in the cystathionine γ-lyase (CSE) knockout (CSE-KO) mice. NaHS administration could improve the cardiac damage and strongly attenuate LPS-induced inflammation and ERS in the SIMD mice. Overall, these findings indicated that H_2S could protect against SIMD by inhibiting inflammation and ERS through the TLR4 signalling pathway. (COI: No)

2P-078

Physiological Studies on the Protective Effect of Melatonin Against Doxorubicin Cardiotoxicity

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Aim of the Study: to study effect of melatonin (MEL) pretreatment on doxorubicin (DXO) cardiotoxicity, using Langendorff model of isolated perfused rat hearts. **Material and Methods:** 24 male Wistar rats were divided into 2 groups; untreated control group and MEL pretreated test group that received melatonin in a dose of 5mg/kg b.w, one hour before heart isolation. Hearts isolated, from both groups, were perfused with 30μM DXO for 60 minutes. The cardiac functions were assessed at end of the 60 min. Cardiac samples were examined by electron microscope. **Results:** DXO perfusion induced marked deterioration in cardiac functions of the controls; there were significant bradycardia, increase in peak tension, and prolongation in all cardiac times. Myocardial flow rate (MFR) was significantly compromised. Ultrastructural examination revealed myofilaments disarray with areas of focal loss and necrosis, hyper-contracted fibers and rarefied mitochondrial. MEL pretreated hearts, at 60 minutes of DXO perfusion, had significantly less bradycardiac response, enhanced cardiac times and higher MFR. Therefore, the observed DXO-induced deterioration in cardiac chronotropy, inotropy, lusitropy and myocardial flow, was greatly attenuated by MEL pretreatment. Also, MEL could protect the myofilaments from degeneration and preserve mitochondrial integrity. **Conclusion:** Single injection of melatonin greatly attenuated both structural and functional insults of doxorubicin on the heart. (COI: No)

2P-079

Optogenetic cardiac pacing in freely-moving mice

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The cardiac pacemaker consists of the pulse generator and leads which deliver electrical stimulation to the heart. However, leads sometimes occurs trouble: perforation of ventricular wall, vein stenosis, cable disconnection. Furthermore, there is a risk that electrical stimulation reaches to the phrenic nerve or skeletal muscle. Thus, leadless and non-electrical pacemaker may improve QOL of patients wearing pacemaker. In this study, we established leadless cardiac pacing in mice using optogenetics, a biological technique to cause light-induced cell excitation.
First, the gene encoding channelrhodopsin2 (ChR2), a light-gated cation channel, was injected into mice using adeno-associated virus (AAV) as a vector. After AAV injection, we were able to confirm expression of ChR2 in the cardiac muscle. To activate ChR2 in cardiac muscle of freely moving mice, a custom-made small LED (470nm, blue light) device was implanted close to cardiac muscle. To record ECG, a transmitter of telemetry system was implanted into abdominal cavity. As a result, beating rate of the heart got into synchronized with blinking blue light.
We were able to leadlessly control heart rate of freely-moving mice using optogenetics. (COI: No)

2P-080

Withdrawn

2P-081

Development of light-controllable nitric oxide releasing small compounds and biological application

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Nitric oxide (NO) is biologically synthesized in human body and mediates various signaling pathway. Because NO is difficult to handle for biological assays, NO releasing small compounds (NO releasers) had been developed for NO researches. Among them, light-controllable NO releasers are quite useful tool because their NO release can be spatiotemporally controlled by light irradiation. Herein, we showed that photoinduced electron transfer (PeT)-driven NO releasers. These compounds composed of a light-harvesting moiety and an NO releasing moiety. Based on this design, we developed a blue light, yellowish-green light, and near-infrared controllable NO releasers. By using these compounds, photocontrollable NO release was realized with visible light irradiation. Also, we achieved photomanipulation of vasodilation under Magnus test condition by using these compounds and a light irradiating apparatus. These compounds could be quite useful tools for NO researches. (COI: Properly Declared)

2P-082

Airway epithelial integrin β4 expression deficiency leads to lung dysplasia in mice

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Purpose: Our previous study found integrin β4 was involved in the susceptibility to asthma. In this study, we plan to explore the role of integrin β4 in lung development.
Methods: Integrin β4 was deleted conditionally in bronchial epithelium during mouse lung development from E7.5 onwards using CCSP promoter-driven Cre. Alterations of lung tissue morphology, differentiation markers and major signaling pathways related to lung development were observed. The impacts of integrin β4 on the interactions between epithelium and ECM were observed in vitro. Animal studies and cell experiments were approved by the Ethics Committee of Xiangya Hospital of Central South University (No: 201803246).
Results: Defects in airway branching morphogenesis in association with impaired epithelial cell adhesion and migration, as well as alveolarization defects were identified. Maturation defects in epithelial cell-specific integrin β4-deleted lungs were associated with decreased expression of TTF1, SHH, BMP4 and 11β-HSD1. Expression of surfactant-associated proteins A, B, and C was decreased by deletion of integrin β4.
Conclusion: Integrin β4 expression deficiency can lead to lung dysplasia, and reduce the ability of proliferation and migration by participating in the skeletal reorganization of BECs, which is closely related to the occurrence and development of bronchopulmonary dysplasia diseases. (Supported by NSFC 81670002) (COI: No)

2P-083

$S1P_2$ aggravates lung fibrosis through altering alveolar macrophage polarization in mice

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Lung fibrosis is a chronic and irreversible scarring disease in the lung with poor prognosis. The pathogenic role of the lysophospholipid mediator sphingosine 1-phosphate (S1P) and its receptor $S1P_2$ in lung fibrosis is unknown. In this study, we used a bleomycin-induced lung fibrosis model to investigate roles of S1P/$S1P_2$ in lung fibrosis. We found that $S1P_2$-null mice showed attenuated bleomycin-induced lung fibrosis and alveolar inflammation compared with wild-type mice. We observed by using $S1P_2^{LacZ/+}$ mice that $S1P_2$ was expressed in alveolar macrophages (MΦs), vascular endothelial cells and alveolar epithelial cells in the lung and that $S1P_2$-expressing cells accumulated in the fibrotic lesions. Bone marrow chimera experiments and pharmacological inhibition of colony stimulating factor-1 receptor indicated that $S1P_2$ most likely in MΦs contributes to the development of lung fibrosis. The gene expression analyses by DNA microarray and real-time qPCR showed that the production of Th2 cytokines was reduced in $S1P_2$-null alveolar MΦs compared with wild-type alveolar MΦs. We also found impaired activation of STAT6 in $S1P_2$-null alveolar MΦs, a transcription factor which is activated in response to the Th2 cytokines. Finally, pharmacological $S1P_2$ blockade in wild-type mice alleviated bleomycin-induced lung fibrosis. In conclusion, $S1P_2$ plays a crucial role in the development of lung fibrosis and $S1P_2$ is a novel therapeutic target for lung fibrosis. (COI: No)

2P-084

Lung Functions and Feno Levels during Phases of Menstrual Cycle in Asthmatic and Healthy Females

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Purpose: Female sex hormones influence status of asthma but conclusions are divergent. We aimed to assess lung function during different phases of menstrual cycle in regularly menstruating women with and without asthma.
Methods: In healthy females(n=39) and asthmatics(n=26) with regular menstrual cycles lung functions(FEV_1,FVC,PEF) and Exhaled breath Nitric oxide(FENO)were measured during menstruation, follicular and luteal phases of menstrual cycle.
Results: The mean age of asthmatics and healthy women were 22.38(±1.44)years and 22.36(± 1.33)years respectively.
In healthy females highest mean FEV_1(2.467±.41)L and FVC(2.73±.4390)L were seen during luteal phase while lowest FEV_1(2.462±.38)L, FVC(2.71L±.438) were seen during follicular phase. In asthmatics highest mean FEV_1 was in follicular phase(2.305L±.3018) while lowest was in luteal phase(2.2904±.308 L) and highest mean FVC(2.76±.43L) and PEF(5.97±.83L/s) were during menstrual phase while lowest FVC(2.65±.37L),PEF(5.90s±.89L/s) were during the luteal phase. In asthmatics FEV1correlated negatively with FENO during all three phases and PEF correlated negatively at follicular and luteal phases. In normal females FeNO correlated negatively with FEV1 at follicular phase and positively in other phases.
Conclusions: Changes in PEF,FEV1,FEV1% follow a distinct pattern in normal women and those with asthma. In asthmatics the changes in lung function are associated with FENO level. (COI: No)

2P-085

The role of miR-126 on LPS-induced acute lung injury in mice

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Aim: To investigate the role of miR-126 on LPS-induced acute lung injury (ALI) and explore the underlying mechanism. **Methods:** Mice were randomly divided into four groups: Control, LPS, miR-126 mimic+LPS and miR-126 antagomir+LPS. Lung tissues were collected 24 h after LPS injection. The permeability was detected by EBA. The expressions of related proteins and genes were detected by WB and qRT-PCR. The cellular adhesion was tested by TER after LPS treated. **Results:** Compared with LPS or miR-126 antagomir +LPS group, miR-126 mimic treatment alleviated the degree of pulmonary edema as showed by a decrease in the lung wet/dry ratio. MiR-126 mimic treatment also reduced the lung permeability and the levels of cytokines as well as the expression of VEGF. MiR-126 mimic treatment reversed LPS-induced a decrease in VE-cadherin and β-catenin protein expression, which was confirmed by the IP result. In addition, miR-126 mimic treatment significantly increased the expression of VE-cadherin/β-catenin and the cellular adhesion when compared with LPS or miR-126 antagomir+LPS treatment, as showed by the results of IF and TER, respectively. **Conclusion:** miR-126 has a protective effect on LPS-induced ALI in mice, which may be mediated by the inhibition of VEGF on VE-cadherin/β-catenin complex.
Key words: Acute lung injury; miR-126; Cadherin; LPS
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2P-086

Chloramphenicol induces autophagy and inhibits the HIF1α pathway in NSCLC cells

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Chloramphenicol (CAP) is a controversial antibiotic that has an excellent bactericidal potential in the Third World countries, whereas its systemic application has been abandoned in developed countries. In recent years, clinicians have reintroduced CAP in clinical practice. In this study, CAP was initially found to repress the oxygen-labile transcription factor, HIF1α in A549 and H1299. Our data showed that CAP significantly reduced HIF1α protein level in hypoxic cells, and furthermore, it suppressed the mRNA levels of VEGF and GLUT1, eventually decreasing VEGF secretion. CAP initiates the autophagy pathway in treated cells, as observed by the increase in formation of Atg12-Atg5 conjugates, elevation of beclin1 and LC3II levels. The acceleration of HIF1α degradation by CAP was completely reverted by autophagic flux blockage. In HIF1α overexpressing H1299 cells, HIF1α/SENP1 protein complex facilitated the deSUMOylation of HIF1α, resulting in escape of HIF1α from proteosomal degradation. CAP inhibited the protein interaction between HIF1α and SENP1, thereby destabilizing HIF1a protein. The enhancement in HIF1α degradation due to CAP was evident during both incubation before hypoxia and treatment after HIF1α accumulation. Growing evidence has indicated that HIF1α plays multiple roles against infections, inflammation, and cancer stemness, and is considered a novel therapeutic target against them. (COI: No)

2P-087(Y-09)

Influence of Tobacco smoking on carboxyhaemglobin levels and blood lipid levels

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Tobacco smoking is a major risk factor for cardiovascular diseases. Smoking builds up high levels of Carbon Monoxide (CO), Carboxyhaemoglobin (COHb%) in the blood, as well as smoking, affects on blood lipid levels. The aim of this study was to examine the effect of tobacco smoking on CO levels, COHb% level, haemoglobin (Hb) level and serum lipid levels among Sri Lankan adult male smokers.
Consenting apparently healthy current adult male smokers (n=50) who consumed ≥05 cigarettes/day from Colombo district Sri Lanka were selected as the study group. Smokers were compared with age-matched nonsmoking males (n=25). Breath CO, COHb%, serum lipid levels and full blood count were measured. Alcohol consumption was recorded as an associated factor.
There was a significantly higher mean CO and COHb% levels of smokers when compared to the nonsmokers ($p<0.05$). Smokers have significantly higher mean values of serum total cholesterol, serum triglyceride, serum very low-density lipoprotein, total cholesterol/ high-density lipoprotein ratio when compared to non-smokers ($p<0.05$) while significantly lower serum HDL levels in smokers ($p<0.05$). Significantly higher Hb levels observed among smokers ($P<0.05$). However there was no significant association between alcohol use and blood lipid levels ($P>0.05$).
Tobacco smokers had higher CO and COHb% levels and altered blood lipid levels when compared to nonsmokers. The high levels of COHb% values might cause to produce higher Hb levels of smokers. (COI: No)

2P-088

Stimulation of nitric oxide production in vascular endothelial cells by *Raphanus sativus* extract

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Cerebrovascular diseases, such as stroke, and heart diseases, such as angina pectoris and myocardial infarction are the leading causes of death. Some vascular diseases occur as the result of decreases in vascular endothelial function. The innermost layer of the vasculature is formed by vascular endothelial cells (VECs), which are critical for nitric oxide (NO) synthesis. In our search for active constituents in farm products with the potential for improving the vascular system, we examined the effect of *Raphanus sativus* cv. Sakurajima Daikon on NO production in VECs. Sakurajima Daikon was certified as the world's biggest radish by the Guinness Book of Records. The radishes regularly weigh about 4 to 5 kg, but large ones weigh around 30 kg, with a girth of approximately 110 cm. In this study, we found that the underlying mechanism for stimulating NO production by Sakurajima Daikon extract involves endothelial-NO-synthase (eNOS) activation by the phosphorylation of Ser1177 and the dephosphorylation of Thr495, which are triggered by elevated concentrations of cytoplasmic Ca^{2+} resulting from the activation of Ca^{2+} channels in VECs. We observed that trigonelline, an active constituent of Sakurajima Daikon, improves NO production in VEC cultures. Importantly, we determined that the NO-production stimulant in extracts of Sakurajima Daikon, the world's biggest radish, is trigonelline. (COI: Properly Declared)

2P-089

The vasodilatory effect of Tiliacorinine 12'-*O*-acetate in rat aorta

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Hypertension is a major risk factor of cardiovascular diseases. Moreover, the natural products are becoming the alternative treatment for hypertension. In this study, we investigated the vasodilatory effect of tiliacorinine 12'-*O*-acetate that is a chemically modified from tiliacorinine in rat aorta by using organ bath technique. All the animal experiments were approved by the Institutional Animal Care and Use Committee with the guidelines for ethical conduct on the care and use of animals as prepared by Chiang Mai University (Permitted number: 15/2560). To clarify the mechanisms of tiliacorinine 12'-*O*-acetate induced vasodilation, the aortic rings were pre-incubated with indomethacin (COX inhibitor), N(ω)-nitro-L-arginine methyl ester (L-NAME, eNOS inhibitor) and 1H-[1,2,4]-oxadiazolo-[4,3,-α]-quinoxalin-1-one (ODQ, sGC inhibitor) before added phenylephrine (PE) induced contraction. Then, tiliacorinine 12'-*O*-acetate was added cumulatively into organ bath. Tiliacorinine 12'-*O*-acetate showed significantly the relaxation in both endothelium intact and denuded rings. In addition, the loss of the vasodilatory effect of this compound showed in the presence of L-NAME and ODQ. The results can be suggested that tiliacorinine 12'-*O*-acetate relaxes the vessels via endothelium dependent and also relaxes smooth muscle cell directly. These data has important to support the medical application of this compound to apply for the alternative treatment of hypertension. (COI: No)

2P-090

Withdrawn

2P-091

Hemodynamic responses to hyperbaric treatment in skeletal muscle of obesity and type 2 diabetes rats

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Hyperbaric treatment at 1.3 atmosphere absolute (ATA) improves glucose tolerance in type 2 diabetes rats. The improvement could be due to changes of skeletal muscular hemodynamics. The aim of this study was to investigate the change of oxygen saturation and blood flow during hyperbaric treatment in the skeletal muscle of type 2 diabetes.

Twenty-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats were used as animal models of obesity with initial type 2 diabetes, whereas age-matched Long-Evans Tokushima Otsuka (LETO) rats were used as healthy controls. Oxygenated- and deoxygenated-hemoglobin in the calf muscle were measured by near-infrared spectroscopy, and consequently, oxygen saturation and total-hemoglobin were calculated at 1.0 and 1.3 ATA (before and during hyperbaric treatment, respectively).

Oxygen saturation significantly increased during hyperbaric treatment in both OLETF and LETO rats. There was no difference in the increasing level between OLETF and LETO rats. In contrast, total-hemoglobin significantly increased during hyperbaric treatment only in OLETF rats.

The difference suggested vascular functional compensation of skeletal muscle in twenty-week-old OLETF rats as an initial symptom of type 2 diabetes. The improvement of glucose tolerance by hyperbaric treatment could be induced by increased blood flow and oxygen saturation. (COI: No)

2P-092

Differential changes of flow-induced vasodilation mechanisms in coronary arteries from SHR and WKY

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Shear stress is the physiological vasodilatory stimulus mediated by endothelium-dependent hyperpolarization (EDH) as well as NO release. Changes in EDH of coronary artery (CoA) and the relevant ionic currents in coronary smooth muscle cells (CoASMCs) in chronic hypertension are poorly studied. Here, we examined the endothelium dependent relaxation responses CoA to NS309, an activator of Ca^{2+}-activated K^+ channels (IK_{Ca} and SK_{Ca}) in endothelial cells. Concentration-dependent relaxation of CoA by NS309 showed lower sensitivity in SHR than WKY, which was not altered by pretreatment with apamin (SK_{Ca} inhibitor). However, the NS309 relaxation was largely abolished by TRAM34 (IK_{Ca} inhibitor), and the TRAM-34-sensitive relaxation was smaller in SHR than WKY. There were no contraction differences in serotonin between WKY and SHR. The endothelial release of K^+ via IK_{Ca} would have relaxing influence via activation of Kir in CoASMCs. Interestingly, the amplitude of I_{Kir} of CoASMCs was higher in SHR than WKY. Consistently, the contraction of CoA by Kir inhibitor was larger in SHR than WKY. In contrast to CoASMCs, I_{Kir} in the skeletal and cerebral arterial myocytes were smaller in SHR. The results suggest functional downregulation of IK_{Ca} in the CoA endothelium of SHR, which might be partly compensated by the increased I_{Kir} in CoASMCs. The physiological implication of the opposite I_{Kir} changes between CoA and other systemic arteries require further investigation. (COI: No)

2P-093

Measurement of pulmonary arterial capacitance in the pathogenesis of pulmonary hypertension in rats

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The following two indices are known to represent the condition of pulmonary arteries, which strongly affects the right ventricular afterload. One is pulmonary vascular resistance (PVR), which represents the resistance of pulmonary capillaries, and the other is pulmonary arterial capacitance (PAC), which represents compliance of pulmonary arteries. Recently, PAC has attracted attention as it has been reported that PAC is more sensitive to pulmonary blood vessel abnormality than PVR. Pulmonary vascular tone is regulated by the sympathetic nervous system and is strongly affected by anesthesia, so we considered that the measurement of PAC in unanesthetized animals is important. Thus, the authors developed a technique to implant a telemetric pressure transducer and a transit time flow probe to continuously measure PAP and SV, respectively, in rats. This made it possible to analyze PAC and PVR over time in various pulmonary arterial hypertension models such as chronic hypoxia, monocrotaline administration, and Semaxanib (Su5416) + chronic hypoxia. In conclusion, this technical development can be a powerful tool for detailed analysis of PAC and PVR responses to some therapeutic treatments in various pulmonary hypertension models. (COI: No)

2P-094

Advanced method for vessel identification and assessment of concurrent dynamic vascular events

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The vasculature undergoes changes in diameter, permeability and blood flow in response to specific stimuli. The dynamics and interdependence of these responses in different vessels are largely unknown. Here we report a non-invasive technique to study dynamic events in different vessel categories by nonliner optical microscopy and an image analysis tool, RVDM (relative velocity, direction, and morphology) allowing the identification of vessel categories by their red blood cell (RBC) parameters. Moreover, Claudin5 promoter-driven green fluorescent protein (GFP) expression is used to distinguish capillary subtypes. Intradermal injection of vascular endothelial growth factor A (VEGFA) is shown to induce leakage of circulating dextran, with vessel-type-dependent kinetics, from capillaries and venules devoid of GFP expression. VEGFA-induced leakage in capillaries coincides with vessel dilatio n and reduced flow velocity. Thus, intravital imaging of non-invasive stimulation combined with RVDM analysis allows for recording and quantification of very rapid events in the vasculature. (COI: No)

2P-095

Resveratrol stimulates Na^+-Ca^{2+}exchanger to reduce cytosolic Ca^{2+}in rat aortic smooth muscle cells

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Background: Resveratrol has well-documented vascular relaxant and anti-hypertensive effect. Here we studied the action of resveratrol in modulating cytosolic [Ca^{2+}] level and ATP-induced Ca^{2+}release from sarcoplasmic reticulum**(SR) in rat aortic smooth muscle cells (ASMCs) and explored the underlying mechanisms.Method and result:**Cytosolic [Ca^{2+}] and SR [Ca^{2+}] in ASMCs were determined by Fluo-4/AM or Mag-Fluo-4/AM, respectively. Resveratrol (20, 50 and 100 μM) caused a rapid and substantial reduction in cytosolic [Ca^{2+}] in ASMCs bathed either in the normal Hank's Balanced Salt Solution (HBSS) or in a Ca^{2+}-free HBSS. Resveratrol pretreatment reduced ATP-induced SR Ca^{2+}release and also lowered SR Ca^{2+}content. In cells bathed in a Na^+-free physiological saline, which favors reverse mode ofNa^+-Ca^{2+}exchanger (NCX),resveratrol induced rises in cytosolic [Ca^{2+}] and SR [Ca^{2+}]. The effect of resveratrol on cytosolic [Ca^{2+}] and SR [Ca^{2+}] were inhibited by a selective NCX inhibitor, SEA0400. **Conclusion:** Resveratrol stimulates NCX to reduce cytosolic [Ca^{2+}] and SR [Ca^{2+}] in ASMCs in normal physiological saline. (COI: No)

2P-096

The involvement of calpain in abnormal vascular smooth muscle contraction induced by SPC and U46619

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Rho-kinase (ROK)-mediated Ca^{2+}-sensitization of vascular smooth muscle (VSM) plays a critical role for abnormal VSM contractions such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC)/Fyn/ROK pathway as a novel signaling pathway for abnormal VSM contraction. As possible downstream targets of Fyn tyrosine kinase, we identified vimentin by focused proteomics in which tyrosine-phosphorylated proteins were concentrated using 4G10 antibody and identified by tandem mass spectrometry. Interestingly, western blot analysis revealed that SPC induced limited proteolysis of vimentin not only in human coronary artery smooth muscle cells (CASMCs) but also in VSM strips of the porcine coronary artery. Since vimentin is reported as the target of calpain, we examined the possible involvement of calpain. In CASMCs, SPC increased calpain activity, which was blocked by PD150606, a calpain inhibitor. Furthermore, PD150606 inhibited the SPC-induced abnormal VSM contraction both in porcine coronary and mouse basilar arteries. PD150606 also inhibited U46619-induced VSM contraction in porcine coronary arteries. Interestingly, PD150606 did not inhibit Ca^{2+}-dependent contraction induced by high K^+ depolarization. These findings suggest the possible involvement of calpain in the signal transduction of Ca^{2+}-sensitization of VSM contraction induced by SPC and U46619. (COI: No)

2P-097

Effects of Capsaiciniod Nonivamide on Obesity-Related Vascular Dysfunction in Obese Rat

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The obesity-related complications become severe important clinical and public health burdens worldwide since obesity is the major risk factor of many pathological conditions; hypertension, inflammation, T2DM and atherosclerosis. Excessive bodyweight and atherogenic lipid profiles lead to enhanced cardiovascular morbidity and mortality. Capsaiciniod Nonivamide (PAVA), the phytochemical that found in hot peppers, is known as a multiple pharmacological agent for anti-inflammation and anti-obesity. Thus, in this study we investigate the effects of PAVA to prevent and reverse HFD-induced obesity in rats. Male SD rats (150-180 g) were divided into 4 groups (1) Normal control; (2) Normal+PAVA 1 mg/kg/d; (3) HFD control and (4) HFD+PAVA 1 mg/kg/d (Permitted number of IACUCs: 50/2559). Each group was received with a ND or HFD for 16 wks. PAVA was administered via SC injection once daily for 4 wks starting from the 16th wk; Blood pressure, heart rate and body weight were recorded. At the end of period, rats were measured body weight and the animals were killed; bloods were obtained to analyses, atherogenic lipid profiles (TC, TG, LDL-c and HDL-c). PAVA significantly attenuated HFD-induced obesity in rats by lowering the body weight, lipid profiles when compared with HFD control group and also improve HDL-c level, heart rate and blood pressure. Our results suggest that PAVA represents an alternative compound that could be a useful therapy against obesity-related complications in CVDs. (COI: No)

2P-098

Deficiency of HIF2α in VSMCs Protects Against Angiotensin II-Induced Abdominal Aortic Aneurysm

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Recent studies suggest that hypoxia may exist in the process of abdominal aortic aneurysms (AAA). However, the underlying mechanisms remain unknown. We hypothesize that HIF2α in VSMCs may abolish AAA formation. We identified that hypoxic niche and HIF2α activation exist in VSMCs from human AAA samples and angiotensin II-induced AAA models in $ApoE^{-/-}$ mice. To investigate the role of VSMC HIF2α in AAA, VSMC-specific HIF2α-deficient mice were generated in $ApoE^{-/-}$ background. No spontaneous AAA formation were observed in either $Hif2\alpha^{\triangle SMC}/ApoE^{-/-}$ or $Hif2\alpha^{F/F}/ApoE^{-/-}$ mice without challenge. However, disruption of HIF2α significantly lowered arterial blood pressure and inhibited the dilation of abdominal aortas in Ang II-treated $ApoE^{-/-}$ mice. There were significant declines for the incidence of AAA formation and rupture in $Hif2\alpha^{\triangle SMC}/ApoE^{-/-}$ mice than in $Hif2\alpha^{F/F}/ApoE^{-/-}$ mice. Morphologically, there was a dramatically decreased infiltration of M1 macrophages and neutrophils in $Hif2\alpha^{\triangle SMC}/ApoE^{-/-}$ mice. Mechanistically, Ang II stimulated the secretion of Cxcl1 from VSMCs and consequent recruitment of M1 macrophages and neutrophils via HIF2α-dependent manner. Finally, inhibition of HIF2α by its specific inhibitor PT2385 abrogated Ang II-induced AAA development in $ApoE^{-/-}$ mice. In summary, disruption of HIF2α in VSMCs abolishes AAA formation at least partially via inhibition of Cxcl1-Cxcr2 axis, thus HIF2α may serve as a potential therapeutic target for AAA disease. (COI: Properly Declared)

2P-099

Intermedin reduces neointima formation by regulating vascular smooth muscle cell phenotype

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Objective: To investigate the role of IMD in neointima formation and the possible mechanism.
Methods and Results: With the rat carotid-artery balloon-injury model, IMD was significantly downregulated in plasma and injured arteries. Exogenous IMD_{1-53} greatly inhibited neointima formation and prevented VSMCs from switching to a synthetic phenotype. With the left common carotid-artery ligation-injury model, IMD-transgenic mice showed less neointima formation than C57BL/6J mice. Platelet-derived growth factor-BB reduced IMD mRNA expression in rat primary cultured VSMCs but increased that of its receptors, calcitonin receptor-like receptor or receptor activity-modifying proteins. Furthermore, platelet-derived growth factor-BB promoted VSMC proliferation and migration and transformed VSMCs to the synthetic phenotype, which was reversed with IMD_{1-53} treatment. Mechanistically, IMD_{1-53} maintained the contractile VSMC phenotype via the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway.
Conclusions: IMD attenuated neointima formation both in the rat model of carotid-artery balloon injury and mouse model of common carotid-artery ligation injury. IMD protection may be mediated by maintaining a VSMC contractile phenotype via the cAMP/PKA pathway. (COI: No)

2P-100

Role of mitochondrial phosphate transporters in vascular calcification

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Inorganic phosphate (P_i) plays an essential role in cell signaling and energy metabolism. However, elevated serum Pi results in a variety of serious disorders including cardiovascular complications. Until now, the underlying molecular mechanisms of how P_i induces vascular calcification have not been clearly elucidated. Here we investigated whether mitochondrial P_i uptake followed by reactive oxygen species (ROS) generation acts a critical role in high Pi-induced vascular calcification in rat aortic smooth muscle cells. Type III Na^+-P_i cotransporters (PiT-1/2) which are the predominant plasmalemmal P_i transporters expressed in vascular smooth muscle, were upregulated by high Pi incubation. Cellular P_i uptake elicited cytosolic alkalinization that further facilitated Pi transport into mitochondrial matrix. Increased mitochondrial P_i uptake accelerated superoxide generation (ROS), upregulation of osteogenic genes and calcific changes in primary rat aortic smooth muscle cells. Vascular calcification by high Pi was effectively prevented by mitoTEMPO, a mitochondrial ROS scavengers. Genetic suppression or pharmacologic blocking of mitochondrial P_i transporters also inhibit ROS generation as well as calcific changes induced by high Pi. We propose that P_i transport across mitochondrial inner membrane could be a novel therapeutic target for vascular calcification and cardiovascular morbidities. (COI: No)

2P-101

Evolutional relationship between hearts and elastic protein connectins

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Vertebrate hearts broadly classified into coronary circulation hearts and sinusoidal circulation hearts. Mammals, birds and crocodiles have coronary circulation hearts that supply oxygen and nutrition into compacta myocardium via blood vessels. On the other hand, amphibians and most of reptiles have sinusoidal circulation hearts that take oxygen and nutrition into spongiosa myocardium directly from heart lumen. To prevent excessive extensions of the heart during diastole that reduce blood flow into coronary vessels, the coronary circulation hearts should have stiffer mechanical property. The expandability of hearts is mainly determined by an elastic protein connectin, which is the largest protein that exists from the Z-line to the M-line of the sarcomere in cardiomyocytes and functions as molecular springs for generations of passive tension during diastole of hearts. To understand the extension restriction of coronary circulation hearts, we investigated the elastic structures of connectin in hearts of various vertebrates. As results, we found that elastic regions of connectin were shorter in coronary circulation hearts with comparison to sinusoidal circulation hearts and the shortening occurred independently among mammals, birds and crocodiles. These results indicated that the extension of coronary circulation hearts were restricted by shortening of the elastic structure of connectin. (COI: No)

2P-102

Changes in the Right Coronary Microvascular Function in Pulmonary Arterial Hypertension

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Purpose: Dysfunction of the right coronary microcirculation might play a large role in the pathogenesis of right ventricle (RV) failure in pulmonary arterial hypertension (PAH). The sugen hypoxia rat model has many of the features of PAH, but the progression varies between strains. Here we compared right coronary microvascular endothelial function in vivo in Sprague-Dawley, Wistar and Goto-Kakizaki (GK) strains following sugen hypoxia treatment. We then investigated if the ghrelin analogue hexarelin prevents the onset of right coronary endothelial dysfunction. **Methods:** Male rats were given sugen 5416 (20mg/kg sc) followed by 3 weeks exposure to chronic hypoxia (10% O_2) and 3 weeks of normoxia. One group of SD rats was treated daily with hexarelin (100μg/kg/day sc). Coronary microangiograms were obtained utilizing synchrotron radiation. **Results:** Microangiography revealed that SD and Wistar rats exhibited a reduced vasodilation to acetylcholine (ACh), indicative of endothelial dysfunction. In contrast to these rat strains, insulin resistant GK rats showed vasoconstrictor responses to both ACh and sodium nitroprusside, indicating endothelial and smooth muscle dysfunction. Hexarelin restored ACh-induced vasodilation up to nearly normal level. **Conclusion:** Right coronary endothelial dysfunction is induced in the early phases of PAH. Insulin resistance evokes smooth muscle dysfunction in PAH. Further, hexarelin is able to prevent early endothelial dysfunction in PAH rats. (COI: No)

2P-103

Decreased Kir and Kv of right coronary artery SMC in pulmonary arterial hypertensive rats

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In vascular smooth muscle, K^+ channels such as voltage-gated K^+ channels (Kv) counterbalance the depolarizing stimuli to set the membrane potential. In addition, inwardly rectifying K^+ channels (Kir) mediate the endothelium dependent hyperpolarization and the relaxation by moderate increase of $[K^+]_{ext}$. Pulmonary arterial hypertension (PAH) induces right ventricle hypertrophy (RVH) and right heart failure (RHF). The risk of right ventricular ischemia is elevated in PAH. Although the previous studies demonstrated dysfunctions of coronary endothelial cells in PAH, the changes of K^+ channel activities in the right coronary artery smooth muscle cells (RCoSCs) are rarely investigated. Here we compared Kv and Kir current densities (I_{Kv} and I_{Kir}) in left (LCoSCs) and RCoSCs from control and monocrotaline-induced PAH rats. Through the whole-cell patch clamp study, we found that the I_{Kir} in control RCoSCs was smaller than that of LCoSCs. While the peak amplitudes of I_{Kv} were not different, their half-inactivation voltage ($V_{1/2,inact}$) was more negative in RCoSCs than LCoSCs. In MCT-3w, the amplitudes of both I_{Kv} and I_{Kir} were selectively decreased in RCoSCs. Also, $V_{1/2,inact}$ of RCoSCs was slightly right-shifted. The RCoSCs-specific decreases of the I_{Kv} and I_{Kir} might partly underlie the functional impairment of coronary blood flow regulation in pathological RVH and RHF. (COI: No)

2P-104(Y-010)

FUNDC2 regulates platelet activation through AKT/GSK-3β/cGMP axis

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Aims: AKT kinase is vital for regulating signal transduction in platelet aggregation. We previously found that mitochondrial protein FUNDC2 mediates AKT phosphorylation and regulates platelet apoptosis. The aim of this study was to evaluate the role of FUNDC2 in platelet activation and aggregation.
Methods and Results: We demonstrated that FUNDC2 deficiency diminished platelet aggregation in response to ADP and thrombin. Consistently, tail bleeding and thrombus formation assays showed that FUNDC2-knockout mice displayed deficiency in hemostasis and thrombosis. Mechanistically, FUNDC2 deficiency impairs the phosphorylation of AKT and downstream GSK-3β. Moreover, cGMP also plays an important role in FUNDC2/AKT-mediated platelet activation and clot retraction of platelet-rich plasma.
Conclusions: This FUNDC2/AKT/GSK-3β/cGMP axis provides new insight for platelet-related diseases. (COI: No)

2P-105

A Mathematical Model of Cardiac Cycle Driven by the Human Ventricular Cell Model

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The cardiac cycle represents most of significant features of blood pumping function of heart. Thus, various kinds of mathematical models have been made to reconstruct the cardiac cycle. Toward this end, we newly used the biophysical human ventricular cell (HuVEC) model (Himeno et al. 2015) to drive the Laplace ventricle placed within a simple circulation model, consisting of afterload, resistant vessel, preload and a Laplace atrium. The sarcomere shortening during systole is usually determined by the crossbridge head sliding along the actin fiber in the cell model, while it is calculated separately by the cyclic volume change of the Laplace ventricle. To couple these two models, a new condition was introduced; the contractile rate of the cell model is equal to the change rate of cellular length calculated from the circulation model. Accordingly, we developed a new algorithm to obtain a common sarcomere shortening that fulfills the above condition. Since the HuVEC model calculates membrane potential, ion homeostasis, muscle contraction as well as the accompanying ATP consumption, we believe that this cardiac cycle model enables us to analyze the pressure-volume trajectory and the energy consumption to drive the blood circulation, refilling mechanism of ventricle during diastole, and the control of blood pressure. (COI: No)

2P-106

Atypical antipsychotic drug olanzapine leads to aggravation of atherosclerosis in apoE-null mice

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Olanzapine, an atypical antipsychotic drug, has therapeutic effect in treating schizophrenia. However, clinical reports indicate that patients taking atypical antipsychotic drugs run high risk to suffer from metabolic syndrome with unclear mechanisms. We investigated the effect of olanzapine on atherosclerosis and involving mechanisms in apolipoprotein E-null (apoE$^{-/-}$) mice. In this study, apoE$^{-/-}$ mice were as *in vivo* models. Daily treatment with olanzapine (3 mg/kg body weight) for 4 weeks increased the mean arterial blood pressure and the whitening of brown adipose tissues in apoE$^{-/-}$ mice. In addition, olanzapine impaired aortic cholesterol homeostasis and exacerbated hyperlipidemia and aortic inflammation, leading to acceleration of atherosclerosis in apoE$^{-/-}$ mice. Moreover, olanzapine-treated apoE$^{-/-}$ mice exhibited more lipid accumulation in liver by upregulating the expression of *de novo* lipid synthesis-related proteins while downregulating the expression of cholesterol clearance- or very low-density lipoprotein secretion-related proteins. Collectively, our findings suggest that olanzapine may exacerbate atherosclerosis by deregulating hepatic lipid metabolism, worsening hyperlipidemia and aorta inflammation, leading to acceleration of atherosclerosis. (COI: Properly Declared)

2P-107

Effect of Total Cholesterol on Blood Pressure and the Difference between Genders

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Rise in cholesterol level lifts risk of vascular disease. It is well known that higher cholesterol increases blood pressure. However, few people know that gender is related to relationship between total cholesterol and blood pressure.

This work aims to compare relationship between total cholesterol and blood pressure, and their classification based on gender.

This research had been approved by the ethic committee. The data was analized using Bivariate correlation method. First analysis was done without gender classification, while second analysis with gender classification.

The cholesterol values spread from 100 to 310 mg/dL, and the blood pressure from 50 to 140 mmHg. From the first analysis, it was obtained that the relationship between blood pressure, either systolic or diastolic, and cholesterol value is linear with positive relation. After gender classification, different results were obtained. For female subjects, blood pressure is inversely proportional to cholesterol value. For male subjects, systolic blood pressure is almost not affected by cholesterol value, while diastolic blood pressure is proportional with cholesterol.

We conclude that for a population with relatively homogen age from 19 to 21 years old, higher cholesterol values does not necessarily yield higher blood pressure in female group, cholesterol value has no correlation with systolic blood pressuer in male group and higher cholesterol value represents higher diastolic blood pressure in male group. (COI: Properly Declared)

2P-108

Withdrawn

2P-110(Y-11)

Genistein and running exercise modulates HDAC3 and the fibrosis markers in OVX rats with NASH

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The incidence of nonalcoholic steatoheptatitis (NASH) in menopausal women has rising nowadays and the effective treatment is still questions. To investigate the treatment effects of genistein and moderate intensity of running exercise in ovariectomized (OVX) rats with NASH. Female SD rats were divided into (1) control, (2) OVX with standard diet, (3) OVX with high fat and high fructose (HFHF) diet for 4 weeks, (4) OVX with HFHF with genistein treatment for 5 weeks (16 mg/kg BW, once daily) and, (5) OVX with HFHF and moderate intensity exercise (running 80%VO_2max, 3 times/week) for 5 weeks. Histopathologic NASH score and fibrosis were analyzed by H&E staining, IL-13 and matrix metalloproteinase-12 (MMP12) using western blot. Serum IL-6 level, free fatty acid (FFA), steatosis using Oil Red O staining, and histone deacetylase 3 (HDAC3) were examined. Steatosis and mild severity of NASH were found in OVX and more severe in OVX with HFHF feeding. FFA, IL-6, IL-13 and MMP12 were higher in OVX and more increased in OVX with HFHF when compared with control. Either genistein or moderate running exercise improved steatosis and pathologic NASH score. Genistein or moderate intensity exercise significantly decreased FFA, IL-6, IL-13 and MMP12 expression while increased HDAC3 when compared with OVX with HFHF. Genistein has effective as moderate intensity exercise in reducing the severity of NASH in OVX rat with HFHF diet via modulated HDAC3 pathway and attenuate fibrosis.

(COI: No)

2P-111

DHA Protects Against Hepatic Steatosis by Activating Sirt1 in Nonalcoholic Fatty Liver Disease Mice

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Purpose: Docosahexaenoic acid (DHA; C22; N-3) shows beneficial effects on Non-alcoholic fatty liver disease (NAFLD). The protein deacetylase Sirtuin1 (Sirt1) increases energy metabolism and decreases lipogenesis. Here, we investigated whether DHA plays a role in protecting against hepatic steatosis via Sirt1.

Methods and results: Both *in vivo* and *in vitro* hepatic steatosis models were used: diet-induced obesity (DIO) model (middle-aged C57BL/6 mice fed a high-fat diet (HFD)) and palmitic acid (PA)-induced lipid accumulation cell model (HepG2 cells). In DIO mice, treatment with DHA for 8 weeks inhibited the lipid accumulation, increased FA oxidation and induced triglyceride export in liver. These changes were accompanied by attenuation of inflammation. Moreover, DHA reversed the HFD-induced reduction of Sirt1 in liver. Interestingly, the beneficial effects of DHA were attenuated by lentivirus-mediated Sirt1 knockdown, accompanied with increased expression of markers of lipogenesis, inflammation and reduced fatty acid oxidation. In HepG2 cells, DHA prevented the accumulation of PA-induced lipid droplets, the decrease of FA oxidation and the reduction of Sirt1 level. Inhibition of Sirt1 by sirtinol partially reversed the beneficial effects of DHA on PA-treated cells.

Conclusions: DHA alleviated hepatic steatosis and reduced inflammation of liver in obese middle-aged mice by mechanisms involving Sirt1 activation. (COI: Properly Declared)

2P-112

Neurosecretory protein GL, a hypothalamic small protein, regulates appetite and energy homeostasis

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We have recently identified a novel cDNA encoding a small secretory protein named neurosecretory protein GL (NPGL) from the avian hypothalamus. In chicks, NPGL increases body weight gain. A genome database search reveals that NPGL is conserved throughout vertebrates. However, the central distribution and functional role of NPGL remains to be elucidated in mammals. In this study, we identified the precursor cDNA encoding NPGL from the mouse hypothalamus. Quantitative RT-PCR and morphological analyses revealed that *NPGL* precursor mRNA is robustly expressed in the mediobasal hypothalamus with NPGL neurons specifically localized to the lateroposterior part of the arcuate nucleus in the hypothalamus. NPGL-immunoreactive fibers were observed in close anatomical contact with pro-opiomelanocortin neurons in the rostral region of the arcuate nucleus. *NPGL* mRNA expression was elevated by 24 h fasting and reduced by feeding of a high fat diet for 5 weeks. These data suggested that NPGL participates in energy homeostasis in mammals. Furthermore, intracerebroventricular injection of mature NPGL increased food intake, pointing to an important role in feeding. Taken together, these findings provide the first report on the distribution of NPGL in the mammalian brain and point to an important role for this neuropeptide in energy homeostasis. (COI: No)

2P-113

Effect of long term high-fat diet and calorie restriction on the hepatic NAD metabolism in mice

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Nnmt, one enzyme in NAD^+ metabolic pathways, methylates nicotinamide to produce N1-methylnicotinamide. It is, however, unknown how hepatic Nnmt as well as NAD^+ metabolism is regulated in response to hyper- and hypo-nutrition status. In this work, we fed C57BL6/J mice either a chow diet (CD), a high-fat diet (HFD), or a 40% CR diet for 16 weeks. As anticipated, HFD mice gained more body mass and liver weight, stored more hepatic lipids and had an impaired glucose tolerance. Conversely, CR mice gained less mass while hepatic lipids and glucose tolerance showed no significance. NAD^+ levels as well as key enzymes in the NAD^+ salvage pathway, Nnmt, Nampt and Nmnat1 mRNA expression and protein abundance were significantly increased in CR mice while no changes were found in HFD-fed mice. Enhanced NAD^+ levels were associated with increased activation of AMPK and sirt1 in CR mice. Interestly, though no hepatic NAD^+ level change was showed in HFD mice, we still found that hepatic Nampt, Nmnat1 and Nrk1 was slightly increased in HFD mice, indicating this increase may be a compensatory mechanism to protect against negative impact of hepatic lipid accumulation. (This work is supported by the National Natural Science Foundation of China (No. 81700773, 81871190) and the National Science Foundation for Postdoctoral Scientists of China (No. 2017M623196). (COI: No)

2P-114

Effect of flaxseed on a inflammatory response in patients with hypercholesterolemia-preliminary data

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Familial hypercholesterolemia is associated with severe abnormalities including: atherosclerosis, dyslipidaemia, oxidative stress, inflammation and endothelium dysfunction. Flaxseed in the diet acts upon blood lipids, lowering cholesterol, and also has anti-oxidative effects. These effects are beneficial for patients suffering from dyslipidaemia, oxidative stress, inflammation and endothelium dysfunction.The protocol consist of four stages: a run-in phase of 10 weeks (patients on a standard diet) and an experimental stage - flaxseed supplementation or placebo (whole-wheat) supplementation, and wash out phase of 10 weeks. Samples of saliva and blood was collected after each phase of the trial, just before routine lipoprotein apheresis, which is usually once every 2 weeks. Saliva myeloperoxidase levels were analyzed and flow cytometry was used for measurement of circulating microparticles. The study adhered to the Principles of the Declaration of Helsinki. The levels of salivary myeloperoxidase were lower after flaxseed supplementation. The number of circulating microparticles: CD105, CD45 and CD41 was smaller after flaxseed supplementation compared with a run in phase Our preliminary observations indicate that that a diet containing flaxseed may improve endothelial cell function and thus contribute to an amelioration of unfavourable effects associated with hypercholesterolemia. There is no actual or potential conflict of interest in relation to this presentation. (COI: No)

2P-115

The hypothalamic feeding-related neuropeptides in the streptozotocin-induced diabetic rat

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We examined the hypothalamic feeding-related neuropeptides gene expressions in the paraventricular nucleus (PVN) and the arcuate nucleus (ARC) in the diabetic rats administered streptozotocin (STZ). STZ (80 mg/kg) was administered intraperitoneally (i.p.) in adult male Wistar rats. Rats were divided into 3 groups: PG1 (<300 mg/dl at light period), PG2 (≧300 mg/dl at light period and <200 mg/dl after fasting for dark period) and PG3 (≧300 mg/dl at light period and >200 mg/dl after fasting for dark period). Two weeks after i.p. administration of STZ, they were decapitated after fasting for 12 hours. The gene expressions of corticotrophin releasing hormone (CRH), thyrotropin-releasing hormone (TRH), proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), neuropeptide Y (NPY), agouti-related protein (AgRP) in the ARC were quantified by using in situ hybridization histochemistry. POMC and CART were significantly decreased in PG2 and PG3 compared to PG1. On the other hand, NPY, AgRP and TRH were significantly increased in PG3 but not PG2 compared to PG1. The gene expression of the hypothalamic ARC anorexigenic neuropeptide decreased in the rats with hyperglycemia after STZ administration but not hyperglycemia after fasting, and no significant change was observed in the orexigenic neuropeptide. (COI: No)

2P-116

Effects of estradiol on an orexigenic function of ghrelin in ovariectomized rats fed high-fat diet

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Estrogen has an ability to control energy balance, food intake, and body fat distribution. However, it is not clear whether estrogen modulates food intake by regulating orexigenic hormone, ghrelin, in high-fat diet (HFD)-induced obesity. In this study, we investigated chronic effects of estrogen on the function of ghrelin in ovariectomized (OVX) rats fed HFD. Female Wistar rats aged 9 weeks were ovariectomized. After 4 weeks, pellets containing either 17β-estradiol (E2) or placebo (Pla) were subcutaneously implanted into the rats. Simultaneously, rats were given HFD (60% of calories from fat). HFD increased energy intake and body weight only in the Pla group, but decreased it in the E2 group during first 2 weeks, resulting in the difference in the body weight between the two groups. Intraperitoneal injection of ghrelin receptor agonist, growth hormone-releasing peptide 6 (GHRP-6, 400 nmol/kg) induced the enhancement of food intake only in the Pla group, but not in the E2 group. Furthermore, the number of the c-Fos-positive neurons in the arcuate nucleus after injection of GHRP-6 was significantly lower in the E2 group compared with the Pla group. However, there was no significant difference in the plasma level of active ghrelin after 17 hr fasting between the two groups. In summary, our results suggest that estrogen replacement attenuates food intake and prevents body weight gain by inhibiting the orexigenic action of ghrelin in OVX rats fed on HFD. (COI: No)

2P-117

Possible involvement of central nesfatin-1 neurons in xenin-induced feeding suppression in rats

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Xenin is a 25-amino acid peptide originally identified from human gastric mucosa. Central and peripherally administered xenin decreased food intake in rodent. Nesfatin-1, the newly identified anorectic neuropeptide, is synthesized in both peripheral tissues and the central nervous system. We examined the central effects of xenin on food intake, water intake and nesfatin-1 like immunoreactivity (Nesfatin-1-LI) neurons in rat. Intracerebroventricular (icv) administration of xenin significantly decreased food intake and water intake. Fos like immunoreactivity (Fos-LI) expressed in the supraoptic nucleus (SON), the paraventricular nucleus (PVN), area postrema, and the nucleus of the solitary tractafter icv administration of xenin. Icv administration of xenin (6μg/rat) caused significant increases the number of Fos-LI in expressing nesfatin-1-LI neurones in the SON and the PVN. Furthermore, decreased food intake induced by central administered xenin was significantly attenuated by pretreatment with icv administration of antisense nesfatin-1. These results indicate that nesfatin-1-expressing neurones in the SON and PVN may play an important role in xenin-induced food suppression in rats. (COI: No)

2P-118

Adrenomedullin enhances chorda tympani nerve responses to sugars in mice

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Recent studies by ours and others showed that there may be a T1Rs-independent [Glucose Transporters(GTs), KATP Channel] pathway for detecting only sugars in addition to a T1Rs-dependent(T1R2/T1R3) pathway which detects not only sugars but artificial sweeteners(AS) as well(Sukumaran et al, 2016). A cephalic phase insulin release(CPIR) can be induced by oral administration of sugars, but not AS in Wild type and T1R3-knockout(KO) mice. And incapacitation of the KATP pathway abolishes CPIR, suggesting a possibility that T1Rs-independent and -dependent pathways may function independently of one another(Glendining et al. 2017). However, details still remain unknown. In the gut enterocytes, expression of GTs were shown to increase after administration with Adrenomedullin(ADM), a biologically active peptide(Fernandez et al., 2005). So, our question is if this would also be the case in taste cells. To answer the question, we examined expression of ADM receptors(ADMRs) in mouse taste buds by using RT-PCR and potential effects of ADM on the GT system by measuring mouse chorda tympani(CT) nerve responses to various tastants. ADMRs were expressed in mouse taste buds and ADM enhanced CT responses to sugars but not to AS and other tastants. Furthermore, ADMRs antagonist AM22-52 inhibited the sweet enhancing effect of ADM. Taken together, our data suggest potential involvement of GTs in T1Rs-independent sweet pathway in taste cells whose expression could be enhanced by ADM. (COI: No)

2P-119

Dietary fat modulation of oral fatty acid sensitivity and preference in young men and women

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Long-term consumption of high-fat diet decreases oral fatty acid sensitivity. However, it is unclear whether dietary fat temporarily modulates oral fatty acid sensitivity. We examined whether oral stimulation of lipid-sensors by fat affects an oleic acid threshold and fat preference by comparison with sucrose stimulation in young healthy men and women at three phases during the menstrual cycle. In addition, we assessed the sex difference or effects of sex hormones on fat taste perception or preference. The fatty acid detection threshold was examined using a three-alternative, forced-choice methodology for oleic acid. The fat preference test was performed by selecting favorite food that contains dietary lipids in different concentrations. Measurements of oleic acid detection threshold and fat preference were repeated after oral stimulation by fat or, sucrose. Free-feeding intake of energy and lipid in buffet-style lunch were measured in the subjects. The oral oleic acid threshold varied during menstrual cycle in women. Short-term stimulation with dietary fat enhanced the oleic acid thresholds in both women and men. In addition, there was a correlation between free-feeding energy intake and the oleic acid threshold after fat stimulation. The present result suggests that oral fatty acid sensitivity decreased after dietary fat stimulation, and mediates total energy intake in meal, while ovarian hormones have effects on oral fatty acid sensitivity in women. (COI: No)

2P-120

Nutritional status of Japanese children with developmental disorders

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Children with developmental disorders are at risk of having an unbalanced diet because of sensory problems, such as hyperesthesia. Moreover, developmentally disabled children often dislike physical exercise, and dietary imbalance and obesity can exacerbate this problem. The current study investigated the caloric intake of developmentally disabled Japanese children, and examined their alimentary characteristics.

Subjects were 67 children (boys : girls = 45:22) who underwent the Hirosaki Five-Year-Old Children Developmental Health Check-up in 2018. We investigated children's nutrition status using the Brief-type Self-administered Diet History Questionnaire 3 years (BDHQ3y). Child psychiatrists diagnosed developmental disorders.

The results indicated that developmentally disabled children had a high rate of obesity. In addition, developmentally disabled children had an excessive intake of salt, lipids, carbohydrates, and a deficient intake of calcium, and iron.

The high rates of obesity and neglect of the nutrition status of children with developmental disorders may adversely affect development. It is necessary for future studies to compare the nutritional status of children with developmental disorders and typically developing children in future. (COI: Properly Declared)

2P-121(Y-12)

The influence of central leptin signalling upon Obesity-induced hypertension

Stephanie Elise Simonds; Jack T Pryor; Tony Tiganis; Michael A Cowley (*Monash University, Australia*)

Excess body fat increases the risk of cardiovascular diseases. Leptin a hormone produced and secreted from white fat cells is significantly higher as body fat is accumulated. Leptin is a crucial regulator of both energy homeostasis and blood pressure. Recent studies have proposed that disinhibition of central leptin signal transduction can slow the progression of dietinduced obesity, insulin resistance and glucose resistance. As such the inhibitory phosphatases responsible for signal inhibition have been touted as a potential target for novel anti obesity drugs. Here using blood pressure radiotelemetry and genetically modified mice with AAV Cre driven phosphatase expression we report the differential cardiovascular effects at different plasma leptin concentrations and a key role of dorsomedial hypothalamic neurons (DMH). At low exogenous leptin conc (2ug/g of BW) food intake is affected whereas at a leptin concentration of 6ug/g of BW blood pressure is significantly elevated (6.8±1.8mmHg). DMH specific knockout of PTP1B significantly elevated heart rate (+70 ±6BPM) whilst TCPTP knockout had no effect on heart rate but did increase systolic blood pressure (+ 5.6±0.8mmHg). Double knockout of PTP1B and TCPTP in DMH elevated both heart rate and blood pressure, triple knockout of PTP1B, TCPTP and SOCS3 had no additional increases. These data demonstrate that leptin induced cardiovascular effects can be controlled with dose and signalling alterations in obesity. (COI: No)

2P-122(Y-13)

FKBP51 defect is resistant to diet induced obesity, inflammation and insulin resistance

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Obesity is associated with a chronic, low-grade inflammation status. The pathogenic mechanisms at the molecular level in obesity-associated inflammation and insulin resistance are not fully understood and need to be elucidated. The 51 KD FK506-binding protein 51 (Fkbp51), encoded by Fkbp5, is one of the of immunophilin family members. FKBP51 null mice are resistant to diet-induced obesity. Therefore, we hypothesize that Fkbp51 is involved within the pathogenic mechanism linking obesity-associated inflammation and insulin resistance. Results of animal study showed that high-fat diet (HFD) feeding induced adipose Fkbp5 mRNA up-regulation in wild-type (WT) mice. Fkbp5-KO can ameliorate the obesity, adipocyte hypertrophy and hyperplasia, insulin resistance, and inflammation induced by HFD feeding in mice. Results of *in vitro* study showed that Fkbp51 expression was progressively increased during 3T3-L1 adipocytes differentiation and played an important regulator of adipogenesis. In human adipose tissues, we found that fat cell size was significantly positively correlated with expression level of Fkbp5 mRNA. Both fat cell size and Fkbp5 mRNA level were positively correlated with the mRNA expression levels of proinflammatory cytokines. In conclusion, Fkbp51 plays a pivotal role to link obesity-associated inflammation and insulin resistance.

Keywords: FKBP51, obesity, insulin resistance, inflammation (COI: No)

2P-123

Leptin is a key regulator of glucose homeostasis in obesity

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Approximately 9 out of 10 people with type-2 diabetes are overweight. Leptin is a hormone secreted from white fat cells that exerts control over both food intake and energy expenditure. Recently we described the central mechanisms underlying the hypertensive effects of hyperleptinemia in obesity. Work presented here used continuous blood glucose monitoring to determine the effects of dorsomedial hypothalamic (DMH) leptin signalling on the glycaemic control of mice. A leptin receptor (LepR) antagonist administered directly into the brain of diet-induced obese (DIO) mice impaired glucose tolerance demonstrating that leptin has the ability to regulate blood glucose levels even in obesity. Knockdown of DMH LepR function using short hairpin adeno-associated virus (AAV) RNA significantly reduced brown adipose tissue (BAT) thermogenesis (-0.8 ± 0.1°C), increased basal blood glucose concentration (14.3 ± 0.6mmol/l to 9.4 ± 0.5mmol/l), and impaired glucose tolerance. Conversely, chemogenic activation of LepR-expressing DMH neurons significantly elevated BAT thermogenesis by 1.5 ± 0.2 °C. This increased BAT temperature was immediately followed by a decrease in blood glucose concentration from 7.6 ± 0.5 mmol/l to 5.8 ± 0.5 mmol/l. Work presented here demonstrates that increasing the activity of LepR-expressing neurons in the DMH can improve glucose tolerance in obesity through an elevation of BAT metabolic activity. (COI: Properly Declared)

2P-124

Visfatin promotes monocyte-endothelial cell adhesion via activation of p38-PI3K-Akt signaling

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Visfatin, an adipocytokine, is preferentially expressed and produced in visceral adipose tissues. Visfatin is known to act as a mediator in several metabolic disorders, such as obesity, diabetes, and cardiovascular diseases. Recent studies have supported the possible role of visfatin in the pathophysiology of cardiovascular complications. One of the key events in the early stage of atherosclerosis is the adhesion of circulating monocytes to endothelial cells. This study aimed to investigate the effect of visfatin on the adhesion of THP-1 monocytes to human vascular endothelial cells and the underlying mechanism. Results showed that visfatin significantly caused the upregulation of intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells, as well as enhanced monocyte adhesion to endothelial cells. Moreover, we found that inhibition of PI3K, Akt, and p38 MAPK activation significantly prevented visfatin-enhanced expression of ICAM-1 and VCAM-1 and monocyte adhesion to endothelial cells. Visfatin enhanced ROS production and IKK/NF-κB activation and then led to upregulation of ICAM-1 and VCAM-1 and enhanced monocyte adhesion to endothelial cells. These effects were also p38/PI3K/Akt-dependent. These results demonstrated that visfatin promoted monocyte-endothelial cell adhesion by increasing ICAM-1 and VCAM-1 expression via the activation of p38/PI3K/Akt signaling and downstream ROS production and IKK/NF-κB activation. (COI: No)

2P-125

Pin1 suppress thermogenesis through promoting the degradation of PRDM16

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Pin 1 is an enzyme that binds to various target proteins and regulates their functions by performing cis-trans isomerization of proline.

Interestingly, Pin1 null mice showed resistance to high fat diet-induced obesity. This phenomenon raises the possibility that Pin 1 enhanced adipogenesis while suppressing heat generation in both brown and beige adipose tissue.

In this study, we show that Pin1 is the negative regulator of thermogenesis through promoting the degradation of PRDM16.

We made the adipocyte-specific Pin1 KO mice (AdipoPin1 KO). Then, both Pin1 ff and AdipoPin1 KO were treated in cold temperature for 6 hr, and both BAT and scWAT were extirpated. Expressions of thermogenic genes induced by cold exposure in AdipoPin1 KO show the higher values, compared to Pin1 ff.

Next, we searched the Pin1 binding partner which was involved in thermogenesis, and identified PRDM16, transcriptional co-activator. When both S-tag Pin1 and Flat-PRDM16 were overexpressed in 293T cells, the association of two proteins was detected. In addition, Pin1 endogenously binds to PRDM16 in BAT. Pin1 overexpression in 293T cells decreased PRDM16 protein, depending on its isomerase activity. Moreover, proteasome inhibitor treatment inhibited the reduction of PRDM16 protein by Pin1 overexpression.

Taken together, Pin1 alleviates thermogenesis through promoting the degradation of PRDM16. If drugs are able to be selectively delivered to adipocytes, Pin1 inhibitors may be useful for treatment of obesity. (COI: No)

2P-127(Y-14)

Effect of Dapagliflozin on Glucose Metabolism and Renal and Hepatic PEPCK Expression in Obese Rats

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Purpose

Sodium glucose cotransporter 2 inhibitor (SGLT2i) is the new interesting anti-diabetic agent that inhibits renal glucose reabsorption. This study investigated the effect of SGLT2i—dapagliflozin on renal and hepatic gluconeogenesis in high-fat diet-induced obese rats.

Method

Male Wistar rats were fed with normal diet or high-fat diet (HF) for 16 weeks. Then, the HF rats were randomized into three groups, and the rats received either vehicle (HF), dapagliflozin (HFD) or metformin (HFM) for 4 weeks. After 20 weeks, metabolic parameters and gluconeogenesis were determined.

Results

Although the fasting plasma glucose (FPG) was not different among the groups, AUCglucose for OGTT was increased in HF group which was ameliorated by dapagliflozin. Urinary glucose excretion was substantially increased by dapagliflozin. The elevated renal PEPCK expression in HF rats was attenuated by dapagliflozin that was associated with the decrease in renal oxidative stress markers, MDA and NOX4, as well as transcription factor CREB. HF group did not show increase in hepatic PEPCK. However, HFD group exhibited the elevation of hepatic PEPCK together with the increase in sirtuin 1 expression.

Conclusion

The glycemic control of dapagliflozin was comparable to that of metformin in obese rats. Although the renal PEPCK was decreased by dapagliflozin, substantial urinary glucose loss seemed to trigger the upregulation of hepatic PEPCK expression via sirtuin 1 as a metabolic compensation to maintain FPG. (COI: No)

2P-128

Tentonin 3/TMEM150C contributes to glucose-stimulated insulin secretion in pancreatic β-cells

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Glucose homeostasis is initially regulated by pancreatic hormone, insulin. Glucose-stimulated insulin secretion in β-cells is composed of two cellular mechanisms; high glucose concentration not only depolarizes membrane potential of the β-cells via ATP-sensitive K^+ channels, but also induces cell inflation, which is sufficient to release insulin granules. However, the molecular identity of the stretch-activated cation (SAC) channel responsible for the latter pathway has not been determined. Here, we demonstrate that Tentonin 3 (TTN3/TMEM150c), a recently identified mechanosensitive channel, contributes to glucose-stimulated insulin secretion by mediating cation influx. TTN3 is expressed specifically in β-cells in mouse pancreas and mediates cation currents to glucose- and hypotonic-stimulations. The glucose-induced depolarization, firing activity, and Ca^{2+} influx of pancreatic β-cells were significantly lower in *Ttn3* knock-out (KO) mice. More importantly, *Ttn3* KO mice showed impaired glucose tolerance with decreased insulin secretion *in vivo*. We therefore conclude that TTN3, as a SAC channel of pancreatic β-cells, contributes to insulin secretion in response to glucose stimulation *in vivo*. (COI: Properly Declared)

2P-129

Cytosolic phospholipase A2 in hypothalamus modulates systemic glucose metabolism differently by meal

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The hypothalamus plays a central role in monitoring and regulating systemic glucose metabolism. Neurons in the hypothalamus receive signals of hormones like insulin and detect blood glucose levels, and maintain glucose homeostasis by controlling metabolism in peripheral tissues. Brain is enriched with phospholipids containing poly-unsaturated fatty acids, which are biologically active in physiological regulations. In this study, we measured distributions and amounts of phospholipids in the hypothalamus by using imaging mass spectrometry (IMS). We found that arachidonic acid-containing phospholipids were decreased by intraperitoneal glucose or insulin injection, which activated cytosolic phospholipase A2(cPLA2) in hypothalamus but not secretory PLA2. Injection of cPLA2 inhibitor into the hypothalamus impaired systemic glucose tolerance, and knockdown of cPLA2 in the ventromedial hypothalamus (VMH) by short-hairpin RNA (shRNA) lowered insulin sensitivity compared to control mice. In contrast, the down-regulation of glucose metabolism by high fat diet (HFD) feeding was improved by the knockdown of cPLA2 in the VMH. Body weight was not different between groups in both regular chow diet (RCD) and HFD. These results suggest that cPLA2 in the VMH plays distinct roles during RCD and HFD feeding, i.e. cPLA2 is necessary for the systemic glucose metabolism during RCD, while it has a deteriorative role in glucose metabolism during HFD. (COI: No)

2P-130

Heterotypic endosomal fusion as an initial trigger for insulin-induced GLUT4 translocation

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The glucose transporter GLUT4 mediates insulin- and exercise-responsive facilitation of glucose uptake via its translocation from intracellular storage compartments to the plasma membrane in the target tissues such as skeletal muscle and adipose tissues. Exercise also enhances insulin sensitivity. However, the trafficking mechanisms controlling GLUT4 mobilization in skeletal muscle remain uncertain due to technical limitations. We herein attempted to reveal intramyofiber GLUT4 trafficking properties with various imaging analyses on isolated skeletal myofibers such as single molecule nanometry and superresolution imaging, and found that 1) GLUT4 molecules are governed by regulatory systems involving static retention and stimulus-dependent liberation, and 2) insulin promptly induced enlargement of very small GLUT4-containing structures and increases in colocalization with transferrin receptor. These findings suggest that insulin initially stimulates endomembrane fusion of GLUT4-containing vesicles with a subset of transferrin receptor-containing endosomes. By visualizing such fusion events using a novel fluorometric assay, we clearly demonstrated the insulin-induced heterotypic endomembrane fusion. Importantly, prior exposure to exercise-mimetic stimuli potentiated this insulin-responsive endomembrane fusion event involving GLUT4–containing vesicles in skeletal muscle, suggesting the endomembranous regulation process to be a potential site related to exercise effects. (COI: No)

2P-131

Exogenous pyruvate maintains glycolysis-TCA cycle flux in Schwann cell under high glucose conditions

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Endogenous pyruvate is a key molecule in energy production, whereas exogenous pyruvate works as an antioxidant. It has been reported that the treatment of diabetic animals with pyruvate ameliorates diabetic retinopathy and nephropathy, whereas the beneficial effects of exogenous pyruvate on diabetic neuropathy remain unclear. This study aimed to clarify the role of exogenous pyruvate in Schwann cells under high glucose conditions. Immortalized adult mouse Schwann cells (IMS32) were exposed to normal (5 mM) and high glucose (>15 mM) conditions in the presence or absence of sodium pyruvate (1 mM) for up to 24 h, and cell viability and glucose metabolism under each culture condition were evaluated.

The high glucose and pyruvate-deficient conditions induced rapid IMS32 cell death with the inhibition of glycolytic flux, mitochondrial respiration and ATP synthesis. The same conditions significantly increased the intracellular contents of polyol pathway products, such as fructose and sorbitol. Moreover, supplementation with TCA cycle intermediates (e.g., 2-oxoglutarate) completely prevented the IMS32 cell death, mitochondrial dysfunction and ATP depletion. These findings suggest that pyruvate deprivation under high glucose conditions escalates glucose flux in the polyol pathway and reduces flux in the glycolysis–TCA cycle in IMS32 cells. These metabolic alterations may decrease ATP production in mitochondria, thereby being a cause of rapid Schwann cell death. (COI: No)

2P-132

Withdrawn

2P-133

Withdrawn

2P-134

Withdrawn

2P-135

Withdrawn

2P-136(Y-15)

Correlation of median nerve parameters with TSH values in hypothyroid patients

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Background

Entrapment neuropathies are most common in hypothyroidism. Nerve conduction parameters are impaired even in newly diagnosed cases of hypothyroid. Thus we wanted to study the correlation between thyroid stimulating hormone (TSH) and nerve conduction study (NCS) parameters in median nerve in newly diagnosed hypothyroid patients.

Method

This cross sectional, descriptive study included newly diagnosed hypothyroid patients (n=30; age: 31.96±9.12). In all subjects NCS were performed in median motor and sensory nerve using Nihon Kohden machine in Neurophysiology lab 2, B.P. Koirala Institute of Health Science. Thyroid function test (TFT) was analysed by ELISA. The association between TSH and NCS parameters were done using Pearson correlation.

Result

In NCS parameters; distal latency of bilateral median motor nerve showed significant positive correlation with TSH (lt; r= 0.426, p= 0.021; rt; r= 0.435, p=0.018) whereas CMAP amplitude and nerve conduction velocity of bilateral median nerve showed significant negative correlation with TSH. Among sensory parameters; onset latency of bilateral median nerve showed significant positive correlation with TSH.

Conclusion

In this study; strong association was found between TSH values and latency of median nerve (sensory and motor) parameter. Early diagnosis and treatment may help in management of neuropathies.

Keywords: Nerve conduction study, Thyroid function test, Thyroid stimulating hormone (COI: No)

2P-137

Role of PCSK9 in lipid metabolic disorders and ovarian dysfunction in polycystic ovary syndrome

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) plays a critical role in the cholesterol metabolism by negatively regulating the low-density lipoprotein receptor (LDLR). Here we intended to elucidate the role of PCSK9 in the pathogenesis of polycystic ovary syndrome (PCOS). We collected serum samples of PCOS patients and normal controls, and checked the PCSK9 level. Furthermore, we establish PCOS model mice, and some of them for rescue group. Their serum lipid profiles, ovarian morphology and function, PCSK9 and LDLR levels were measured. Serum PCSK9 levels were higher in PCOS patients than controls. The PCOS mice exhibited significantly increased serum levels of total cholesterol (TC) and LDL-C. Moreover, the serum PCSK9 level was significantly increased in PCOS mice, which positively correlated with LDL-C and TC. In both liver and ovary of PCOS mice, PCSK9 mRNA and protein levels were significantly increased, but LDLR levels were significantly decreased. Furthermore, alirocumab inhibiting PCSK9 may partly increase in LDLR expression in PCOS mice, also ameliorated the lipid metabolic disorders and pathological changes of ovarian morphology and function. Abnormal high expression of PCSK9 in the blood, liver and ovary might play an important role in PCOS pathogenesis by affecting lipid metabolism and ovarian function, and alirocumab may partly reverse the pathological changes of PCOS, suggesting a possibility of PCSK9 as a new target for diagnosis and treatment of PCOS. (COI: No)

2P-138

Norepinephrine inhibits Th17 cells via beta2-adrenoreceptor signaling in collagen-induced arthritis

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Purpose: Norepinephrine (NE), a neurotransmitter released from the sympathetic nerves, has been involved in rheumatoid arthritis (RA). However, role of NE in RA is divergent. Herein, we demonstrated that NE exerts an anti-inflammatory property in collagen-induced arthritis (CIA), a mouse model of RA, by inhibiting T-helper (Th)17 cells via β2-adrenoreceptor (β2-AR) signaling.
Methods: CIA was prepared by intradermal injection of collagen type II in tail base of DBA1/J mice. On the 41st day post-immunization, $CD4^+$ T cells from the spleen were purified using magnetic cell sorting and activated with anti-CD3 anti-CD28 antibodies. Th17 cells were polarized from the $CD4^+$ T cells using various antibodies and cytokines.
Results: A co-expression of CD4 and β2-AR was observed in spleens of both intact and CIA mice. β2-AR expression in the ankle and spleen was downregulated in CIA mice. CIA induced increases in interleukin (IL)-17 and IL-22 production, $CD25^-IL\text{-}17^+$ cell percentage and ROR-γt exprssion in $CD4^+$ T cells. NE reduced the CIA-induced $CD4^+$ T cell shift towards Th17 phenotype and the β2-AR antagonist ICI118551 blocked the NE effect. Moreover, the β2-AR agonist terbutaline inhibited CIA-induced $CD4^+$ T cell proliferation and shift towards Th17 phenotype, and the protein kinase A (PKA) inhibitor H-89 abolished the agonist effect.
Conclusion: NE inhibits Th17 cell differentiation and function in CIA condition by activation of β2-AR/PKA signaling. (COI: No)

2P-139

Roles of macrophages and PAI-1 in diabetic delayed bone repair in female mice

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Delayed fracture healing is major clinical issue in diabetic patients. However, the details in the mechanisms by which diabetic state induces delayed bone repair still remain unclear. Here, we investigated the roles of macrophages and plasminogen activator inhibitor-1 (PAI-1) in diabetic delayed bone repair after femoral bone injury using streptozotocin (STZ)-induced diabetic and/or PAI-1-deficient female mice. STZ treatment significantly decreased the number of macrophages, but not neutrophils, at the damaged site on day 2 after femoral bone injury in mice. STZ treatment significantly reduced the mRNA levels of macrophage colony-stimulating factor, inducible nitric oxide synthase (iNOS), interleukin (IL)-6 and CD206 in the damaged femur on day 2 after bone injury. Moreover, STZ treatment attenuated a decrease in the number of hematopoietic stem cells in bone marrow from damaged femurs induced by bone injury. On the other hand, PAI-1 deficiency significantly blunted the number of macrophages at the damaged site and macrophage phagocytosis decreased by diabetic state on day 2 after bone injury. PAI-1 deficiency did not affect the mRNA levels of iNOS and IL-6 in macrophages decreased by diabetic state from the bone marrow of the damaged femurs. In conclusion, we demonstrated that diabetic state decreases accumulation and phagocytosis of macrophages at the damaged site during the early bone repair process after femoral bone injury through PAI-1 related mechanisms in female mice. (COI: No)

2P-140

Mechanical allodynia caused by peripheral nerve hyperexcitability in adult-onset hypothyroid mice

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Approximately 50% of adult-onset hypothyroid patients suffer from sensory symptoms including pain, which may be caused by peripheral neuropathy. Aδ and C fiber play crucial roles in pain conduction. Their excitability can be altered by voltage-gated ion channel expression. Although thyroid hormone is known to regulate the expression of voltage-gated potassium channel (Kv1.1) in brain, the mechanism behind the pain in hypothyroidism is still unknown. We generated adult-onset hypothyroid mouse model by administrating 50 ppm propylthiouracil (PTU) for 5 weeks. The mechanical allodynia, examined by von Frey test, was observed during PTU exposure and recovered after termination of PTU treatment. Then compound action potentials of sciatic nerves were analyzed. Conduction velocities and thresholds were measured by single stimulation. The latency delay after repetitive stimulation was also measured. No significant changes in conduction velocities or threshold was observed in hypothyroid group. However, latency delay in Aδ and C fibers were less in hypothyroid group at 4th and 5th week of PTU exposure, indicating that conduction block occurrence may decrease in nociceptive fibers. Kv1.1 protein levels decreased significantly in sciatic nerves of hypothyroid group at 4th and 5th week. These results indicate that adult-onset hypothyroidism in mice causes mechanical allodynia due to hyperactivity of peripheral nerves, and reduction of Kv1.1 may be involved in such alteration. (COI: No)

2P-141

Ketogenic diet induces slow-type shift of skeletal muscle in male rat

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Purpose. Ketogenic diet (KD) is high fat diet with extremely low or absence of carbohydrate in the total nutrient. The KD is reported to modulate animal behavior and brain function, thus utilize for treatment of drug-resistant epilepsy, whereas its influence on the peripheral tissue is largely unknown. Skeletal muscles comprise about 40 % of total body mass and dietary fat can influence the skeletal muscle function. Therefore, we aimed to clarify whether the KD affects the muscle performance and fiber type composition in rat. **Methods.** Weight-matched male Wistar rats (8-w)were assigned to either control (CON, n = 7) or KD (n = 6) groups. CON and KD animals were fed with control diet (10% protein, 10% fat, 80% carbohydrate) and KD (10% protein, 90% fat), respectively. After a month, the animals were anesthetized, and the soleus muscle was harvested to measure the muscle contractile function. The muscles were then weighted used to analyze myosin heavy chain (MyHC) composition. The blood ketone was also measured. **Results.** Body weight was significantly decreased in KD. Blood ketone concentration in KD was significantly higher than CON. Tetanic tension at 80 Hz in KD was significantly lower than CON without difference in the twitch and fatigue rate. Type I MyHC proportion in KD was significantly higher than CON. Muscle weight did not differ between groups. **Conclusion.** Consuming KD stimulated muscle fiber type transformation from fast to slow without gain of body weight in male rat. (COI: No)

2P-142

Administration of xylooligosaccharides from rice husk delayed the progression of diabetic rat model

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Rice husk (RH) is one of agricultural wastes obtained after rice milling process. Xylooligosaccharides (XOS) is known as prebiotics that can enhance the growth and/or activity of beneficial gut bacteria. Several studies indicate that the composition of gut microbiota may be involved in progression of insulin resistance in diabetes. This study aimed to evaluate the anti-diabetic effect of XOS from RH using a diabetic rat model induced by high-fat diet and streptozotocin injection. Diabetic rats were randomly assigned to receive vehicle (DMC), XOS (DM-XOS), and metformin (DMM), along with high-fat diet for 16 weeks. An additional group of rats fed with normal diet plus vehicle (NDC) and normal diet plus XOS were also included. Blood chemical parameters and lipopolysaccharides (LPS), gut permeability and cecum SCFA were examined at the end of study. Supplementation with XOS successfully decreased not only the fasting plasma glucose, insulin and leptin levels but also LPS level in DM-XOS compared to DMC group. In addition, XOS supplement significantly reversed the changed gut permeability and total SCFAs content, mainly propionate and butyrate in cecal content. This study demonstrates the efficacy of XOS from RH in the prevention of diabetic progression by maintenance of endotoxemia and gut microbiota. The findings reveal the benefits of agricultural waste and also display an opportunity to add its value through the development as functional food, particularly for diabetes. (COI: No)

2P-143

Improvement of organ bath technique as *ex vivo* systems in the insulin secretion assay

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[Purpose] Prevalence of diabetes mellitus has been increasing worldwide. To assess new drugs for diabetes mellitus, it is important to evaluate the insulin secretion by new compounds. For the insulin assay, we reported analysis of insulin secretion from isolated pancreas by the organ bath technique [Exp Anim. 2018, 67: 15-22.]. However, the insulin levels were diminished with assay time when 1 μM Glucagon-like peptide-1 (GLP-1) an inducer of insulin was added to organ bath, speculating breakdown of insulin by pancreatic proteases. In this study, we searched the appropriate condition in the organ bath technique to measure insulin from isolated rat pancreas preparations.
[Methods/Results] Isolated rat pancreas were suspended in organ bath and were incubated in 2.3 mL of Tyrode's solution with or without soybean Trypsin inhibitor (TI), and harvest and replace new solution four times every 20 minutes. Insulin in the solutions were measured by ELISA. In the absence of TI, the insulin levels in the solution gradually decreased. The insulin levels were not diminished with TI. These findings indicated that TI significantly inhibit the activity of the protease trypsin, and thus, the insulin turn-over was not changed in the solution.
[Conclusions]
In this study, we found TI is indispensable in the organ bath technique for the insulin assay, enabling to measure insulin level stably. The organ bath technique could be a useful tool for assay of insulin secretion. (COI: No)

2P-144

Responsiveness of vomeronasal cells to a male-attractant, imorin in the newt, *Cynops pyrrhogaster*

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The male red-bellied newt (*Cynops pyrrhogaster*) approaches the female's cloaca prior to performing courtship behavior, as if he is using some released substance to gauge whether she is sexually receptive. Therefore, we investigated whether such a female sexual attractiveness pheromone exists. Recently we found that a tripeptide with amino acid sequence Ala-Glu-Phe is secreted by the ciliary cells in the epithelium of the proximal portion of the oviduct of sexually developed newts and confirmed that this is the major active substance in water in which sexually developed female newts have been kept. The active substance was designated imorin. Imorin only attracted sexually developed male newts, but not the sexually undeveloped male and sexually developed or undeveloped female newts. The responsiveness of sexually developed males to imorin was confirmed at the cellular level as well: imorin enhanced the EOG response in the vomeronasal (VN) organ and increased in number the VN epithelial cells in which intracellular Ca2+ concentrations were elevated. Such effects of imorin were not conspicuous in the VN cells in the sexually undeveloped male and sexually developed or undeveloped female newts. The responsiveness to imorin was revealed to be hormone- and sex -dependent. (COI: No)

2P-145

Uterine environment regulates nurturing behavior in the offspring with prolactin as a key factor

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The physiological mechanism of nurturing behavior is largely unknown. Here we show that the mother's nurturing behavior is regulated by factors present during her own fetal development. This was studied by using CIN85 knockout ($Cin85^{-/-}$) mice which display a defect in nurturing behavior towards her newborn mice. Our first finding that $Cin85^{-/-}$ born from heterozygous mouse show a normal nurturing behavior implied the uterine environment being the regulator for the next generation. Surprisingly, when WT embryos were transplanted into the fallopian tubes of $Cin85^{-/-}$ mice, they also exhibited inhibited nurturing behavior as adults. Conversely, when $Cin85^{-/-}$ embryos were transplanted into the fallopian tubes of WT mice, the resultant pups exhibited normal nurturing behaviors as adults. Also we found that $Cin85^{-/-}$ mother mice had reduced pituitary hormone prolactin (PRL) secretion as a result of excessive dopamine signaling in the brain. When PRL was administered to $Cin85^{-/-}$ mice during late pregnancy, a higher proportion of the resultant pups exhibited nurturing behaviors as adults. This correlates with our findings that neural circuitry associated with nurturing behaviors was less active in pups born to $Cin85^{-/-}$ mothers, but PRL administration to mothers restored neural activity to normal levels. In conclusion, perinatally secreted maternal PRL affects the expression of nurturing behaviors not only in a mother, but also in her pups when they have reached adulthood. (COI: No)

2P-146

Effect of maternal high-fat diet and exercise during gestation on placental signaling

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Placenta plays important role in balancing maternal nutrient supply and fetal need during gestation. Here, we determined the effects of maternal high-fat (HF) diet and exercise during gestation on placental signaling. **Methods:** pregnant Sprague-Dawley rats were maintained on chow (CH) or HF diet through gestation. On gestation day (GD) 3, dams were given free access to running wheels (RW) or remained sedentary (SD) during gestation, resulting in four groups: CH-SD, CH-RW, HF-SD, HF-RW. **Results:** Maternal HF diet increased dams' adiposity while prenatal exercise reduced adiposity in HF dams. Plasma leptin and insulin levels were increased in HF-SD dams, and prenatal exercise brought these hormone levels back to normal. On GD 20, placentas were collected. Maternal HF diet activated placental ERK, STAT3 and 4EBP1 branch of mTORC1 signaling, prenatal exercise normalized the effects of maternal HF diet on STAT3 and 4EBP1 activation but not ERK. And prenatal exercise down-regulated the rpS6 branch of mTORC1 signaling regardless of maternal diet. **Conclusions:** our data suggest that prenatal exercise improves dams' metabolic phenotype, however, HF diet and exercise during gestation differentially affect placental nutrient- and energy-sensing signaling in this model. (This study is supported by: the National Natural Science Foundation of China (No. 81801459, 81741079, 31300966), the Fundamental Research Funds for the Central Universities, China (No. 1191329825, xjj2017141). (COI: No)

2P-147

Fetal heart rate variability: a biomarker for evolving fetal hypoxic-ischaemic brain injury

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Purpose: Many cases of cerebral palsy after preterm birth are associated with hypoxia-ischaemia (HI) before birth. We assessed the utility of fetal heart rate (FHR) and FHR variability (FHRV) to predict evolving neural injury *in utero*.**Methods:** Preterm fetal sheep (0.7 gestation) were surgically instrumented and underwent sham HI (n=8) or HI (25 min of umbilical cord occlusion) (n=8), with 21 days post-HI monitoring of FHR, FHRV and electroencephalographic (EEG) activity. Very low frequency (VLF), low frequency (LF) and high frequency (HF) FHRV power were calculated. Sample entropy (SampEn) was calculated as the complexity. The laboratory light phase was 06.00-18.00h. Sheep were fed *ad libitum*. **Results:** HI was associated with transient suppression of VLF (0-8h), LF (0-3h) and HF (0-1h) power, followed by return to control and then a striking secondary reduction from ~18-72h. In contrast, SampEN was markedly increased from 0–48h post-HI ($P<0.05$), falling to control values in the last week, with increased nocturnal circadian activity and greater diurnal falls. Intriguingly, the circadian rhythms in VLF, LF and HF became markedly exaggerated after HI, with a significantly greater diurnal fall between 06.00-12.00h ($P<0.05$) > 7days post-HI. The magnitude of the circadian rhythm correlated with the severity of EEG suppression. **Conclusion:** FHRV indices reflect evolving preterm neural injury. Their diurnal patterns may be particularly useful for assessing long-term recovery after HI. (COI: No)

2P-148

Evaluation of spontaneous behaviors on an elevated plus maze using bisphenol A exposure model

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Elevated plus maze test (EPM) using experimental animals is widely used as an evaluation method of anxiety level. That is mainly evaluated by the parameter such as an open-arm staying time. However, general spontaneous behaviors (such as ambulation, rearing, and grooming) on EPM have not been focused much attention. This time, we conducted the EPM and the open-field test (OFT), and the score of the same parameter was compared. Next, using the exposure model of bisphenol A (BPA) which is one of the endocrine-disrupting chemicals, and the impact of BPA on the scores in both tests was evaluated. In males, a significant positive correlation was confirmed in all three parameters between the two tests. In females, three parameters showed a positive correlation, but they did not reach significance. In the BPA exposure model, only the grooming, the result between EPM and OFT had no correlation at all. BPA exposure let the correlation disappear. In the comparison between groups, the effect of BPA exposure in males was seen on the OFT. Increasing of rearing and decreasing of grooming were observed. In females, effects were seen only in EPM. In BPA-exposed rats, ambulation and rearing showed high values, grooming showed low one. The manner of impact on spontaneous behavior by BPA exposure depended on experimental method and gender. It is useful to evaluate by combining EPM and OFT as a new strategy for behavioral testing in the field of toxicology. (COI: No)

2P-149

Genistein and daidzein augments thyroid hormone-mediated dendritogenesis of cerebellar Purkinje cell

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Thyroid hormones (THs) play critical roles in brain development. Our previous study have shown that soybean isoflavones especially genistein and daidzein modulate the TH receptor (TR)-mediated action. However, their effects on TH-mediated brain development have not yet been extensively studied. Thus, the aims of this study are to examine the effects of genistein and daidzein on Purkinje cell dendrite arborization in primary cultures.

Cerebella of P0 C57BL/6J mice were cultured for 17 days. Immunocytochemistry was performed to quantify the Purkinje cells dendrite area and mRNA expression level was examined by quantitative real-time RT-PCR.

We found that genistein and daidzein (10^{-5} M) augmented TH-induced and estradiol-induced Purkinje cell dendrite arborization, which were suppressed by 4-OHT (4-hydroxy tamoxifen). Genistein and daidzein (10^{-5} M) also increased mRNA expression level of TH responsive genes including *Mbp*, *Bdnf*, *Rc3*, *Nt3*, and *Hr*. Moreover, genistein and daidazein also increased mRNA expression level *of Syn1* and *Psd95* that are involved in synaptic plasticity.

These findings indicate that genistein and daidzein augmented TH-mediated Purkinje cell dendrite arborization and synaptic plasticity via genomic action on nuclear TR. As 4-OHT, which does not bind to TR, suppressed such action, indicating that estrogen receptors may be also involved in Purkinje cell development. Further study is required to clarify the mechanism.

Keywords: isoflavone, cerebellum, T4, T3. (COI: No)

2P-150

Positive effects of reduced nocturnal screen light on sleep in bedtime phone users

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Bedtime screen light exposure is one of the environmental factors that may increase the risk of sleep disturbance by suppressing melatonin secretion and increasing alertness level at night. In this study, the effects of reduced screen light, both white light and short-wave light, on melatonin secretion and sleep quality were investigated. Young adults from 18 to 29 years old were recruited into white light group (N=16) and blue-light group (N=8). In two non-consecutive nights, subjects in both groups experienced a control night with smartphone use at 70% of full screen light emission (control white light, CWL), and the other night with 30% of full screen light emission (decreased white light, DWL group) or CWL with blue light blocking goggles on (BL-Blocking group) respectively, between 9-11 pm before sleep. Urine melatonin concentration in next morning was tested. Sleep quality was monitored by Karolinska Sleepiness scale (KSS) questionnaire that were collected before sleep at night (KSS-night) and after waking up in the next morning (KSS-morning). Melatonin concentration and sleep quality in both DWL night and BL-blocking night were significantly increased when compared with those in CWL night. Our results suggest that the lower white light screen emission and blue light blocking goggles can both decrease negative effect of melatonin suppression and promote sleep quality in bedtime phone user. (COI: No)

2P-151

Association of sex and sex hormones with the functional brain network at rest

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Although a number of studies have been reported sex difference of the brain structures and functions, few studies have taken the effects of menstrual cycle and prenatal sex hormones into account. We investigated brain functional connectivity organization using resting-state functional MRI obtainedfrom right-handed healthy subjects (100 males, 100 females). Functional connectivity was calculated by cross-correlation coefficients between each gray matter voxel (6×6×6 mm) pair. Each voxel node was classified into 4 types (global hub, local hub, global node, and local node) based on normalized α-centrality calculation with the two parameters, one corresponds to degree centrality and the other corresponds to eigenvector centrality. The percentage of global hubs in the frontal and temporal regions for the *mean* male brain was significantly larger than that for the *mean* female brain, and the value in the occipital and cerebellar regions for males was significantly lower than that for females. Further, we found the significant effects of prenatal sex hormones estimated by the right hand 2D:4D digit ratio on the brain network organization for both males and females. For females, the effects of menstrual cycle on the brain network organization were larger for the low digit ratio group than that for the high digit ratio group. Further, the sex difference could not be due to the female brain network variability related to menstrual cycle. (COI: No)

2P-152

The relationships between embryogenic outcome and membrane potential of mouse ova

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Morphological inspection is the most commonly used technique to pick quality oocytes and embryos for artificial fertilization. To raise reproductive ratio, a new selection method from new point of view is needed. The membrane potential reflects expression of ion channels and completeness of cell membrane, it may evaluate oocyte quality. We previously showed that there was wide dispersion of membrane potential among eggs without morphological difference, using GII and 4-cell stage eggs. It implied this technique could be applied for quality selection.
In this study, we analyzed the relationships between embryogenic outcome and membrane potential of mouse ova. Oocytes were collected from hyperovulated 4-week old female mice. After insemination, single and 2-cell phase embryos were used to measure membrane potential. And we measured membrane potential with voltage-sensitive fluorescent dye after direct recording using single electrode. Some of embryos performed positive membrane potential and good morphological characteristics, could reach blastocysts.But most of embryos which performed near zero voltage stopped development. The near zero voltage embryos are possible to be scratched during conventional protocol. This method may be applicable to ignore damaged embryos. And the effect of mechanical damage on membrane potential will be discussed. All animal experiments were planned toward institutional guidelines and reviewed by institutional animal care and use committee. (COI: No)

2P-153

Regulation of hyperactivation by interactions among oviductal hormones in hamster sperm

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In mammals, only capacitated sperm are able to bind to an oocyte. During capacitation, sperm exhibits two quality changes such as hyperactivation and acrosome reaction. Hyperactivation is changes of motility to swim in oviductal fluids and to pass through zona pellucida. Acrosome reaction is an exocytosis to release enzymes which digest zona pellucida and cumulus cell layers. Additionally, systems to bind to an oocyte are opened by acrosome reaction. Recently, it has been demonstrated that some oviductal hormones regulate sperm capacitation. In the present study, I show that hamster sperm hyperactivation are regulated by interactions among some oviductal hormones. Progesterone, serotonin and melatonin enhanced hyperactivation through each specific receptor. Moreover, progesterone and serotonin stimulate Ca signals associated with PLC/IP_3 receptor/soluble adenylate cyclase and cAMP signals associated with transmembrane adenylate cyclase. Effects of progesterone and melatonin on hyperactivation are suppressed by estradiol via estrogen receptor. In addition, effects of progesterone and serotonin on hyperactivation are suppressed by GABA via $GABA_A$ receptor. Generally, progesterone, serotonin and melatonin increase after ovulation although estradiol and GABA increase before ovulation. Therefore, these results show that hamster sperm hyperactivation are regulated by interactions among progesterone, serotonin, melatonin, estradiol and GABA. (COI: Properly Declared)

2P-154

Proteomics analysis of whole testis in cordycepin treatment in streptozotocin-induced diabetic mice

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Diabetes mellitus is a metabolic disorder that contributes to multi-organ complication including male reproductive function. The aim of this study was to identify differential proteins expressed in testis of streptozotocin-induced diabetic mice after cordycepin treatement. Twenty-four mice were randomly divided into four groups; group 1, normal control mice; group 2, normal mice treated with cordycepin (24 mg/kg); group 3, diabetic control mice and group 4, diabetic mice treated with cordycepin (24 mg/kg). All animals were treated for 14 consecutive days. Total proteins were extracted from whole testis tissue and analyzed by LC-MS/MS. A total of 366 proteins were differentially expressed between groups. The 67 proteins uniquely found in cordycepin treated diabetic mice played role in metabolism, membrane transport, organismal system and human diseases. However, 5 proteins uniquely detected cordycepin treated normal mice were involved in genetic information processing and cellular process. The finding of the present study provides a rich resource for further studies and helps in better understanding mechanisms of cordycepin in testis of normal and diabetic mice. (COI: No)

2P-155(Y-16)

Overexpression of Anthrax toxin receptor 2 (ANTXR2) promotes early development of endometriosis

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Endometriosis is one of the most common gynecological diseases in women of reproductive age that reduces life quality and fertility of patients. One of critical steps for endometriosis development is that retrograded endometrial tissues need to adhere to peritoneal cavity. However, the underlying mechanism is still unclear. Herein, bioinformatics analysis and our bench works have revealed that Anthrax toxin receptor 2 (ANTXR2) was overexpressed in ectopic endometriotic tissues. Since extracellular matrix is the endogenous ligand for ANTXR2, we hypothesize that ANTXR2 might play a role in cell adhesion. Indeed, functional assay has shown that loss of ANTXR2 reduced cell adhesive ability in ectopic stromal cells. Furthermore, hypoxia-increasing cell adhesive ability in eutopic stromal cell was mediated by ANTXR2. Due to signaling pathway induced by ANTXR2 is still a mystery, our findings have shown that ANTXR2 inhibited Hippo pathway and resulted in YAP1 activation. In addition, inhibition of YAP1 function reduced cell adhesive ability in ectopic stromal cell. More importantly, pharmacological blocking of ANTXR2 has demonstrated to block cell adhesion, inhibit YAP1 downstream target gene expressions and reduce endometriotic lesion formation in the animal model of endometriosis. Taken together, our findings suggest that ANTXR2 may contribute to pathogenesis of endometriosis via regulation of adhesive process and block its function may have clinical application in the future. (COI: No)

2P-156

Promoting follicle development by inducing ovarian angiogenesis

Kouji Komatsu; Satoru Masubuchi (*Department of Physiology, Aichi Medical University, Japan*)

Ovarian follicle development is coordinated by multiple factors, which sustain periodic ovulation. After the secondary follicular stage, follicle development is mainly regulated by gonadotropins. However, primordial and primary follicle development operates independently of gonadotropins, and the regulatory mechanisms that govern these stages remain poorly understood. To elucidate these regulatory mechanisms, we developed mouse ovarian tissue culture and live imaging analysis methods using a transgenic mouse model containing the transgenes *oogenesin1 (Oog1) pro3.9*. *Oog1* is an oocyte-specific gene in the ovaries; it is expressed after the start of meiosis. *Oog1pro3.9* mice contain a transgene that connects the *Oog1* promoter to the green fluorescein protein AcGFP1. First, we developed a method for live imaging analysis wherein we used changes in the AcGFP1 signal to observe primordial follicle activation under culture conditions. As a result, we confirmed that the activation of dormant primordial follicles depended on the concentration of fetal bovine serum in the culture medium. Next, we induced ovarian angiogenesis *in vivo* by transplanting a biodegradable hydrogel containing recombinant vascular endothelial growth factor (VEGF). Our results indicated that the number of activated primordial follicles increased at 5 days post-transplantation. These results show that angiogenesis in the ovary might be a key event for activating dormant primordial follicle development. (COI: Properly Declared)

2P-157

Repression of COUP-TFII by proinflammatory cytokines contributes to endometriotic lymphangiogenesis

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Endometriosis is a common gynecological disease that affects 8-10% women of reproductive age. It is characterized as the presence of endometriotic lesions outside the uterine cavity and often causes symptoms in patients such as pelvic pains, dyspareunia, and even infertility. Greater angiogenic and lymphangiogenic processes have been found in ectopic lesions. However, the underlying mechanism is still uncharacterized. In present study, we found VEGF-C is highly secreted by endometriotic stromal cells. Elevation of VEGF-C in endometriotic stromal cells is mediated by derepression of chicken ovalbumin upstream promoter- transcription factor II (COUP-TFII) dependent transcriptional regulation. Further investigation reveals that level of COUP-TFII is suppressed by proinflammatory cytokines. Additionally, we also demonstrated that functional VEGF-C can be transported by exosomes, a 30-100 nm-size extracellular vesicles derived from the endosomal compartments, to enhance the tube formation abilities of lymphatic endothelial cells (LECs). Blockage of VEGF-C signaling by a highly selective inhibitor for VEGFR-2/3, Lenvatinib, abolished loss of COUP-TFII-mediated lymphangiogenesis in endometriosis both in *in vitro* and *in vivo* models. Herein, we have unveiled the novel mechanism of VEGF-C transportation by extracellular vesicles to communicate between endometriotic cells and LECs and demonstrate the regulatory status of VEGF-C involved in the pathophysiology of endometriosis. (COI: Properly Declared)

2P-158

Effects of exposure to mild hyperbaric oxygen on the outcome of infertility treatment

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Purpose: Low metabolism in the uterus and/or ovary may be one of the factors responsible for infertility because it reduces the ability of fertilized eggs to remain in the uterus. Exposure to mild hyperbaric oxygen has a possibility to enhance oxygen supply to cells and tissues, thus improving metabolism. This study aimed to examine whether exposure to mild hyperbaric oxygen improves the outcome of infertility treatment. Methods: Thirty-seven women(aged 39,3 ±3.7 years) with intractable infertility who had previously received over five embryo transfers with low pregnancy rate(4.9%) and without birth underwent some cycles of mild hyperbaric oxygen at 1266 hPa with 36% oxygen before receiving an additional embryo transfer. Results: Thirteen women achieved clinical pregnancy with the rate of 13.8 %. Five women gave birth after *in vitro* fertilization treatment Two women achieved natural conception and gave birth. One woman had an extra-uterine pregnancy, and five women miscarried. Conclusions: Exposure to mild hyperbaric oxygen is effective for improved outcomes of infertility treatment. The results suggest a possibility of low metabolism due to insufficiency of oxygen utilization in female reproductive organs as a cause of human infertility. We need to study more intensively the mechanism of blood circulation of the female reproductive organs. (COI: No)

2P-159

Insufficient in utero prolactin exposure causes impaired maternal behavior in the offspring

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Maternal behavior is important for the preservation of a species especially in mammals. However, our knowledge to these behaviors is insufficient. Recently, by using CIN85 (Cbl-interacting protein of 85kDa) knockout mouse, we have reported about the possibility of prolactin signaling during fetal development as one of the factors determining the expression of maternal behaviors after maturation. In this study, plasma prolactin (PRL) concentration of the wild type mother mice during pregnancy is first investigated. At late pregnancy, PRL concentration is constantly at a basal level and elevated significantly from day 19 of the pregnancy until parturition. To reduce exposure of PRL to their fetuses, wild type mouse was injected subcutaneously with bromocriptine during late pregnancy. Mice born to bromocriptine-injected mothers matured and produced their own pups, however, the survival rate of their pups is lower compare to the mice born to saline-injected mothers as a control. These results suggest that prenatal maternal PRL secretion is very important in determining the expression of maternal behaviors in the next generation. (COI: No)

2P-160

Dominantly expressed Serpina3n suppresses the phenotypes of osteoblasts of female mice

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[Objectives] It is well known that sex differences exist about the severity of osteoporosis and bone metabolism. However, the clinical evidence suggest that factors other than sex hormones might be related to sex differences of bone metabolism. We therefore performed the comparative gene expression analyses between female and male osteoblasts.

[Methods & Results] We identified Serpina3n, a novel serine protease inhibitor, as the gene whose expression was the highest ratio of female to male. A reduction in endogenous levels of Serpina3n by siRNA significantly enhanced the mRNA levels of osteoblastic genes in both male and female osteoblasts. Moreover, Serpina3n overexpression significantly suppressed the mRNA levels of those genes in MC3T3-E1 cells. Serpina3n overexpression did not affect Osterix, ALP and osteocalcin mRNA levels enhanced by bone morphogenetic protein (BMP)-2 in ST2 cells, adipogenic differentiation in ST2 and 3T3-L1 cells, receptor activator of nuclear factor-k B ligand (RANKL)-induced osteoclast formation in RAW264.7 cells, although it significantly suppressed mineralization in ST2 cells differentiated into osteoblasts.**[Conclusions]** In conclusion, we first found Serpina3n as the most female osteoblast-dominant gene. Serpina3n exerts a suppression of the osteoblast phenotypes in differentiated osteoblasts, which might partly explain sex differences of the osteoblast phenotypes in mice. (COI: No)

2P-161

The role of CTCF in the mammalian cochlea

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The inner ear is essential for hearing and balance. Its formation is dependent on tight regulation of spatiotemporal expression of genes. Epigenetic factors play crucial roles in regulating various organs development. However, the roles of higher-order chromatin organization and its regulators in early otic development, hair cell differentiation, and its maintenance are not known. CCTC-binding factor (CTCF) is a highly conserved 11-zinc finger protein which regulates the three-dimensional architecture of chromatin and is known to involve in various gene regulation processes. To determine the roles of CTCF in inner ear development and hearing function, we analyzed Ctcf conditional knockout mice (Pax2-Cre; Ctcffl/fl, Gfi1-Cre; Ctcffl/fl). In Pax2-Cre; Ctcflox/lox mice, oticneurogenesis is severely malformed due to the loss of Neurog1 expression. This loss of Neurog1 expression by Ctcfknockdown demonstrates its association with changes in histone modification. In Gfi1-Cre;Ctcflox/lox, hair cell differentiation remains unaffected. After three weeks of birth, deficiency of Ctcf resulted in the degeneration of stereociliarybundles and hair cells leading to a severe hearing loss. These results suggest the dual roles of CTCF in otic neurogenesis and hearing function by modulating histone modification in the Neurg1 locus and maintenance of hair cells in the mouse cochlea. (COI: Properly Declared)

2P-162

Electric axon guidance in embryonic retina: Regulation of integrin activities by extracellular Ca^{2+}

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Growing axons are directed not only by chemical signals but also by electric fields in a process known as galvanotropism. During embryonic development, retinal ganglion cell (RGC) axons are directed by endogenous positive direct current (DC) potentials generated by neuroepithelial cells' sodium transport (Yamashita, ***BBRC***, 2013). However, there is no experimental evidence for the cell surface molecule that is responsible for the electric axon guidance. Here I show that regulation of integrin activities by the extracellular Ca^{2+} plays a pivotal role in electric axon orientation. Retinal strips of chick embryos were cultured in the electric field of the same strength as that *in vivo* (15 mV/mm). They were embedded in Matrigel®, since Matrigel® and the inner limiting membrane, on which RGC axons extend expressing integrin a6b1, contain the extracellular matrix proteins, laminin and collagen, to which integrins bind. RGC axons extended towards the cathode, and monoclonal anti-chicken integrin b1 antibodies TASC and W1B10 significantly enhanced the cathodal growth in a dose-dependent manner. Since integrin b1 subunit contains a Ca^{2+}-dependent negative regulatory site and Mn^{2+} occupies this site to activate integrin, retinal strips were cultured in the presence of Mn^{2+} and it abolished the electric effect. These results suggested that binding of the extracellular Ca^{2+} to the negative regulatory site of integrin b1 subunit regulates integrin activities to direct axons. (COI: No)

2P-163

Improvement of motor function induced by skeletal muscle contraction in spinal cord injury rats

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PURPOSE: Treadmill training after spinal cord injury (SCI) has been shown to recover locomotive function with elevation of BDNF in spinal cord in a rat model. However, the contribution of muscle contraction to this observation has not been elucidated. The purpose of this study was to investigate the role of muscle contraction in motor function recovery after SCI, with a focus on BDNF. METHODS: Using a weight drop SCI (T9 level) rat model (Sprague-Dawley), percutaneous electrical muscle stimulation (ES) was applied to both limbs immediately after SCI for 4 weeks. Motor function was assessed by BBB score, inclined plane, and Rotarod. One week post-SCI, TUNEL-positive cells in the spinal cord and BDNF in both the spinal cord and lower limb muscles were assessed. Four weeks post-SCI, the cavity volume of the epicenter and pGAP43 in the spinal cord were measured. The sham, SCI, and SCI+ES groups were compared. RESULTS: The BBB score and inclined plane test improved in SCI+ES compared to SCI, 4 weeks post-SCI. Also, a decrease in the cavity volume and an increase in pGAP43 were observed in SCI+ES. Electrical muscle stimulation decreased the numbers of TUNEL-positive cells in the epicenter and increased the levels of BDNF in the spinal cord and lower limb muscles 1 week post-SCI. CONCLUSIONS: Electrical muscle stimulation improved the motor function of SCI rat model. The muscle contraction induced BDNF was considered to associate to this function recovery. (COI: Properly Declared)

2P-164

TRPV4 is functionally expressed in cultured mouse Schwann cells

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Schwann cells (SCs) are the primary glial cells in peripheral nerves system. Sensing and detecting the changes in the external microenvironment is important for retaining the SCs' phenotypic plasticity. TRP channels were reported as sensors for temperature, osmotic pressure, volume, stretch, and vibration, and they are expressed and activated throughout the body. However, there are few reports about TRP channels in SCs.

SCs were isolated from sciatic nerves (SCN) from adult mice, and physiological analyse were performed. Intracellular calcium concentrations were increased by the TRPV4 activator GSK1016790A, suggesting that TRPV4 is functionally expressed in SCs in adult mice. However, we didn't find any difference in the expression of MAG, P0, MBP proteins, which are the main myelin structure proteins, between WT and TRPV4-KO adult mice. SCN transection- or crush-induced Wallerian degeneration was evaluated by western bloting and immunostaining in WT mice and TRPV4-KO mice. The amount of P0 protein was larger in TRPV4-KO mice after SCN transection or crush injury. However, there was no significant difference in the expression of neurofilament (NF) and autophagosomal marker LC3-II. Increased expression of P0 protein could affect the transaction or crush injury of SCN. (COI: Properly Declared)

2P-165

Spontaneous network activity in the embryonic CNS analyzed with voltage-sensitive dye recording

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In the developing central nervous system (CNS), spontaneous activity appears well before the brain responds to external sensory inputs. One of the earliest activities exhibited a large-scale correlated wave, which was observed in different species including the chick, rat, and mouse embryo. This spontaneous wave, termed the depolarization wave, showed several outstanding characteristics. (1) The wave was generated by multiple regions rather than a single, fixed pacemaker. The origin of the spontaneous wave changed with development. (2) The wave was highly correlated across a large number of neurons and propagated over a wide region of the CNS (maximally extending to the lumbosacral cord and to the forebrain). (3) The wave was mediated by multiple neurotransmitters and possibly gap junctions, and the primary mediator switched from acetylcholine to glutamate as development proceeded. (4) The wave was expressed during a particular period of development (chick: E4-E8, rat: E13-E16?, mouse: E11-E14). (5) The wave was under homeostatic control, which may act to regulate and maintain the overall excitability of the network. *In ovo* blockade of the wave in the chick embryo demonstrated that the wave plays a significant role in the early process of synaptic network formation. (COI: Properly Declared)

2P-166

Optical analysis of functional development of the mouse vestibular nucleus

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One major challenge in developmental neurobiology is clarifying when and how the brain is functionally organized during embryogenesis. Although such investigations are significant, they have been hampered by the limited conventional electrophysiological means available for immature neurons and assessing spatio-temporal patterns of neural responses. In the present study, a multiple-site optical recording technique with a voltage-sensitive dye was applied to the mouse embryo, and functional organization of the vestibular nucleus was examined. Stimulation of the vestibular nerve in E12-13 mouse brainstems elicited fast and slow optical signals, which corresponded to the action potential and the excitatory postsynaptic potential (EPSP), respectively. The EPSP was mediated by glutamate and was sensitive to extracellular Mg^{2+}, which suppresses the NMDA receptor. In the E13 embryo, the EPSP-related signals were detected from the region extending longitudinally to the levels rostral and caudal to the vestibular ganglion, with high signals concentrated in the caudal region. At E12, the EPSP was lower and generally restricted to the caudal region even when extracellular $Mg^{2+}$was removed to enhance the glutamate receptor function. These results suggest that the developmental sequence of functional synaptic expression is different between the vestibular subnuclei, and that the EPSP initially appears in the caudal vestibular nucleus in the mouse embryo. (COI: Properly Declared)

2P-167

Sexual differentiation of the preoptic area by estrogen-induced cell migration through Rac1 pathway

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The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) is larger in male rats than in females. It seems that incomplete differentiation of this nucleus, as well as the other sexually dimorphic nucleus, causes gender dysphoria. However, the mechanism for the establishment of sexual dimorphism remains largely unknown, except that estrogen aromatized from androgen during perinatal period cause the masculinization of the nucleus. Transgenic rats were generated that express EGFP under the control of an estrogen receptor (ER) alfa gene promoter 0/B, and this EGFP expression was shown as a live, specific marker for the SDN-POA neurons (0/B-SDN). Recently, we have visualized the nucleogenesis of the 0/B-SDN *in vitro* using the organotypic brain slice cultures and time-lapse imaging. These results suggested that scattering neural migration by estrogen was critical role for the masculinization of the SDN-POA. In the present study, we examined whether the actin dynamics, through Rac1 pathway, could be involved in the sexual differentiation of the SDN-POA using this method. 17beta-estradiol in the culture medium masculinized the 0/B-SDN in embryonic brain slice cultures. On the other hand, Rac1 inhibitor prevented the estradiol-induced masculinization of the 0/B-SDN. These results propose the regulation of the neural migration mediated by ERα/Rac1/cofilin/actin pathway is crucial for the establishment of sexual dimorphism of the SDN-POA. (COI: No)

2P-168

Neuronal differentiation induced by vitamin K and generation of derivatives to treat brain diseases

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Purpose: Vitamin K has an important role in blood coagulation and bone formation, but its role in the brain has not been elucidated. We have shown that vitamin K is present in high concentrations in brain and is converted in brain tissue [*Nature* (2010), *JBC* (2013)]. Therefore, we concluded that vitamin K is important in maintaining the function of the cranial nervous system and aimed to elucidate the physiological action of vitamin K in the brain.
Methods and Results : First, we found that vitamin K homologues induced neuronal differentiation from neural progenitors primarily cultured from mouse embryos. Next, we elucidated the neuronal differentiation induction mechanism. Various studies using the L-type Ca^{2+} channel inhibitor verapamil suggested that the L-type Ca^{2+} channel is strongly involved in the vitamin K-dependent mechanism underlying the induction of differentiation into neurons. Structures that strongly induce differentiation into neurons were analyzed using over 40 types of vitamin K derivatives. Hence, we found that both the naphthoquinone ring and isoprenyl side chain structure of vitamin K were important. Furthermore, vitamin K derivatives that had *m*-methylphenyl group introduced of the side chain showed two-fold differentiation activity. [*JMC* (2015, 2017)].
Conclusions: Based on these results, we hope to contribute to research leading to the prevention and treatment of neurodegenerative diseases by clarifying the physiological role of vitamin K in the brain. (COI: No)

2P-169

Intranasal IGF-1 reduced neonatal LPS-induced behavioral deficits and inflammation in juvenile rats

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Aims: This study was to investigate whether intranasal IGF-1 protects against neonatal lipopolysaccharide (LPS)-induced brain neuronal injury and neurobehavioral dysfunction in juvenile rats.
Methods: Recombinant human IGF-1 (rhIGF-1) at a dose of 50 μg/pup was administered intranasally 1 hour following intracerebral injection of LPS (1 mg/kg) in postnatal day 5 (P5) Sprague-Dawley rat pups. Neurobehavioral tests were carried out from P7 to P21, and brain neuronal injury was examined at P21.
Results: Our results showed that intranasal rhIGF-1 treatment attenuated neonatal LPS-induced central catecholaminergic neuronal injury and motor behavioral disturbances, including locomotion, beam walking test and gait analysis in juvenile rats. Intranasal rhIGF-1 administration also attenuated neonatal LPS-induced elevation of IL-1β levels and numbers of activated microglia, and cyclooxygenase-2+ cells, which were double labeled with TH+ cells in the substantia nigra, ventral tegmental area, olfactory bulb and locus coeruleus of the P21 rat brain.
Conclusions: These results suggest that IGF-1 provide a protection against neonatal LPS exposure-induced central catecholaminergic neuronal injury and motor behavioral disturbances, and that the protective effects are associated with the inhibition of microglia activation and the reduction of neuronal oxidative stress by the suppression of the neuronal cyclooxygenase-2 expression. (COI: No)

2P-170

Early exercise inhibits inflammation and promotes neuroprotection in intracerebral hemorrhage rats

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The present study examined the effect of early exercise on brain damage and recovery of motor function following intracerebral hemorrhage (ICH) in rats. Subjects were randomly assigned to no training after ICH (ICH), no training after sham surgery (SHAM), early treadmill exercise after ICH (ICH + ET), and late treadmill exercise after ICH (ICH + LT) groups. The ICH + ET and ICH + LT groups were trained for 7 consecutive days starting on day 2 or day 9 after surgery, respectively. At day 16 post-surgery, the brain was removed, and lesion volume, cortical thickness, neuronal number, dendritic length, and dendritic complexity were analyzed. Expression levels of IL-1b, TGF-b1, and IGF-1 mRNAs in ipsilateral sensorimotor cortex were measured by RT-PCR. Cortical thickness and neuronal number were significantly higher in the ICH + ET group than the ICH and ICH + LT groups. The length and complexity of dendrites were also significantly greater in the ICH + ET group compared to the ICH and ICH + LT groups. Expression of IL-1b mRNA was significantly lower in the ICH + ET group than the ICH group. Collectively, these results suggest that early treadmill exercise after ICH promotes recovery of sensorimotor function by preventing neuronal death and ensuing cortical atrophy, and by preserving dendritic structure compared to late treadmill exercise and no exercise. Early exercise may prevent neurodegeneration and functional loss by inhibiting neuroinflammation. (COI: No)

2P-171

Glial cells missing 1 promote cell differentiation and angiogenesis in the mammalian brain

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Although *glial cell missing* (*gcm*) plays a critical role in the glial cell development in *Drosophila*, the function of *Gcm1*, one of the orthologues of *gcm* in mammals, in the brain remains elusive. Overexpression of *Gcm1* by retrovirus injected in the lateral ventricle was shown to promote the differentiation of neural precursor cells into astrocytes (Iwasaki et al., Development 130: 6027-6035, 2003). However, the function of *Gcm1* in the mammalian brain remains to be investigated because *Gcm1*-deficient mouse embryos are lethal around E9.5 due to the placental dysfunction. In this study, we performed *in utero* electroporation (IUEP) studies to overexpress *Gcm1* together with GFP in neural precursor cells at E14.5 and found that *Gcm1* significantly promoted the GFAP(+) and S100β(+) astrocytic differentiation. Next, we investigated the differentiation into oligodendrocyte lineage cells by immunostaining the *Gcm1* electroporated brains against Olig2, a marker of oligodendrocyte progenitor cells and mature oligodendrocytes. We observed a significant increase in the number of Olig2(+) cells both in GFP(+) and in GFP(-) populations. Furthermore, we also noticed that *Gcm1* overexpression resulted in more angiogenesis. On the other hand, we revealed that the *Gcm1* was increased the expression level in the injury area of the brain. Thus, our results suggest that *Gcm1* plays an important role in cell differentiation, proliferation, and angiogenesis in the mammalian brain development and repair. (COI: No)

2P-172

The effect of forced limb training of rats under photochemically induced focal cerebral ischemia

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The constraint-induced movement therapy is regarded as an effective treatment for impaired upper limb movement after stroke. Moreover, the forced impaired limb use (FLU) task has been found to improve neural function during the reaching task component of the motor skills training (MST) program. The aim of this study was to investigate the effects of the FLU and MST for forelimb function of cerebral ischemic stroke. We evaluated in a model of photochemically induced focal cerebral ischemia.
The rats were divided into three groups; a non-exercise (n = 10) , trained using the single-pellet reaching and FLU tasks (n = 12), and trained using only the FLU task (n = 10). Rehabilitation took place on days 4–10 after the surgery. We assessed motor function using the wire hang test, forelimb-placing test, beam-walking test, and single-pellet reaching test. All tests were performed before surgery and at day 1, 4, 10, 14 after surgery. The animals in all groups were severely paralyzed on day 1 after surgery.
The scores on the wire hang and forelimb-placing test were significantly higher in the FLU+MST and the FLU compared with non-Ex. ($P < 0.05$). Moreover the scores on the single-pellet reaching test were significantly higher in the FLU+MST than FLU and non-Ex. ($P < 0.05$).
These data indicate that the FLU+MST promotes recovery of upper limb function. Particularly, the MST appears to be effective for treating gross motor dysfunction but not fine motor dysfunction. (COI: No)

2P-173

Role of SAD-A kinase in radial neuronal migration during development of cerebral cortex

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SAD kinases are involved in presynaptic vesicle clustering. Mouse homologues SAD-A and SAD-B have been considered to be functionally redundant, because previous study reported *Sada*$^{-/-}$ and *Sadb*$^{-/-}$ double mutant mice showed perinatal lethality due to polarity defects while their single mutants did not show any apparent phenotype. To elucidate the non-redundant role, we generated *Sada*$^{-/-}$ mice and backcrossed them to a C57BL/6N background. These mice died within a few days after birth. The cortical lamination of *Sada*$^{-/-}$ mice showed disorganized pattern. Birth date analysis showed that the percentage of BrdU-positive cells in the superficial layer of *Sada*$^{-/-}$ mice labeled at E14.5 was significantly decreased but that in the middle layer was increased when compared to those in *Sada*$^{+/+}$ mice. In utero electropolation technique using pCAG-EGFP vector confirmed the aberrant radial migration in the mutant brains. In the experiment of SAD-A knockdown, a lot of SAD-A-depleted neurons remained in the lower part of the cortical plate. The neurites of cultured hippocampal neurons in *Sada*$^{-/-}$ mice could differentiate into axons and dendrites, although the average length of their axons was shorter than that of the wild type. In contrast, *Sadb*$^{-/-}$ mice did not show neonatal lethality and their radial migration appears to be normal. These results suggest that SAD-A but not SAD-B regulates radial neuronal migration in the developing brain. (COI: No)

2P-174

Voluntary and forced rehabilitation to promote motor palsy recovery in intracerebral hemorrhage rats

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The psychological factors with rehabilitation such as the motivation promotes motor recovery, but it is still unknown about the mechanism. Previously, we demonstrated that motivational rehabilitation had a greater effect for motor recovery than the forced exercise, however the details of the mechanisms were unclear.The purpose of this study was to clarify the reason of the benefit by the voluntary exercise for the body function recovery in ICH rat. Male SD rats were injected with collagenase into striatum to induce ICH, and divided into three groups: forced treadmill exercise (F-Ex., n=8), voluntary wheel running (V-Ex., n=8) and no exercise (Non-Ex., n=10). Motor functions were assessed by motor deficit score (MDS) and beam walk test (2.4 cm, 1.0 cm wide). The levels of corticosterone and ΔFosB in the nucleus accumbens were compared among three groups in the non-surgery rats because of checking the conditions of stress and motivation through the each exercise. The recovery of the trained groups was prominent than the Non-Ex. group in all tests. Furthermore, there were greater scores in the V-Ex. group than the F-Ex. group (1.0cm beam walk and MDS) ($P<0.05$). The level of corticosterone was higher in the F-Ex. group than the V-ex. group ($P<0.05$). The expression of the ΔFosB protein tended to be higher in the V-Ex. group.These data suggested that the voluntary exercise promotes the recovery of the motor function by the lower stress and high motivation. (COI: Properly Declared)

2P-175

Alteration of gut microbiota and cerebellar structures in Glyphosate-exposure rat

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Glyphosate (GP) is the most widely used herbicide in the world. Although scientific institutions worldwide have concluded that there is no indication of any human health concern, recent studies have shown that GP exposure in prenatal is associated with neurodevelopmental disorders like autism spectrum disorder, ASD. In this study, we investigated the neurodevelopmental alteration in GP-exposed rat pups and the alteration in gut microbiota in GP-administrated pregnant rats. GP was administrated 250 mg/kg to gestational day 15, G15. The stools from the rats were collected in G6, 13, 17, 20. The samples were prepared and purified by using QIAmp DNA Stool Mini Kit. Purified DNA was then subjected to 16S rRNA sequencing using MiSeq (Illumina), and the data were then analyzed using a DADA2 package as described by Callahan et al., 2016. The cerebellums of the rat pups stained with the immunofluorescent antibody were observed using the laser confocal microscopy. The result reveals that the gut microbiota in pregnant G20 rats is different in between the control and the GP-exposed rat. The ratio of the phylum *Bacteroidetes* to the phylum *Firmicutes* of the GP-exposed rats was significantly more increased than the control ones. Moreover, the Purkinje cells of the cerebellum of GP-exposed rat pups at P14 showed decreased to the control. We suggest that prenatal glyphosate-exposure would change the gut microbiota and affect the cerebellar structures of offspring. (COI: No)

2P-176

Analysis of rat fetal movement before and after anesthetic drug using non-anesthesia pregnant rat

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Body movement analysis of human fetus has been studying using ultrasonic tomographic imaging. However, that of rat fetus has not been much studying and there was no data of rat fetal movement. In the previous study, we created the equipment for analysis of rat fetal movement using the ultrasonic tomographic imaging in the non-anesthesia pregnant rat. So, we investigate the comparison of rat fetal movement and human baby movement. We observed the fetal movement of the total observation time (10 minutes) by using ultrasonic tomographic imaging system (Honda electrical Co, HS-1600V) in the embryonic day15 (E15), E17, E19, E21 of Wistar rat fetus. We recognized two kinds of fetal movements in the late embryonic stage. One is a body movement, and the other is a twitching movement like a reflex. The total body movement time of the total observation time was 2.9 sec (E15), 231 sec (E17), 100 sec (E19) and 47 sec (E21). The twitching movement first appeared E15 (0.05 sec), and the twitching movement was 2.6 sec (E17), 7.8 sec (E19) and 13.2 sec (E21). The body movement was depressed by anesthetic drug application (GABA + adrenaline blocker + kappa-receptor agonist), but the twitching movement was partially blocked by the anesthetic drug. These results suggested that 1) the peak value of body movement was E17 and the peak value of twitching movement was E21 in late embryonic period; 2) the body movement was more dependent on the inhibitory network than the twitching movement. (COI: No)

2P-177

Altered gut flora and cerebellar development abnormalities in VPA rat model of ASD

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Recent studies have shown that the gut-brain axis has a critical role in elucidating autism spectrum disorder (ASD). In this research, we investigate the relationship in between gut flora of pregnant dam and the cerebellar development of the pups using valproate (VPA) to induce ASD. We prepared ASD model rat by administrating 400~600 mg/kg of valproate into the pregnant dam on gestational day 15, G15. The stool samples were collected throughout the pregnancy and purified by QIAmp DNA Stool Mini Kit, then subjected to 16S rRNA sequencing using MiSeq (Illumina). Data analysis was done using R console with DADA2 pipeline as described by Callahan et al., 2016. In addition, cerebellar slices of infantile pups (post-natal day 3, P3 ~ P14) were prepared accordingly to investigate cerebellar development abnormalities in pups. The result reveals that the ratio of the phylum *Bacteroidetes* and *Firmicutes* is different in vehicle and VPA-dam. An increase in the phylum *Firmicutes* in VPA-dam suggests that inflammation occurs in their gastrointestinal system. Furthermore, we observed defects in Purkinje cells and cerebellar cortex hyperplasia. Moreover, an increase in microglial, confirmed with Iba 1 immunostaining, that responds to inflammation implies that proinflammatory cytokines interfere with the cerebellar development. This reveals an interesting insight that perhaps VPA induces ASD through inflammation by disrupted gut flora. (COI: No)

2P-178

Histological analysis of peripheral nerve injury in methylmercury-exposed rat

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Methylmercury (MeHg) is known as the causal substrate of Minamata disease, which induces central and peripheral nerve injury. MeHg induced neural degeneration have been investigated mainly by using brain, however, MeHg induced neural injury in peripheral nervous system (PNS) is not well investigated. To clarify the aspects of neural injury in PNS induced by MeHg, 9 weeks Wistar male rats were exposed by MeHgCl solution orally for 5 days and not exposed for subsequent 2 days (Day 7). This cycle was continued to the other week again (Day 14). In day 7 and 14, rats were fixed and their DRG, motor and sensory fibers were cryosectioned. Co-staining of axonal marker NF and myelin marker MBP showed axonal degeneration in sensory, but not in motor fiber in Day 14. The neurons in DRG also degenerated in Day 14. DRG has many different subtypes of neurons (sense pain, pressure, itch, etc.) so that neurons were classified by different markers such as NF, PLXNC1, TrkA and TH, however, these classified staining did not show any differences of degeneration between each subtypes. We also analyzed another cell type such as microglia (Iba1), macrophage (CD68), astrocyte (GFAP), fibroblast (Vimentin), epithelial cell (CD31) and Schwann cell (SOX10). Microglia, macrophage and Schwann cell were significantly increased. These results suggest that MeHg affects peripheral neural degeneration in DRG and axonal degeneration in sensory fiber but not in motor fiber with concerting several cells. (COI: No)

2P-179

The role of Cdon in differentiation of mouse embryonic stem cells into motor neurons

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In-vitro neuronal differentiation of pluripotent stem cells has become a widely used tool in disease modeling and prospective regenerative medicine. Thus it is important to define molecular mechanisms underlying motor neurons (MNs) specification and maturation. Cdon enhances Shh signaling activity which is crucial for MNs specification. However, the exact role of Cdon in MNs differentiation is still unclear. Here we demonstrated Cdon' function in MNs differentiation by using *in vitro* differentiation system. When the generation of neurons was induced in response to Shh, Cdon-deficient embryonic stem cells (ESCs) exhibit impaired expression of markers for MNs specification while markers for dorsal interneuron specification are significantly increased, compared to WT ESCs. In contrast, reactivation of Shh signaling by a Shh agonist SAG restored fully the expression of specification markers in Cdon-deficient ESCs. To examine the functional maturity of MNs, the electrophysiological properties of neurons derived from WT and Cdon-deficient ESCs were analyzed using patch-clamp technique. Neurons derived from Cdon-deficient ESCs can fire single action potentials (APs) to depolarizing currents, but are unable to elicit repetitive trains of APs, unlike neurons derived from WT ESCs. Electrophysiological properties in these cells were partially recovered by exogenous SAG treatment. Taken together, these data suggest that Cdon plays a critical role in generating functional motor neurons. (COI: No)

2P-180

PlexinA1 is crucial for the midline crossing of callosal axons during corpus callosum development

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In the mouse cortical midline during the development of corpus callosum (CC), the guidepost structures express axon guidance molecules to instruct neurons about the proper direction of axonal elongation. Neuropilin 1 (Npn1), a high affinity receptor for class 3 semaphorins, localized on the cingulate pioneers has a crucial role in the midline crossing through the interactions with semaphorins like Sema3C. However, it remains unproved which type of Plexin acts as a component of Npn1-containing receptor complex. To examine if PlexinA1 is involved in CC development, CC phenotype was examined in PlexinA1-deficient BALB/c mice at P0.5. All of the PlexinA1-deficient mice showed partial or complete agenesis of CC. Our immunohistochemistry (IHC) revealed the expression of PlexinA1 on the dorsal side of callosal axons crossing the cortical midline at E17.5. The IHC confirmed the specific localization of Sema3C in the guideposts such as subcallosal sling. To examine the role of PlexinA1 in the midline crossing, the extension of cingulate axons across the midline was traced by both IHC of Npn1 and DiI anterograde axonal tracing. Both methods showed that the incidence in midline crossing of cingulate axons at E17.5 was significantly lower in PlexinA1-deficient brain compared with WT. The axon guidance assay with cingulate cortical explants indicated that PlexinA1 acts as a functional receptor mediating both the repulsive activity of Sema3A and the attractive activity of Sema3C. (COI: No)

2P-181

The maintenance of adult neural stem cells by *Klf5* gene

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Klf5 is one of the Krüppel-like factor (Klf) family, which is an ortholog of the Drosophila melanogaster gene Krüppel. Our previous data suggested that the shortening of the cell cycle length by overexpressing *Klf5* gene promotes the proliferation of neural precursor cells (NPCs) in the mouse brain development.

Embryonic neural stem cells (NSCs) increase the number of themselves vigorously, provide neurons at midgestation, generate glial cells at postnatal stage and eventually, the part of embryonic NSCs provide adult NSCs. Adult NSCs, which show the self-renewal and multipotent capabilities, reside quiescent throughout life. The NSCs and neurogenesis system in the adult stage was involved in the learning and the emotional disorder. Considering our data, we hypothesized that the regulation of NSCs' self-renewing by the expression of *Klf5* gene alters the memory and the emotion. In addition, it is reported that the protein expression levels of *Klf5* gene was decreased in the postmortem brain of the schizophrenia patient. We evaluate higher brain function, mental states and behavior of mice by using behavioral test battery. Our data suggests that overexpressing *Klf5* gene modifies behavior and memory. (COI: No)

2P-182

Upregulation of heat shock factor and Factor XIII-A after optic nerve injury in zebrafish

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Purpose: Factor XIII A-subunit (FXIII-A) was originally identified as a plasma transglutaminase that promotes fibrin stabilization. In addition, cellular FXIII, homodimer of FXIII-A (FXIII-A2), upregulates in the optic nerve and retina after nerve lesion in zebrafish. Here, we focused on the mechanism of FXIII-A activation and heat shock factor (HSF) involved in optic nerve regeneration using zebrafish nerve injury models. **Methods**: Changes of mRNA levels of HSF, FXIII-A and other regeneration associate molecules were analyzed using damaged optic nerve and retina by quantitative real-time PCR. Plasma FXIII activator, thrombin expression in damaged optic nerve and retina were analyzed by RT-PCR. Sequences of FXIII-A clones derived from intact or injured retina were analysed using 5'RACE PCR products. **Results:** The expression of HSF and FXIII-A started to increase in zebrafish retina and optic nerve within a few hours after optic nerve injury. However, thrombin mRNA was undetectable. Sequence analysis of 5'RACE PCR products showed that most of clones derived from intact retina showed full sequence of FXIII-A. However, many clones derived from injured retina showed shorter sequences of FXIII-A which lacked the coding region of activation peptides but HSF binding motif. **Conclusions**: These results suggest that the expression of heat shock factor might be involved in the direct expression of activated FXIII-A protein in zebrafish retina and optic nerve after nerve injury. (COI: Properly Declared)

2P-183

Oligodendrocyte progenitor cells during development and upon sensory loss in mouse visual cortex

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Oligodendrocyte progenitor cells (OPCs) are widely distributed as the principal proliferative cell type in the postnatal cortex, and continue to proliferate and produce newly myelinating cells throughout the lifetime. It is unclear how OPCs self-renew or differentiate into oligodendrocytes (OLs) and how sensory deprivation affects cell proliferation and differentiation. Using immunofluorescence methods and confocal microscopic analyses, we examined developmental changes in the distribution of OPCs and the effects of binocular enucleation (BE) in primary visual cortex (V1). Proliferative cells increased from postnatal day (P) 22 to P25 (i.e., near the onset of the critical period for ocular dominance plasticity, ODP). BE at P15 notably increased proliferated OPCs under cell cycle in lower layer 6 at P25. At P30, the most proliferated cells exited the cell cycle, where BE increased the number of dormant cells without affecting that of differentiated cells. This increase in dormant cells was mediated by sonic hedgehog (Shh). Many of the proliferated OPCs differentiated into mature OLs at P50. At this age, CNPase-immunopositive OL somas were slightly shifted toward the white matter in BE mice. Overall, these results suggest that V1 has a sensitive period of OPC cell cycle phase around the ODP critical period, where sensory loss further promoted undifferentiated OPC generation in the deep layer, resulting in an increase in dormant OPCs via Shh and later in mature OL expansion. (COI: No)

2P-184

Enhanced neuronal migration through activated glia promotes post-stroke neuronal regeneration

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New neurons are continuously generated in the ventricular-subventricular zone (V-SVZ) throughout life. After brain injury, the immature new neurons referred to as neuroblasts migrate toward the lesion. However, the ability of the mammalian brain to regenerate neuronal circuits for functional recovery is quite limited. Using a mouse model for ischemic stroke in which the main lesion was in the lateral striatum, we found that neuroblast migration toward the lateral striatum was restricted by insult-activated astrocytes in and around the lesion. Although the neuroblasts used Slit1-Robo2 signaling to disrupt the actin cytoskeleton in the activated astrocytes at the site of contact for their migration toward the lesion, their Slit1 expression level rapidly and progressively decreased as they got further away from the V-SVZ. We next examined whether lentivirus-mediated Slit1 overexpression in neuroblasts could promote their migration toward the lesion. Slit1-overexpressing neuroblasts transplanted into the post-stroke brain could migrate closer to the lesion. These neuroblasts matured into striatal neurons within 4 weeks and efficiently regenerate neuronal circuit, resulting in functional recovery in the post-stroke mice. Therefore, these results suggest that the appropriate positioning of new neurons will be critical for functional neuronal regeneration in stem cell-based therapies for brain injury. (COI: No)

2P-185

Postnatal development of core fields in the mouse auditory cortex

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The rodent is often used as a model for studying the developmental plasticity of auditory cortex. Acoustic exposure in a postnatal period induces alterations in sound representation in core fields of auditory cortex. Although the plasticity is well studied, the postnatal development of the rodent auditory cortex has seldom been the subject of study. In rats, while some studies showed that the size of the auditory cortex decreased over time after reaching a transient peak at P16, one study found monotonic increase in size of the primary auditory cortex (A1) from P11, until reaching the adult size by P13-P14. To address this discrepancy, here we examined the postnatal development of the core area of the mouse auditory cortex (A1 and the anterior auditory field, AAF) from P14, at a macroscopic level, using an imaging technique. We recorded the initial response of A1 and AAF to 4-kHz- and 16-kHz-tones, and measured the distance between the centroids of initial responses to the tones for estimation of the size of A1 and AAF. We found that while A1 size increased from P14 to reach a stable value at about P21, AAF size showed little change. The line connecting the two centroids in a field is taken as the frequency axis of the field; and we found that the angle between frequency axis in A1 and AAF exhibited progressive decrease during postnatal development. However, the low frequency end of A1 and AAF appeared to drift apart. (COI: No)

2P-186

Moduration of CRMP2 Accelerates Motor Function Recovery from Brain Damage

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Brain damage mainly caused by stroke is a severe neurological condition, which may lead to paralysis and compromise work capacity and self-care. No pharmacological intervention that could foster recovery and complement current rehabilitation has yet been established as effective. Restoration of motor impairment after brain damage is considered to be the result of compensative neural plasticity in intact brain regions, mediated by the reorganization of cortical motor maps. Experience-dependent synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid) receptor (AMPAR) delivery underlies behaviors that require neural plasticity such as learning. We found that a small compound, edonerpic-maleate (also known as T-817MA), facilitated experience-driven synaptic glutamate AMPA receptor delivery and resulted in the acceleration of motor function recovery after motor cortex cryoinjury in mice in a training-dependent manner through cortical reorganization. Edonerpic bound to collapsin-response-mediator-protein 2 (CRMP2), a downstream molecule of semaphorin, and is thought to be related to synaptic plasticity and learning. Edonerpic failed to facilitate experience-driven synaptic glutamate AMPA receptor delivery and augment recovery in CRMP2-deficient mice. Thus, edonerpic-maleate, a neural plasticity enhancer, could be a clinically potent small compound to accelerate rehabilitation after brain damage (Abe, Jitsuki et al., Science 2018). (COI: No)

2P-187

Neurochemical differentiation of hypothalamic MCH neurons derived from mouse embryonic stem cells

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Melanin-concentrating hormone (MCH)-producing neurons are one of the major peptidergic cell groups in the hypothalamus and play an important role in sleep regulation and energy homeostasis. Although numerous studies have characterized histological and physiological properties of the MCH system, the molecular pathways underlying development of MCH neurons are largely unknown. Here we report that a three-dimensional, hypothalamic differentiation culture of mouse embryonic stem cells (mESCs) (Wataya *et al.*, 2008, *PNAS*) can generate MCH-positive neurons, which share common neurochemical properties with native MCH neurons. Immunohistochemical analysis showed that mESC-derived MCH neurons co-expressed a GABAergic marker (GAD67), a glutamatergic marker (VGLUT2), or other neuropeptides (CART and nesfatin-1), all of which are expressed in native MCH neurons. Among these molecules, CART was detected in almost all MCH neurons generated under default culture condition of mESCs, but addition of a sonic hedgehog (Shh) pathway agonist promoted induction of $MCH^{+}/CART^{-}$ neurons. Since half of MCH neurons *in vivo* express CART, our data suggest that this neurochemical heterogeneity of native MCH neurons is attributable to Shh signaling during hypothalamic development. Thus, this study provides the first evidence that mESC culture can recapitulate neurochemical differentiation of MCH neurons *in vivo* and therefore is a useful platform for studying this developmental process. (COI: No)

2P-188

Accelerated climbing fiber synapse elimination in cerebellar Purkinje cells lacking protocadherin 10

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Recent studies suggest that the cerebellum may be involved in the etiology of autism spectrum disorder (ASD). However, it remains unknown whether alteration in cerebellar neural circuit development is related to ASD. In the present study, we focused on an ASD-related gene, protocadherin 10 (*Pcdh10*), which is reported to be expressed in PCs. We investigated whether *Pcdh10* is involved in elimination of redundant climbing fiber (CF) to Purkinje cell (PC) synapses in the developing mouse cerebellum. We generated mice that express Pcdh10-DIO-tdTomato, and crossed them with GluD2-Cre mice to delete Pcdh10 specifically from PCs. The expression of tdTomato in the conditional knockout (cKO) mice confirmed the expression of *Pcdh10* in PCs, which was restricted mainly in a subset of aldolase C (Aldoc)-expressing PCs. We estimated the number of CFs innervating each PC by whole-cell recordings in cerebellar slices at various postnatal days from heterozygous Aldoc-tdTomato mice, heterozygous Pcdh10-cKO mice, or homozygous Pcdh10-cKO mice. We found delayed CF synapse elimination in Aldoc-positive or Pcdh10-positive PCs. By contrast, CF synapse elimination in Pcdh10-cKO PCs was significantly accelerated than that of heterozygous-Aldoc or Pcdh10-positive PCs. Pcdh10-cKO mice had a mild motor deficit and exhibited repetitive behavior in marble burying tests. These results suggest that Pcdh10 partially delays CF synapse elimination, which might be related to abnormal repetitive behavior. (COI: Properly Declared)

2P-189

Vesicular GABA Uptake can be Rate-Limiting for Recovery of IPSCs from Synaptic Depression

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Synaptic efficacy plays crucial roles in neuronal circuit operation and synaptic plasticity. Presynaptic determinants of synaptic efficacy are neurotransmitter content in synaptic vesicles and the number of vesicles undergoing exocytosis at a time. Bursts of presynaptic firings depress synaptic efficacy, mainly due to depletion of releasable vesicles, whereas recovery from strong depression is initiated by endocytic vesicle retrieval followed by refilling of vesicles with neurotransmitter. We washed out presynaptic cytosolic GABA to induce a rundown of IPSCs at cerebellar inhibitory cell pairs in slices from rats, then allowed fast recovery by elevating GABA concentration using photo-uncaging. The time course of this recovery coincided with that of IPSCs from activity-dependent depression induced by a train of high-frequency stimulation. We conclude that vesicular GABA uptake can be a limiting step for the recovery of inhibitory neurotransmission from synaptic depression. (COI: No)

2P-190

M1 receptor-mediated presynaptic inhibition of IPSCs in basal forebrain cholinergic neurons

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A whole-cell patch-clamp study was carried out, using brain slices obtained from young (P12-20) rats, to elucidate cholinergic modulation of excitatory transmission onto cholinergic neurons in the basal forebrain (BF). Cholinergic neurons within BF were identified with Cy3-192IgG. Excitatory postsynaptic currents (EPSCs), mediated by non-NMDA glutamate receptors, were evoked by focal electrical stimulation. Bath application of carbachol (CCh), a muscarinic acetylcholine receptor agonist, inhibited the EPSCs in a concentration-dependent manner between 1-30 μM. CCh-induced inhibition of EPSCs was antagonized by pirenzepine (1 μM), a M1 muscarinic receptor antagonist, whereas MT-7 (100 nM), another muscarinic receptor antagonist that blocks cell surface M1 receptors, had little or no effect on the CCh-induced inhibition. CCh increased paired pulse ratio. CCh-induced inhibition of EPSCs was significantly smaller in the presence of ω- conotoxin GVIA (3 μM) than that without ω- conotoxin GVIA, an N-type calcium channel blocker, whereas CCh still inhibited EPSCs in the presence of ω-agatoxin TK (200 nM), a P/Q-type calcium channel blocker. These findings suggest that activation of presynaptic M1 receptors, mainly localized intracellularly, selectively block N-type calcium channels, thereby inhibiting glutamate release onto BF cholinergic neurons. (COI: No)

2P-191

Construction Rules of the Axospinous Synapses Revealed by FIB-SEM Imaging

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Axospinous synapses are excitatory synaptic contacts established on dendritic spines of postsynaptic neurons. These synapses are found at specific synaptic connections in specific brain regions and highly variable in ultrastructural features even among synapses of the same connection. The synapse normally consists of mitochondria, synaptic vesicles and an active zone in a presynaptic axonal varicosity and a postsynaptic density and smooth endoplasmic reticula (spine apparatuses) in a spine. However, commonality and uniqueness in these structural features among and across synaptic connections are remain unclear.

Here, by focused ion beam-scanning electron microscopy (FIB-SEM), we quantitatively examined morphological features of multiple axospinous synapse connections in dentate gyrus and hippocampus; dentate gyrus middle molecular layer synapses, CA1 stratum lacunosum synapses and CA1 stratum radiatum synapses. The all three synaptic populations showed statistically significant positive correlation between the volume of presynaptic varicosity (pre) and the number of synaptic vesicles (sv), the volume of pre and the volume of spine (sp) and the volume of pre and the volume of sp. Interestingly, slope values and/or y-intercepts of the regression lines are often unique in individual synaptic populations. These results demonstrate commonalities and uniqueness in the construction rules of the individual axospinous synapses among and across synaptic connections. (COI: No)

2P-192

Analysis of the central circadian clock in AVP neuron-specific VGAT deficient mice

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The hypothalamic suprachiasmatic nucleus (SCN) serves as the master circadian clock in the mammals. Recently, we have shown that arginine vasopressin (AVP) producing GABAergic neurons in the dorsomedial SCN have a pacemaking function to determine the length of the circadian cycle. In this study, to investigate a functional role of the GABAergic synaptic output from AVP neurons in controlling the circadian rhythmicity, we have analyzed AVP neuron-specific vesicular GABA transporter-deficient mice (*AVP-Vgat*$^{-/-}$ mice).

First, we found that miniature GABAergic synaptic currents (mGPSCs) with smaller amplitude were more frequently detected in the light phase than in the dark phase from control AVP neurons. However, such a circadian change of the mGPSCs occurrence could not be seen in *AVP-Vgat*$^{-/-}$ mice, suggesting that the smaller mGPSCs derived from AVP neuronal terminals are eliminated in the mutant mice. In constant dark condition, *AVP-Vgat*$^{-/-}$ mice showed marked lengthening more than 5 hours and also a splitting pattern in activity time of behavioral circadian rhythm. However, the circadian oscillations of PER2:LUC luminescence in explants, and Per1 and AVP transcription in vivo were not altered in *AVP-Vgat*$^{-/-}$ mice. This line of observation suggests that weakened neuronal activity coupling in the SCN induces the alteration of behavioral circadian rhythm even though the coupling of clock gene rhythms is not affected. (COI: No)

2P-193

Regulation of reciprocal current in the mouse accessory olfactory bulb by vasopressin V1a receptors

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Central vasopressin (AVP) facilitates social recognition and modulates numerous complex social behaviors in mammals. Recently, vasopressin neurons were reported to exist in the accessory olfactory bulb (AOB). The AOB has been demonstrated to be a critical site for mating-induced mate recognition in female mice. The effect of AVP, however, on the synaptic transmission between dendrites in the AOB of female mice is largely unknown.

To address this issue, we previously measured synaptic currents (IPSCs) from mitral cells in the AOB, We have demonstrated that AVP significantly reduced the IPSCs through AVP V1a receptors. The reciprocal transmission, however, contains both glutamatergic transmission from mitral to granule cells and GABAergic transmission from granule to mitral cells. Thus, it is unclear whether AVP acts on the excitatory and/or the inhibitory transmissions.

In the present study, we have given attention to the effect of V1a receptor activation on GABAergic trasmission. AOB slices were prepared from 23- to 35-day-old Balb/c mice. Using the patch-clamp technique in whole-cell configuration, the current responses of mitral cells were recorded in the presence of antagonists for glutamatergic transmission, CNQX (10 μM) and AP5 (50 μM). An extracellular application of vasopressin did not affect the magnitude of the response of mitral cells to GABA (10 μM and 100 μM), suggesting that AVP modulate the GABAergic transmission not through a postsynaptic mechanism. (COI: No)

2P-194

The activity of metabotropic glutamate receptor affects drebrin localization in dendritic spines

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The group I metabotropic glutamate receptor (mGluR) is an important regulator of function and structure of dendritic spines. Since dendritic spine structure is governed by actin cytoskeleton, mGluR activity may link to dynamics of actin cytoskeleton. However, the intracellular mechanism of mGluR to actin cytoskeleton still remains unknown. We have demonstrated that drebrin an F-actin-binding protein is critical for spine morphogenesis and plasticity. Drebrin has binding motifs for Homer that is scaffolding protein of mGluR. So, we propose that mGluR activity regulates dendritic spine structure through drebrin-Homer interaction. To elucidate this hypothesis, using cultured hippocampal neurons, we have previously examined the relationship between mGluR activity and localization of drebrin and Homer in dendritic spines after mGluR5 agonist CHPG treatment, and reported elsewhere that the number of spines in which drebrin and Homer were co-localized was significantly increased. In this study we examined the effect of CHPG treatment on neuron number, dendritic length and drebrin cluster number using high-content analysis of cultured hippocampal neurons. We found that CHPG increased the density of drebrin cluster in neurons in a dose-dependent manner. Thus, the activity of mGluR5 affects drebrin localization in dendritic spines. We now introduce Homer 1a a dominant negative Homer in neurons to examine if drebrin-Homer interaction is important for the mGluR function. (COI: No)

2P-195

Dopamine induced long-lasting calcium increase in orexin neurons via D_1-like receptor

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Orexin neurons in the hypothalamus have a critical role in the regulation of sleep-wakefulness. Although some extracellular bioactive substances modulating the neurons have been reported so far, most of the studies depended on electrophysiological recordings monitoring for only few minutes. Therefore, the mechanisms of the state change regulation of sleep-wakefulness, which last for seconds to hours, is still elusive.

We previously found dopamine (DA) induced long-lasting intracellular calcium ($[Ca^{2+}]_i$) increase for more than an hour in orexin neurons. Then we further investigated the long-lasting effect, searching receptors involved in using selective antagonists. We made an acute brain slice from transgenic mice expressing Ca^{2+} indicator, Yellow Cameleon-Nano50, exclusively in orexin neurons. We applied an antagonist for 10 min and subsequently applied DA for 2 min, then monitored $[Ca^{2+}]_i$ activity.

As a result, we found dopamine D_1-like receptor antagonist, SCH-23390, suppressed the long-lasting $[Ca^{2+}]_i$ increase. However, SCH did not suppressed the initial transient increase of $[Ca^{2+}]_i$, and adrenaline α_1 receptor antagonist, Prazosin, suppressed it. Taken together, the long-lasting effect of DA in orexin neurons can be achieved by combination of at least 2 types of receptors. Now we are challenging to reveal the physiological role of the long-lasting effect *in vivo*. (COI: NO)

2P-196

Drebrin depletion affects stability of microtubules in dendrites

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Dynamic microtubules have an important role in the maintenance of dendritic spines and inhibition of microtubule growth or depletion of EB3, a microtubule plus-end binding protein, affect spine morphology. Drebrin regulates actin dynamics and has a critical role in synaptic plasticity. Microtubules are known to enter into spines in response to Ca2+ influx through synaptic NMDA receptors (NMDARs) and drebrin which directly binds to EB3 regulates this entry. These facts suggest that microtubule dynamics couple with actin dynamics which is regulated by drebrin. Here, to investigate if drebrin regulates microtubules dynamics in dendrites, we used cultured neurons derived from drebrin knockout (DXKO) mice. Microtubule-associated protein 2 (MAP2) binds to microtubules and modulates microtubule stability. As MAP2 and EB3 binds directly, we first observed MAP2 immunoreactivity using wild-type (WT) neurons and DXKO neurons. We detected less MAP2 positive neurons in DXKO neurons and also found that application of AP-5, an NMDAR antagonist, induced less immunoreactivity of MAP2 in WT neurons. This result suggests low functionality of NMDARs in DXKO neurons. We therefore examined the accumulation and expression level of NMDAR subunits and found that DXKO neurons had different pattern of their accumulation and expression level compared to WT neurons. Taken together, drebrin depletion alters NMDAR function and affects MAP2 immunoreactivity which might affect stability of microtubules. (COI: No)

2P-197

Induction of electrophysiologically-active brain organoids showing human midbrain-specific structure

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Human midbrains have distinct structure from cerebral cortex, and damages to this region give rise to specific diseases, such as Parkinson disease, progressive supranuclear palsy, and corticobasal degeneration, which mechanism still remain unknown. We propose new method to induce brain organoids, from human pluripotent stem cells, which mimics the structure of substantia nigra, the specific structure of midbrains, composed of electrophysiologically active neurons. Our findings will contribute to reveal currently-unknown mechanism under many midbrain-degenerating diseases. (COI: No)

2P-198

C1ql1-Bai3 Signaling Dynamically Modulates Climbing Fiber Synapses in Adult Cerebellum

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Purkinje cells (PCs), which send sole outputs from the cerebellar cortex, receive two excitatory inputs, parallel fibers (PFs) and climbing fibers (CF: axons of inferior olivary neurons). Though multiple CF inputs innervate immature PCs, they are eliminated during neonatal period leaving only one strong CF input. Recently, we found that C1ql1, a C1q-family protein secreted from CF terminals, bound to brain-specific angiogenesis inhibitor 3 (BAI3), which belongs to the adhesion G protein-coupled receptor, expressed in PCs. The C1ql1-BAI3 signaling not only facilitate elimination of loser CFs, but also strengthen a single winner CF (Kakegawa et al., ***Neuron***, 2015). Interestingly, C1ql1 and Bai3 remain expressed in adult cerebellum, suggesting their continuous roles in regulation of CFs. Here, to clarify the function of C1ql1-Bai3 in adult cerebellum, we knocked out or overexpressed Bai3 in PCs, which had already established a 1:1 innervation pattern with winner CFs. We found that winner CF synapses were weakened by knockdown of *Bai3* in adult PCs. Unexpectedly, overexpression of Bai3 induced re-innervation of PCs by surplus CF inputs even in adulthood. These results indicate that appropriate levels of C1ql1-Bai3 are essential to maintain 1:1 innervation pattern at CF-PC synapses throughout life. Though how C1ql1-Bai3 signaling is regulated remains to be clarified, it may serve as a mechanism underlying activity-dependent morphological changes at synapses in adult cerebellum. (COI: No)

2P-199

Layer 5 sublayer-dependent excitatory-inhibitory connections in the rat frontal cortex

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In the rodent frontal cortex layer 5 (L5) is divided into the upper (L5a) and lower (L5b) sublayers on the basis of more thalamic inputs in L5b than in L5a. Both sublayers contains pyramidal cells projecting to the pontine nuclei, corticopontine (CPn) cells. L5a CPn cells are longer in oblique branches of the apical dendrites and more extended in the basal dendrites than L5b CPn cells. Connected pairs of L5b CPn cells are more reciprocal than those of L5a CPn cells. These differences indicate that L5a and L5b CPn cells are different in local connections including GABAergic inhibitions. Low threshold spike (LTS) cells, a subtype of L5 GABAergic cells, express somatostatin and ascend their axons to layer 1 (L1), called Martinotti cells. L5 LTS cells more often connect to CPn cells than to other pyramidal cells. Therefore, we compared the connections of L5a and L5b LTS/CPn pairs, using paired whole-cell recordings and morphological reconstruction of the recorded cells. The axon collaterals in L1 were longer in L5a LTS cells than in L5b LTS cells. The spatial overlaps of L5a LTS to CPn cells were found not only in basal and apical oblique dendrites but also in apical dendritic tufts in L1. On the other hand, L5b LTS cell axons selectively targeted the basal and apical oblique dendrites of L5b CPn cells. These results suggest that the dendritic domains inhibited by LTS cells are different between L5a and L5b CPn cells. (COI: No)

2P-200

Phasic inhibition in the interval of carbachol-induced β oscillation in rat hippocampal

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The epilepsy is suppressed in REM period in a pilocarpine model, and it is promoted in a tetanus toxin model. Theta rhythm is thought to be involved. In the sleep period, β rhythm is also induced as well as θ rhythm. The effect of β rhythm on the occurrence of the epilepsy has not yet been clarified. Beta rhythm can be intermittently induced in rat hippocampal slices with the application of the cholinergic agent carbachol (CCh). The rhythm is called CIBO. The epileptic discharges are induced with the application of $GABA_A$ receptor antagonist in hippocampal slices. Our previous work indicated that $GABA_A$ antagonist-induced epileptic discharges (GIED) were not induced when CIBO was induced in hippocampal slices. The suppression mechanism of GIED by CIBO has not yet been clarified. We hypothesized that in the interval of CIBO the suppression of the induction of GIED is induced. To confirm the hypothesis, we had paired pulse stimulation at the *stratum radiatum* with the stimulation electrode. The present data were obtained from 30 hippocampal slices (450μm thick) of male Wistar rat. We had paired pulse stimulations with the interval of 10 msec at the several phases of the interval.The stimulation induced paired-pulse inhibition (PPI). PPI around at 180° was mostly suppressed. These results suggest that disinhibition will occur in a short time in the interval of CIBO. In results, β rhythm will suppress the induction the epileptic discharges. (COI: No)

2P-201

Exendin-4 promotes myelination in a co-culture of DRG neurons and immortalized Schwann cells IFRS1

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Besides its insulinotropic actions on pancreas, the localization of glucagon-like peptide-1 receptor (GLP-1R) at the nervous system suggests neuroprotective properties of GLP-1. Exendin-4 (Ex-4), a GLP-1R agonist, has been shown toprevent the deterioration of neurons and glial cells following axonal injury and in a variety of neurodegenerative disorders.In this study, we investigated the effects of Ex-4 on myelination in co-cultured adult rat DRG neurons and immortalized Schwann cells IFRS1. DRG neurons were maintained in a serum-free culture medium for 7 days, and then co-cultured with IFRS1 cells under a serum-free condition in the presence of 50 μg/mL ascorbic acid and different concentrations (0, 10, or 100 nM) of Ex-4 for up to 21 days.Under a phase-contrast microscope, treatment with Ex-4 dose-dependently enhanced the movement of IFRS1 cells toward the neurites growing from DRG neurons at 14 days of co-culture. Immunofluorescence and Western blotting performed at 21 days of co-culture revealed that Ex-4 significantly increased the number of myelin protein zero (MPZ)-immunoreactive IFRS1 cells surrounding beta III tubulin-immunoreactive neurites, and up-regulated the protein expression of peripheral myelin protein 22 and MPZ. Moreover, Western blotting carried out at 3 days of co-culture resulted in Ex-4-induced phosphorylation of AKT, suggesting that Ex-4 accelerates the myelination process in the DRG neuron–IFRS1 co-culture via activating PI3 kinase/AKT pathway. (COI: No)

2P-202

Loss-of-function of glial ABCA1 increases the risk for pathogenesis of glaucoma

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Glaucoma is progressive optic neuropathy which is characterized by damages or degeneration of retinal ganglion cells (RGCs). One of the most important risk factors is an elevated intraocular pressure (IOP) that was thought to be the main cause glaucoma. However, it has become apparent that many risk factors other than IOP are involved in the etiology of glaucoma. Recent large scale genome wide association studies (GWAS) have identified that single nucleotide polymorphism (SNP) of *ABCA1* gene is the highest risk for glaucoma. However, its pathogenic mechanisms are totally unclear. First, it is unclear whether or not ABCA1 affects IOP. Second, there are no data indicating which of gain-of-neurotoxicity or loss-of-function of ABCA1 is involved in glaucoma. Third, it is unknown which type of cells contributes to glaucoma. We analyzed conventional ABCA1 knockout (KO) mice and found that IOP was not changed. We also found that ABCA1 was highly enriched in astrocytes of ocular tissues. To further clarifying the role of astrocytic ABCA1, we generated astrocyte-specific ABCA1 knockout (cKO) mice. The cKO mice showed significant increase in the number of apoptotic RGCs and reduction in visual function at middle-age (12 months old). Taken together, our data showed that (1) ABCA1 has no impact on IOP; (2) loss-of-function of ABCA1 is involved in glaucoma; and (3) ABCA1 in glial cells contributes to pathogenesis of glaucoma. (COI: Properly Declared)

2P-203

Müller glial swelling activates TRPV4 and triggers photoreceptor cell death at body temperature

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Using region-specific injection of hyaluronic acid, we developed a mouse model of acute retinal detachment (RD) to investigate molecular mechanisms of photoreceptor cell death triggered by RD. We focused on the TRPV4 ion channel, which functions as a thermosensor, osmosensor and/or mechanosensor. Following RD, the number of apoptotic photoreceptors was reduced by ~50% in TRPV4KO mice relative to wild type mice, indicating the possible involvement of TRPV4 activation in RD-induced photoreceptor cell death. Furthermore, TRPV4 expressed in Müller glial cells can be activated by mechanical stimuli caused by RD-induced swelling of these cells, resulting in release of the cytokine MCP-1, which is reported as a mediator of Müller glia -derived strong mediator for RD-induced photoreceptor death. We also found that the TRPV4 activation by the Müller glial swelling was potentiated by body temperature. Taken together, our results suggest that RD adversely impacts photoreceptor viability via TRPV4-dependent cytokine release from Müller glial cells and that TRPV4 is part of a novel molecular pathway that could exacerbate the effects of hypoxia on photoreceptor survival following RD. (COI: No)

2P-204

Stress-Induced Microglial Activation Occurs through a beta-Adrenergic Receptor

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In the present study, we investigated the underlying mechanism of brain microglial activation by acute stress. We first looked at the spatial distribution of the noradrenaline synthesizing enzyme, DBH, in comparison with noradrenaline receptors—b1, b2, and b3 adrenergic receptors (b1-AR, b2-AR, and b3-AR)—after which we examined the effects of the b-blocker propranolol and a-blockers prazocin and yohimbine on stress-induced microglial activation. Finally, we compared stress-induced microglial activation between wild-type (WT) mice and double-knockout (DKO) mice lacking b1-AR and b2-AR. The results demonstrated that (1) microglial activation occurred in most studied brain regions, including the hippocampus (HC), thalamus (TM), and hypothalamus (HT); (2) within these three brain regions, the noradrenaline-synthesizing enzyme DBH was densely stained in the neuronal fibers; (3) b1-AR and b2-AR, but not b3-AR, are detected in the whole brain, and b1-AR and b2-AR are colocalized with microglial cells, as observed by laser scanning microscopy; (4) b-blocker treatment inhibited microglial activation in terms of morphology and count through the whole brain; a-blockers did not show such effect; (5) unlike WT mice, DKO mice exhibited substantial inhibition of stress-induced microglial activation in the brain. In the present study, we demonstrate that neurons/microglia may interact with noradrenaline via b1-AR and b2-AR. (COI: No)

2P-205

Electrophysiological approach with ex vivo trigeminal ganglia to clarify neuron-glia interactions

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Various receptors and channels have been reported to be involved in trigeminal ganglia (TG) neurons excitation followed by satellite glial cells (SGCs) activation. The P2X7 receptor (P2X7-R) expressing in SGCs is one of the possible mechanism for glia-neuron interactions. We have established the patch-clamp recording from TG neurons in ex vivo prepared TG to evaluate the involvement of SGCs-neuron interaction in TG in orofacial pain processing. Whole-cell patch-clamp recording was performed from TG neurons in whole or sliced TG from naïve or nerve injury (NI) rats using the microscope equipped with Nomarski optics and an infrared-sensitive video camera. We applied BzATP, the selective P2X7-R agonist, to activate the SGCs. RMP and rheobase (T) were recorded and compared with those after vehicle application.

The relative value (vs. before application) of both RMP and T were significantly different between BzATP- and vehicle-applied groups in naïve rats. Conversely, RMP before BzATP application was significantly depolarized in NI rats compared with that in naïve rats, and BzATP application did not have it depolarize more.

TG neurons are assumed to be tightly encircled with SGCs under the ex vivo preparation of TG, and our results indicate SGCs activation via P2X7-R modulate the excitability of TG neurons. This patch-clamp recording with ex vivo preparation can be a useful strategy to clarify the mechanisms underlying SGCs-neuron functional interactions. (COI: No)

2P-206

The role of primary somatosensory cortex in causing mirror image pain

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Peripheral nerve injury causes maladaptive plasticity in the central nervous system and induces chronic pain. In addition to the injured limb, abnormal pain sensation can appear in the limb contralateral to the injury, called mirror image pain. However, the mechanism of causing this disease is still not elucidated in the central nervous system. We previously reported that chronic pain in the hindlimb of mice increased the neuronal activity and induced astrocyte-dependent synaptic remodeling in contralateral primary somatosensory cortex (cont-S1).

Since synaptic remodeling in cont-S1 has critical roles in the induction of chronic pain, cortical reorganization in the S1 ipsilateral to the injured limb (ipsi-S1) may also accompany mirror image pain. To elucidate this, we investigated the activity in ipsi-S1 using *in vivo* 2-photon Ca^{2+} imaging under chronic pain conditions. Following peripheral nerve ligation (PSL), the Ca^{2+} transients in layer 1 inhibitory neurons and astrocytes were increased via callosal inputs from cont-S1, while the Ca^{2+} transients in layer 2/3 pyramidal neurons were decreased. When local inhibitory circuits in ipsi-S1 were blocked, astrocyte-dependent spine turnover rate was increased, and the threshold of mechanical stimuli in the intact hindpaw contralateral to the PSL site was decreased. Thus, our data sugested that activation of cortical astrocytes in ipsi-S1 prime the induction of spine plasticity and mirror image pain after peripheral nerve injury. (COI: No)

2P-207

Visualization of spatiotemporal interaction of neurons and astrocytes

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P2Y1 receptor (P2Y1) is one of Gq-protein coupled receptors, which is thought to play a central role in astrocytic Ca^{2+} signals. P2Y1 receptor-mediated Ca^{2+} signals is enhanced in pathophysiological conditions, such as Alzheimer' disease and epilepsy. However, role of the P2Y1-mediated Ca^{2+} signals in disease is still largely unknown. To elucidate functional significance of such P2Y1-mediated Ca^{2+} signals in astrocytes, we increased the expression of P2Y1 selectively into astrocytes using Tet-off conditional transgenic mice line (astrocyte P2Y1 overexpression mice) and introduced red fluorescent genetically-encoded Ca^{2+} indicator (GECI), R-CaMP2 or jRGECO1a and green fluorescent GECI, GCaMP6f, into neurons and astrocytes, respectively. In acute hippocampal slices of astrocyte P2Y1 overexpression mice, electrical stimulation of the Schaffer collateral resulted in a rapid Ca^{2+} signal in dendrites of CA1 neurons, which was followed by a slow-onset Ca^{2+} signal in astrocytes with a few seconds delay. The astrocytic Ca^{2+} signals required both neuronal excitation and ATP/P2Y1, suggesting existence of ATP-mediated signals from neurons to astrocytes. Furthermore, duration of dendritic Ca^{2+} signal was longer in astrocyte P2Y1 overexpression, suggesting existence of signals from astrocytes to neurons, resulting in enhancement of synaptic transmission. Overall, our data suggest that enhancement of P2Y1-mediated Ca^{2+} signals in astrocytes result in increase in dendritic excitations. (COI: No)

2P-208

Activation of TRPV4 induced significant ATP release in Müller glia

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Müller glia are important to regulate neuronal excitabilities by releasing gliotransmitters. There are several gliotransmitters such as glutamate, D-serine and adenosine tri-phosphate (ATP). Among them, ATP is a major gliotransmitter to suppress the activity of retinal ganglion cells (RGCs). Although, it has reported that volume changes in Müller glia or its membrane stretch induced ATP release, the molecular mechanisms have not been revealed. Transient receptor potential vanilloid 4 (TRPV4) is a non-selective cation channel that is activated by multiple ligands including membrane stretch, hypotonic stimuli, physical temperature (27-34℃), and so on. We have previously demonstrated that the activation of TRPV4 induced ATP release in brain astrocytes. Since, Müller glia are functionally relevant to the astrocytes, it is expected that the effects of TRPV4 on ATP release could be consistent with Müller glia. Here, we addressed whether the activation of Müller glial TRPV4 can induce ATP release. Using the extracellular ATP imaging and a patch clamp-based biosensor method, we demonstrated that the application of TRPV4 ligands (a synthetic ligand and hypotonic stimuli) induced strong ATP release from Müller glia, and the released ATP followed by TRPV4 activation suppressed RGC excitability in the acute retinal slices. Our results indicate that the TRPV4 is a key molecule to induce ATP release from Muller glia, and suppresses neuronal excitability in RGCs. (COI: No)

2P-209

Excitatory synaptic transmission is reduced by astrocytes previously exposed to amyloid β 1-40

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Synaptic transmission in the central nerve system is fundamental machinery that governs memory and consciousness. In Alzheimer's disease (AD) brain, the neurological abnormalities are gradually accelerated with the amyloid β (Aβ) exposure. A major glial cell type in brain, so called astrocyte, also plays important roles in the synaptic transmission. Therefore, it is possible that the astrocytic vulnerability caused by the Aβ-exposure would involve in synaptic dysfunctions in AD brain. To investigate this hypothesis, we evaluated synaptic transmission of single neurons co-cultured with the astrocytes pre-exposed to $Aβ_{1-40}$ for 3 days. In this culture system, neuron was not exposed to $Aβ_{1-40}$ but only astrocyte was exposed. Excitatory synaptic transmission was recorded at 13-16 day in vitro using the patch clamp method. As a result, excitatory postsynaptic current and the number of glutamatergic synapses were significantly decreased in hippocampal neurons with co-culturing of astrocytes that had previously exposed $Aβ_{1-40}$. However, the vesicular release probability was significantly increased in such abnormal condition. Taken together, these phenotypes resembled the general characteristics of the immature synapses. On the basis of these findings, our data indicate that synaptogenesis is unable to be well established by which astrocyte was experienced the $Aβ_{1-40}$ exposure.Our data also propose that only exclusion of $Aβ_{1-40}$ from brain may be insufficient for a therapy for AD. (COI: No)

2P-210

Acute stress induced the alterations of astrocytes and glutamate receptors in the hippocampus of rat

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Exposure to stress has been known to disturb the morphology and function of glial cells especially astrocytes that have been implicated in cognitive and mood disturbances. Several previous studies have revealed that a decrease in glial fibrillary acidic protein (GFAP) following exposure to chronic stress is associated with mood disorder. In this study, we aimed to determine how acute stress altered the expression of astrocytes and proteins expressed by astrocytes such as amino acid transporters 1 and 2 (EAAT1, EAAT2) in the hippocampus. Adult male rats were restrained to a single session of stress. We then measured the immunoreactive materials of GFAP, EAAT1 and EAAT2 by using a cumulative threshold spectra analysis in 6 subregions of the hippocampus. Our analysis demonstrated that acute stress significantly reduced GFAP thresholded staining in 4 of 6 subregions determined. Additionally, we found that there was a reduction of EAAT1 expression, while there was a significant decrease in EAAT2 level in one region of the hippocampus. Collectively, the results indicated that acute stress can induce morphological changes of astrocytes related to alterations in the expression of glutamate receptors in multiple regions of the hippocampus. (COI: No)

2P-211

Visualizing the Interaction of Immune Cells and Peripheral Sensory Fibers in Mice Neuropathic Model

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Neuropathic pain is pain caused by a damage of the somatosensory system. Cumulative literatures suggest that immune system is involved in the generation and maintenance of neuropathic pain. However, what type of the immune cells are implicated, and when and how they interact with peripheral nerves are still unclear. We used intra-vital 2-photon microscopic methods to observe these interactions in transgenic self-fluorescent mice that underwent spared nerve injury of the sciatic nerve (SNI). Two series of experiments were performed. In the first series of experiment, FITC-dextran was intravenously injected into Nav1.8-tdTomato mice. A wave of activated macrophages moved from deep dermis to the nerve plexus in the junction of the epidermis and dermis, starting the first day after SNI. In the next 2 days, immune cells continued their attacks. In the second series of experiment, AAV1-CAG-tdTomato was injected into the 4th lumbar dorsal root ganglion of Iba1-GFP mice to labeled a few nerve fibers. The interaction between the nerve fibers and the Iba1-labeled immune cells in the toe tips was longitudinally observed. The immune cells were also found to move to the sub-epidermal junction to interact with the nerve fibers starting the first day after and to continue many days after SNI. These data suggested that the interaction of immune cells with the degenerating peripheral sensory fibers may be associated with the initiation of neuropathic pain. (COI: No)

2P-212

Tonic release of D-serine through Best1 channel is critical for long term depression

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Bestrophin1 channel (Best1), which is known as Calcium-activated anion channel, is permeable to glutamate or GABA in astrocytes. Here, we tested whether Best1 is also permeable to D-serine, which is even smaller than glutamate and known as co-agonist for NMDA receptors (NMDARs) that is critical for synaptic plasticity and memory. Indeed, co-release of D-serine and glutamate via Best1 in Ca^{2+}- and pore-dependent manner was detected with sniffer patch. In CA1 of hippocampal brain slice, NMDAR-mediated tonic current was eliminated in Best1$^{-/-}$ mice. NMDA to AMPA amplitude ratio of evoked EPSCs has decreased in Best1$^{-/-}$ mice and general knock-down condition, which is recovered by exogenous D-serine and glial Best1 rescue, respectively. In synaptic plasticity and memory test, Best1$^{-/-}$ mice showed impairments in long-term depression (LTD) and reversal learning which is rescued by exogenous D-serine, but not long-term potentiation (LTP) or acquisition. Altogether, these results indicate that Best1-mediated D-serine release regulates synaptic plasticity and behavioral flexibility. (COI: No)

2P-213 (Y-17)

TRPA1 channel is critical for gliotransmitter release from astrocyte by eliciting calcium entry

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Most of studies on astrocytic intracellular Ca^{2+} signals and release of gliotransmitters have focused on Ca^{2+} release from intracellular calcium stores. However, the molecular identify of Ca^{2+} entry and gliotransmitter release by mechanical stimulation are largely unknown. Here, we report that astrocytic TRPA1 channel can be activated by mechanical stimulation and provides Ca^{2+} that is required for gliotransmitter release from astrocytes. We performed Ca^{2+} imaging and whole cell patch clamp upon mechanical stimulation to cultured solitary astrocytes, and found that mechanical stimulation-induced Ca^{2+} entry and current were reduced in TRPA1 shRNA-transfected astrocytes. Furthermore, using sniffer patch technique, we showed that glutamate and ATP release from TRPA1 shRNA-transfected astrocytes were significantly decreased. Based on these findings, we propose that Ca^{2+} entry via TRPA1 channel is the major source of Ca^{2+} for gliotransmitter release from astrocytes. (COI: No)

2P-215

Astrocytes mediate persistent respiratory augmentation in the recovery phase after hypoxic exposure

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Purpose: Respiration is augmented in response to hypoxia, and the respiratory augmentation persists for a while even in the post-hypoxic recovery phase. However, the mechanism of post-hypoxic persistent respiratory augmentation has not been fully elucidated. We aimed to clarify whether astrocytes are involved in the central mechanism of post-hypoxic persistent respiratory augmentation.

Method: Medulla-spinal cord preparations isolated from newborn rats were superfused firstly with oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF), secondly with hypoxic (95% N_2, 5% CO_2) aCSF for 5 min, and thirdly again with oxygenated aCSF for 20 min. We compared respiratory frequencies in the post-hypoxic recovery phase in preparations without and with administration of arundic acid (500 μM), a blocker of astrocytic activation.

Results: Hypoxia increased respiratory frequency in both groups. During superfusion with oxygenated aCSF after hypoxic exposure, respiratory frequency in the group without arundic acid was still persistently increased. On the other hand, respiratory frequency in the group with arundic acid was gradually decreased in the post-hypoxic phase, and it was eventually depressed and less than that of the pre-hypoxic phase in the later post-hypoxic phase.

Conclusions: We conclude that astrocytes play an active role in persistence of post-hypoxic respiratory augmentation. (COI: No)

2P-216

AQP4 involvement in normalization of extracellular potassium after acute ischemic stroke

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The mechanisms underlying metabolic waste clearance and extracellular ion homeostasis in the brain have not been fully understood. The glymphatic model proposes that extracellular fluid movement in the brain is regulated by aquaporin 4 (AQP4), the water channel expressed in predominantly in astrocytes, and noradrenaline levels (Iliff et al., 2012; Xie et al., 2013). We found that systemic adrenergic receptor blockade facilitates the normalization of extracellular potassium concentration after acute ischemic stroke and mitigates the resultant tissue damage. To test if AQP4 plays a role in this treatment mechanism, we evaluated the expression of AQP4 at various time points after stroke by histology and immunoblot. (COI: Properly Declared)

2P-217

Efficacy of *Cinnamomi Cortex* & Coumarin on cold allodynia by oxaliplatin : modulating spinal gila

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In the pathogenesis of neuropathic pain, activation of glial cells and increase of pro-inflammatory cytokines release in the spinal cord play a crucial role. A single injection of oxaliplatin (6 mg/kg, i.p.) can induce acute peripheral neuropathy. *Cinnamomi Cortex* (*C. Cortex*) has been used in East Asia to treat various pain symptoms. Anti-nociceptive effect of Coumarin on variety of pain was reported. We investigated whether and how *C. Cortex* and Coumarin alleviate oxaliplatin-induced cold and mechanical allodynia in SD rats. Significant pain behaviors were observed three days after an oxaliplatin injection. *C. Cortex* (200 mg/kg) and Coumarin (10 mg/kg) were orally administrated for five consecutive days after an oxaliplatin injection. Behavioral studies revealed that both *C. Cortex* and Coumarin have potent relieving effects against oxaliplatin-induced cold allodynia by increasing the tail withdrawal latency against cold stimuli, whereas only *C. Cortex* partially suppressed oxaliplatin-induced mechanical allodynia. IHC studies showed that*C. Cortex* and Coumarin inhibited the activation of spinal astrocytes and microglia by oxaliplatin treatment. In addition, orally treated *C. Cortex* down-regulated up-regulated pro-inflammatory cytokines, interleukin1β and tumor necrosis factorα, in the spinal cord after an oxaliplatin injection. Coumarin decreased increase of tumor necrosis factorα after an oxaliplatin treatment (0.1 mM) on primary cultured astrocytes. (COI: No)

2P-218

Pioglitazone reversed the developmental programming of fructose in the astrocytic glucose metabolism

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Excessive maternal high fructose diet (HFD) during pregnancy and lactation impairs the learning and memory in female offspring. Whether this impairment is due to altered cerebral energy metabolism is largely unknown. Cerebral energy metabolism changes dramatically during fetal and postnatal development, whereby maternal nutrition is a likely key factor. Astrocytes, a non-neuronal cell type in the brain, are considered to mediate high energy demands of neurons via lactate. In this study, primary hippocampal astrocyte-enriched cultures were obtained from female offspring of maternal regular diet (ND) and maternal HFD groups to evaluate the maternal HFD altered astrocytic glucose metabolism. Glycolytic capacity as well as mitochondrial respiration and electron transport chain were suppressed in the HFD group. Western blots and immunofluorescent images further indicated that the glucose transporter 1 (GLUT1) was downregulated whereas the insulin receptor A (IRA) and the p85 subunit of phosphatidylinositide 3-kinases (PI3K) were upregulated in the HFD group. Pioglitazone, which is known to increase astrocytic glucose metabolism, effectively reversed the suppressed glycolysis and lactate release was restored. Moreover, pioglitazone also normalized OXPHOS with an increase of cytosolic adenosine triphosphate (ATP). Together, these results suggest that maternal HFD impairs astrocytic glucose uptake and the downstream metabolic pathway that can be reversed by pioglitazone. (COI: No)

2P-219

Microglial activation caused by lipopolysaccharide and trimethyltin administration in the rat brain

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We have studied microglial activation in the rat brain by administration of lipopolysaccharide (LPS), the main component of the outer membrane of Gram-negative bacteria, and of trimethyltin (TMT), a potent neurotoxic chemical. Intraperitoneal injection of LPS (E.coli O111:B4; 1 or 3 mg/kg) caused an increase in the concentration of plasma corticosterone of male Wistar-Kyoto rats (180-220 g). Besides, there were the pimonidazole (hypoxic probe)-positive regions and cells in the amygdala, hippocampus and paraventricular nucleus of the brain after the LPS injection. Immuno-histochemical investigation has revealed that the LPS injection significantly caused an increase in the number of IBA-1 positive cells in the paraventricular nucleus 24-hour after. In the hippocampus, a tendency was also observed for increase in the number of the IBA-1 immuno-positive cells. No OX-42(cd11b)-positive cells have been found. On the other hands, intraperitoneal injection of TMT (8 mg/kg) induced the OX-42 (cd11b) immune-positive cells in the hippocampus. The present study suggests that the pimonidazole-positive cells in the hippocampus may be more vulnerable than other cells to cytokines and/or corticosterone released by LPS administration. Microglial activation by LPS or TMT observed in this study is thought to be closely linked to inflammatory reactions. Further study needs to ensure the type of the activated microglia by immunostaining with antibodies for tmem119 and other specific markers. (COI: No)

2P-220

Brain area-dependent astrocyte heterogeneity detected in mice by dopamine receptor expressions

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Dopaminergic neurons in substantia nigra pars compacta (SNc) send their axons to striatum wherein both dopamine D1 and D2 receptors (D1R, D2R) are expressed, while extending their dendrites into the adjacent nucleus pars reticulata (SNr) wherein D1R is predominantly expressed. However, the physiological role of the different release modes, axonal and dendritic dopamine release, has yet to be fully understood. Recently, we reported that GFAP-positive astrocytes acutely dissociated from adult mouse SNr express abundant D1R, and not D2R, particularly in their fine processes, whereas those in striatum are either D1R-positive or D1R-negative (Nagatomo et al., ***Front. Neuroanat.*** 2017). Then, how is D2R expression in these astrocytes? To examine this, we conducted triple immunocytochemical analyses using anti-D1R and D2R antibodies, both of which were verified by mice lacking D1R or D2R, together with anti-GFAP antibody. Results show unexpected DR expression patterns in the astrocytes. In a separate study, we have reported that astrocytes can release glycine in response to dopamine via GlyT1 (Shibasaki et al., ***J. Neurochem.*** 2017). Since all GABAergic SNr neurons tested ceased firings in response to glycine, SNr astrocytes could well exert a role in hypothetical transmitter-mediated, neuron-glia-neuron information processing, although further study is required for understanding the role of DR expressions in striatal astrocytes. (COI: No)

2P-221

Social defeat stress reduces newly born oligodendrocytes and induces anxiety-like behavior in mice

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Investigation of glial cell properties and neuron-glial interactions is important for understanding higher brain function and neurological disorders caused by glial abnormalities. Growing number of studies demonstrates that myelin remodeling by oligodendrocytes (OLs) in adult mice is involved in motor learning and social interaction, in which myelination is dependent on neuronal activity. To assess whether psychosocial experience during the period of development affects OL genesis or its property, we applied chronic social defeat stress to adolescent mice, a model for the examination of stress-related disorders in rodents.

We found that the social defeat stress led to decrease in the number of newly born OLs in the prefrontal cortex. In addition, we also found that the number of PLP+ mature OLs was decreased in the corpus callosum. These socially defeated mice showed anxiety-like behavior detected in the open field test. Taken together, these findings suggest that myelin remodeling by OLs in psychosocial environments plays roles in mental disorder. In addition, we will present the data to know whether drug treatment that modulates OL generation in socially defeated mice can rescue the behavioral abnormalities. (COI: No)

2P-222

Rediscovery of GIT1 hetero mice as more practical model for hyperactivity

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As the number of ADHD patients are increasing each year, with incredible amount of social costs, we still do not understand the core mechanisms in the ADHD. The traits of ADHD could be explained by inattentiveness, hyperactivity and impulsiveness. To understand the underlying mechanism of ADHD, we have used GIT1 (G protein-coupled receptor kinase interacting protein-1) KO (knock out) mice on the previous study. However, because GIT1 KO mice have small brain and body size, with high-rate of postnatal lethality, there were a lot of difficulties to use KO type. During the investigation, we have observed that GIT1 HE (Hetero) mice showed hyperactivity than the GIT1 WT mice. In immunohistochemistry, GIT1 HE mice showed significantly decreased amount of GABA than the WT mice. Also, from In vivo microdialysis, decreased level of GABA is observed. In slice recordings on the cerebellum of GIT1 mice, tonic inhibition is also decreased. Taken these results together, even HE mice had not been studied, for they are known to show no differences with WT mice, we have suggested that HE mice can be more useful hyperactive model mice with significant difference of physiological traits. (COI: No)

2P-223

Functional connectivity changes after rTMS used to detect plasticity decline associated with obesity

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It is known that obese patients often suffer from impairments in the peripheral nervous system. It has recently been reported that obesity also involves the central nervous system. We hypothesized that the obesity declines cortical plasticity in the central nervous system, and that the decline can be detected by using the combination of repetitive transcranial magnetic stimulation (rTMS) and functional connectivity changes. rTMS to a cortical region induces changes in cortical excitability for minutes to hours, and also results in changes in "interhemispheric" functional connectivity, the resting-state functional connectivity between the stimulated region and the symmetrically corresponding region in the other side of the hemispheres. In the present study, resting-state functional MRI was measured before and after quadripulse stimulation (QPS), a type of rTMS, applied to the first finger representation in the left primary motor cortex (M1), and the changes in the interhemispheric functional connectivity were calculated. A cluster of connectivity changes was observed in the stimulated region in the central sulcus. Preliminary results indicated that the magnitude of the functional connectivity changes tended to correlate with the body mass index. These results suggest that the changes in interhemispheric functional connectivity induced by QPS can be used to detect cortical plasticity decline associated with obesity. (COI: Properly Declared)

2P-224

Visualization of the activation pattern causality during pain chronification using DREADD-MEMRI

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Functional MRI (fMRI) is a powerful tool in revealing wide-spread activation of the brain associated with pain state. In preclinical researches, manganese-enhanced magnetic resonance imaging (MEMRI) provides a snap-shot of the history of the brain activation during the period spent with spontaneous pain in an anesthetics-free setting. We reported that intraplantar injection of formalin in mice results in high MEMRI signals in the limbic system, such as the nucleus accumbens, the insular cortex and the amygdala, at 6 h after the injection. To reveal the causal link between these regions in establishment of chronic pain state, we combined MEMRI with artificial manipulation of neuronal activities using chemogenetics. Adeno-associated virus vectors for expression of the designer receptors exclusively activated by designer drugs (DREADDs) were injected into the right amygdala of the mice 5 weeks before MEMRI scanning. Repeated activation of DREADDs by clozapine-N-oxide significantly reduced manganese accumulation selectively in the caudate putamen, the nucleus accumbens, the ventral tegmental area and the substantia nigra. These results support the notion that the amygdala, which receives direct and indirect nociceptive information, plays pivotal role in the dynamic transition process from acute to chronic pain in the brain. (COI: No)

2P-225

Correlation analysis of sister mitral and tufted cells

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Multiple mitral and tufted cells, the projection neurons of the olfactory bulb, innervate the same glomerulus and receive input from the same population of olfactory sensory neurons; these cells have been termed 'sister' mitral/tufted cells. One question is whether sister cells within one glomerulus receive different inhibitory inputs via interneurons, resulting in different odorant response properties. To address this question, we performed two-photon calcium imaging of mitral and tufted cells and examined the correlation of calcium signals within one cell or among sister cells. We used either Tbet-cre or Pcdh21-cre transgenic mouse lines to express GCaMP6 selectively in mitral and tufted cells and compared signals between different cells that could be visually confirmed to innervate the same glomerulus, or between different compartments of the same cell. We found that the correlation is high within one cell and among sister cells, especially during the odorant-evoked activation period. To examine response across multiple odorants in sister cells, we presented larger numbers of odorants and; we found that sister cells showed similar activation profiles. However, a principal component analysis revealed some differences in the temporal response patterns among different sister cells. These results indicate that while the response properties of sister cells are largely similar, there may be some modest differences among them. (COI: No)

2P-226

Novel fluoropolymer nanosheets extending *in vivo* two-photon imaging of living mouse brain

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In the field of neurosciences, *in vivo* two-photon microscopy technique has become widely used for deep tissue imaging. For observing deeper regions of living mouse brains by this microscopy, open skull methods have been usually employed: a hole was dug in the skull of the mouse and then was sealed with a glass cover slip to make a window for observations. For making cranial windows, however, this operation was so tricky and requires a high-level skill. In material sciences, polymer thin films, or nanosheets, have been proposed as novel materials for bioimaging and surgical applications. In this study, we utilized newly-developed nanosheets, PEO-CYTOP nanosheets with water-retention effect and adhesive surface hydrophilized. They were composed of a layer of amorphous fluoropolymer CYTOP (Asahi Glass Co., Ltd.) and poly-dimethylpolysiloxane (PDMS) attaching polyethylene oxide(PEO). Instead of glass cover slips, PEO-CYTOP nanosheets enabled to seal larger cranial windows in living mice. In addition, nanosheets might not disturb excitation laser lights and fluorescent emission lights in the optical path. Thus, we successfully achieved deep brain imaging in living mice with a high spatial resolution. We hope that an adhesion stability of nanosheets facilitate long-term imaging. In future, the combination of nanosheets with another technique like adaptive optics probably can realize "*in vivo*" long-term deep imaging with wider-field area in various living organs in other animals. (COI: No)

2P-227

Wide-field imaging of neural activity with high spatial resolution

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In vivo wide-field imaging of neural activity at cellular and subcellular resolutions is a current challenge in neuroscience. To address this issue, we developed two optical methods. First, we developed a micro-opto-mechanical device that rotates within the post-objective space between the objective and brain tissue. Two-photon microscopy with this device enabled sub-second sequential calcium imaging of left and right mouse sensory forelimb areas 6 mm apart. When imaging the rostral and caudal motor forelimb areas (RFA and CFA) 2 mm apart, we found high pairwise correlations in spontaneous activity between RFA and CFA. While mice performed a forelimb-movement task, the layer 2/3 population activity between RFA and CFA covaried across trials. Second, to achieve a high spatio-temporal resolution, we combined an 8K ultra-high-definition camera with spinning-disk one-photon confocal microscopy. This combination allowed us to image a 1 mm^2 field with a pixel resolution of 0.21 μm at 60 fps. When we imaged the CFA layer 1 in a behaving mouse, calcium transients were detected in presynaptic boutons of thalamocortical axons sparsely labeled with GCaMP6s. Axonal boutons with highly correlated activity were detected over the 1 mm^2 field, and were probably distributed on multiple axonal arbors originating from the same thalamic neuron. These methods will be powerful tools to clarify the correlation structure in wide cortical areas at neural population and synaptic levels. (COI: Properly Declared)

2P-228

3-D visualization of avian brainstem auditory circuits using Brainbow labeling and tissue clearing

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Interaural time difference (ITD), a cue for sound source localization, is detected in auditory brainstem circuits at each sound frequency. In avian brainstem, the characteristic arborization patterns of axonal projection from nucleus magnocellularis (NM) to nucleus laminaris (NL) are involved in ITD detection. The developmental mechanisms of this circuit are, however, still not understood well due to the labor-intensive process of single axon tracing. In this study, we established an experimental system which enables 3-D visualization of whole avian auditory brainstem circuits using Brainbow labeling and tissue clearing method. Plasmid vectors which randomly express three-kinds of fluorescent proteins were introduced into NM progenitor cells at embryonic day 1.5 (E1.5) by in *ovo* electroporation method. At E12, the 1.5 mm-thick brainstem slices are cleared with CUBIC protocol and imaged under confocal-microscopy. We found that the NM neurons were labeled with various colors and the individual axons were traceable using the color differences. The traced axons showed topographic representation of sound frequency (tonotopic organization) and characteristic branching pattern (delay line). In addition, we could also label oligodendrocytes which contribute to ITD detection using MBP promoter. From the above, our system enables genetic manipulation and 3-D analysis of auditory brainstem circuits and would facilitate further understanding of the developmental mechanisms. (COI: No)

2P-229

Anesthesia alters orientation and direction selective properties in mouse superior colliculus

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Superior colliculus (SC) is a brainstem center which plays key roles in generating spatial attention and mediating the signal for sensory-motor translation. Recent studies using *in vivo* imaging techniques reveal that the superficial layer of the SC (sSC) has orientation column like functional cell organization. However, it is still in debated about whether such a functional cell organization really exists in the sSC or not.

In this study, we developed chronically stable animal preparation for *in vivo* imaging from the SC. We delivered GCaMP6f, calcium sensitive fluorescent indicator, to the sSC neurons by injecting Adeno-associated virus (AAV) vector (AAV1-hSyn-GCaMP6f) after removing small part of the cortex that overlaid on the SC. To achieve long-term optical access to the SC and make a stable imaging window, small glass cube was implanted on the SC surface. Neuronal population response was recorded by *in vivo* two-photon calcium imaging. Here, we found that the light-isoflurane anesthesia extensively alters the orientation selective response properties in the sSC Specifically, neurons in light-isoflurane anesthetized condition showed stronger and sharper orientation selectivity. This result indicates that the orientation selectivity and generation of the orientation column-like structure might vary with wakefulness of the animal. (COI: No)

2P-230

Analysis of a novel higher visual area, ECT, in the mouse ventral stream

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The visual cortex of mice is a useful model for investigating the mammalian visual system. In primates, higher visual areas are classified into two parts, the dorsal stream ("where" pathway) and ventral stream ("what" pathway). The ventral stream is known to include a part of the temporal cortex. In rodents, some cortical areas adjacent to the primary visual area (V1) in the occipital cortex are thought to be comparable to the ventral stream in primates, although the whole picture of the mouse ventral stream has never been elucidated. Here, we conducted large-scale Ca2+ imaging of temporal cortex of GCaMP transgenic mice in awake condition. We found that moving object visual stimuli induced response not only in known higher visual areas, but also in ectorhinal cortex (ECT) located in ventral to the auditory cortex. This is the first evidence that ECT has a distinct visual response. By analyzing response positions in each area, we found that while the retinotopic map in the ECT is ambiguous, response positions for moving object stimuli in ECT were shifted depending on object size; from posterior to anterior parts of the ECT as the stimulus size was enlarged. We analyzed neuronal responses by using two-photon Ca2+ imaging and found that ECT neurons show selectivity to object size. In conclusion, our findings reveal that mouse ventral visual stream extends to more ventral than previously assumed, and that neurons in the ventral visual area represent object size. (COI: No)

2P-231

An fMRI Study of Brain Network Involved in Elderly Teeth Tapping

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We examined brain activities during a simple teeth tapping by fMRI in an elderly dentulous (ED) group and an elderly edentulous group with (EEd) or without denture wearing, and analyzed the functional network connections. A general linear model analysis revealed that teeth tapping induced activation at various foci (p<0.05, corrected), including the primary sensory cortex (SI), primary motor cortex (MI), supplementary motor cortex (SMC)/premotor cortex (PMA), insula cortex, cerebellum, thalamus, and basal ganglia (BG).Group comparison between ED and EEd revealed that activities decreased in the SI,MI, SMC/PMA, thalamus, BG, and insular cortex (p<0.05, uncorrected). A conjunction analysis among all groups revealed that teeth tapping commonly activates the SI, MI, SMC/PMA, cerebellum, VPM nucleiin the thalamus, and putamen (p<0.05, corrected). These areas have been cited as regions primary involved in voluntary movements.The PPI analyses showed that tapping with denture wearing enhanced effective connectivity from S1 to VPM nuclei in the thalamus, putamen, cerebellum, dorsolateral part of the prefrontal cortex,and SMC (p<0.05), and that may be a consequence of sensory modulation of connectivity between regions and networks implicated in voluntary movements. All these results support an idea that sensory (periodontal) inputs can significantly control teeth tapping *via* feedforward control of intended movements as well as *via* feedback control of ongoing movements. (COI: No)

2P-232

Hippocampus abnormalities evaluated by density imaging in COPD patients

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Neurite Orientation Desperation and Density Imaging (NODDI) is a diffusion magnetic resonance imaging (MRI) technique for estimating microstructural complexity of dendritic density and dispersion in human brain on clinical MRI scanner, and NODDI provides specific information regarding pathological processes in the gray matter. In this study, the amygdala (AMG) and hippocampus (HI) microstructure and volume measurements were assessed with NODDI and voxel-based morphometry in healthy controls and patients with chronic obstructive respiratory disease (COPD) who has been reported having a mild cognitive impairment, and we sought to test relationship between AMG-HI abnormalities and cognitive function measured with mini mental state examination (MMSE). Significant HI volume reduction with higher dendritic density was observed in COPD compared with controls. In COPD, there was no correlation between the left HI volume and MMSE score, however, there was a significant negative correlation between dendritic density and MMSE, indicating that patietns with lower cognitive function had higher dendritic density. It has been reported that long exposure of hypoxia and emotional stress including breathlessness could affect on HI pathology. It could be suggested that dendritic density of the HI measured with NODDI can detect gray matter changes more sensitive manner, and may be useful as a barometer of cognitive decline. (COI: No)

2P-233

Relationship between Resting-State Functional Connectivity and cognitive function

Akira Yoshikawa[1]; Yuri Masaoka[1]; Masaki Yoshida[2]; Nobuyoshi Koiwa[3]; Satomi Kubota[1,4]; Ryo Manabe[1,5]; Natsuko Iizuka[1,4]; Masahiro Ida[6]; Masahiko Izumizaki[1] ([1]*Department of Physiology, Showa University School of Medicine, Japan*; [2]*Department of Ophthalmology, Jikei University School of Medicine, Japan*; [3]*Human Arts and Sciences Research Center, University of Human Arts and Sciences, Japan*; [4]*Department of Neurology, Showa University School of Medicine, Japan*; [5]*Department of Medicine, Division of Respiratory Medicine and Allergology, Showa University School of Medicine, Japan*; [6]*Department of Radiology, Comprehensive Stroke Center, Ebara Hospital, Japan*)

Resting-state functional magnetic resonance imaging (rs-fMRI) connectivity analysis is one of the valuable way to represent functional changes in human brain. Default Mode Network (DMN) is well known to observe in the rs-fMRI, closely associated with self-related mental task. In this study, we investigate the relationship between rs-fMRI connectivity and cognitive function measured with Mini-Mental State Examination (MMSE). Subjects were twenty-five healthy elderly (74.4y, male: 13, female: 12) with no history of brain diseases, and an informed consent was obtained for all subjects. Subjects were measured rs-fMRI with clinical 3T scanner (MAGNETOM Trio A Tim System, Siemens), and then divided into two groups: subjects with normal MMSE scores (N-MMSE) and subjects with low MMSE scores (L-MMSE). DMN-related areas were extracted 32 nodes from Automated Anatomical Labeling (AAL), and compared rs-fMRI connectivity paired within 32 brain areas between two groups using Network-Based Statistic. Nine pairs of regions including a pair of superior frontal gyrus medial (SFM) - angular gyrus were significantly higher connectivity in H-MMSE compared with L-MMSE. In addition, SFM-angular gyrus had significant positive correlation with MMSE. Our results suggest the link between SFM and angular gyrus may react as first sign for changing cognitive function. (COI: No)

2P-234

Decoder construction for MEG signals in a subitizing task

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It has been known that humans can rapidly and accurately enumerate small sets of items, which is called subitizing. Neurofeedback training may alter the subitizing performance, but the point has not been investigated. In this study, to prepare a neurofeedback training system for that purpose, we applied MEG to measure brain activity during subitizing and constructed a decoder. We specifically focused on alpha band, because the frequency is known to play a role in successful visual object recognition (Mima et al., 2001) or visual target detection (Thut et al., 2006). The set of 1-8 white square dots that appeared in random positions was presented on a gray background. The subjects were asked to report the number of dots by pressing corresponding buttons. A 306-channel Elekta Neuromag MEG scanner was used. We computed the power-spectrum density and cross-spectrum density. Using these alpha-band spectra, power spectrum, imaginary part of coherence, phase lag index, and weighted phase lag index, we constructed a decoder (Sparse Multinominal Logistic Regression: SMLR) to discriminate the trials were subitizing or counting.

The decoders with the imaginary part of coherence and phase lag index showed significantly better (p<0.05) discrimination rate (74.2%, 66.3%) than theoretical chance rate.

The relatively high discrimination rate suggested that the decoders are useful to prepare the neurofeedback training system for subitizing. (COI: No)

2P-235

Application of a Spatiotemporal Neural Network to Segment Low Contrast Calcium Fluorescence Images

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Calcium fluorescence imaging stacks capture the changes in calcium concentration of neural cells over time. This data can be analysed to gain insight into the mechanisms of neural cells and subsequently the brain. However extracting the regions of interest (ROI) in low contrast fluorescence image stacks is a complex problem which takes a significant amount of time when performed manually. Due to the low contrast of the images and frame to frame temporal dependency, conventional 2D segmentation approaches do not perform well. The U-Net which is a deep learning neural network architecture renown for its ability to segment biomedical images, is adapted to process image sequence snippets rather than a 2D summary image of the image sequence. This adaption makes use of the Long Short Term Memory (LSTM) unit to take spatiotemporal features into account. The spatiotemporal U-Net may not be able to surpass human capability but does result in significantly faster segmentations. (COI: No)

2P-236

Circuitry changes in Parkinson's disease assessed by qAIM-MRI

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Despite the biochemical characteristics of Parkinson's disease (PD) have been well described, the physiological properties of PD are unclear. In PD, highly synchronized activities were observed in cortico-basal ganglia loops, however, the meanings and the mechanisms have been argued. In addition, it is not fully understood how and where neuronal activities synchronize in PD. For elucidating these issues, we have recorded the history of the neuronal activities in entire brain volume in control mice and MPTP mice model of PD, and have analyzed the correlation among the neuronal activities in the brain regions including cortex, basal ganglia, and thalamus. For recording the history of the neuronal activities in entire brain regions, we used one MRI technique, quantitative activation-induced manganese-enhanced MRI (qAIM-MRI), which is based on the use of Mn^{2+} as a surrogate marker of Ca^{2+} influx to neurons (Kikuta et al., 2015).
In PD model mice, the activities in many regions, especially in striatum and cortex, showed strong correlation. The interhemispheric correlation was also observed in striatum and cortex. Our findings can lead to elucidation of the mechanisms of the highly synchronized brain activities in PD patients. (COI: No)

2P-237

Positron Emission Tomography Tracer for AMPA receptors Characterizes Psychiatric Disorders in Human

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The glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor(AMPAR) plays central roles in neuronal functions. However, clinical translation of AMPA knowledge is limited due to the inability to visualize AMPAR in the living human brain. Here we developed a positron emission tomography(PET) tracer for AMPARs, named compound A, and showed its specific binding to AMPARs. Logan graphical analysis in first-in-human PET study with healthy participants revealed reversible binding of compound A. Further, compound A revealed systemic reduction of AMPARs in patients with depression, while patients with schizophrenia exhibited focal decrease of AMPARs in parahippocampal and cingulate gyrus. These decreases were significantly correlated with their symptomatology scores in both disorders. Thus, compound A could be a useful tool to study biological base of psychiatric disease, and expected to be a novel diagnostic drug in the clinical setting. (COI: No)

2P-238 (Y-18)

Molecular profiling of the subthalamic nucleus

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The basal ganglia is a group of brain regions which are interconnected in a highly sophisticated manner. Among the interacting functional units in the basal ganglia, the subthalamic nucleus (STN) is considered as the sole excitatory nucleus and plays a critical role as the information hub of the network. However, the current understanding of cell types existing in the STN lacks systematic investigation. Therefore, we have taken advantages of advanced cell labelling techniques such as multiplexed fluorescent *in situ* hybridization, immunofluorescence, and neuronal tracing to delineate molecular characteristics of the STN. We molecularly profiled the conventional cell types, such as neuronal/non-neuronal cells, and glutamatergic/GABAergic neurons, quantitatively in the STN using high-resolution microscopy and machine learning techniques. We also examined various types of neurotransmitter receptors to infer the topography of information convergence on the STN. In particular, the subunits of AMPA receptor and GABA receptor are densely expressed in the STN, the spatial distribution pattern of which reveals the balance between excitatory and inhibitory inputs. These basic characterization of the STN would enable cell-type-specific connectivity studies, and thus allow the deeper understanding of circuit functions behind neurological disorders. (COI: Properly Declared)

2P-239

Dynamics of local networks in the motor cortex during sleep and wakefulness

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Macroscopic cortical dynamics such as electroencephalogram reflects sleep/wake states and is associated with several sleep-related brain functions. The dynamics of local cortical networks during sleep is poorly understood, however, despite the local regulation of sleep in the cortex. Using optical observation of neural activity and a sparse network-based approach, we investigated the spatio-temporal structure of local networks in the superficial layer of the motor cortex during sleep/wake states. Individual neural activity in both excitatory and inhibitory neurons was, on average, decreased during NREM sleep and increased during REM sleep, compared with wakefulness. Neural connectivity decreased during NREM sleep and increased during REM sleep. Inhibitory networks exhibited a prominent increase in connectivity with non-adjacent neurons during REM sleep.
These findings indicate that neurons in the superficial local networks of the motor cortex are only sparsely connected during NREM sleep and densely connected during REM sleep, which might contribute to acquiring and maintaining new memories. (COI: No)

2P-240

Relation between Montreal Cognitive Assessment and amygdala-hippocampus volumes in the elderly

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Morphological brain changes in the amygdala (AMG) and hippocampus (HI) are important index for evaluating memory and cognitive functions, have been investigated across the stages of Mild Cognitive Impairment (MCI). Our previous study reported that the AMG volume was associated with Japanese version of Montreal Cognitive Assessment (MoCA-J) scores in 18 elderly subjects with self-reported non-complain about mild cognitive deficits (age from 60 to 83 years old). In this study, we tested which MoCA-J sub-domains including assessments of visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall and orientation (time and place) functions would relate to AMG and HI volumes. Scores of recalling words task was significantly correlated with bilateral HI and the right AMG volumes, and scores of naming task was linked to the left AMG (all $r > 0.5$, $P<0.05$). Recalling words and naming objects tasks may need memory retrieval and linguistic functions, and may require an interaction with the prefrontal regions which play roles for executive function and decision making. Declining of above two MoCA-J sub-scores might be first sign of morphological changes of AMG and HI, might be used for an index of early diagnosis of MCI. (COI: No)

2P-241

Sensory integration and behavioral choice regulated by the metabotropic glutamate receptor

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Animals integrate multiple sensory inputs to make decisions. The sensory integrations and decisions, which are controlled by the excitation/inhibition states of the nervous system, remain unclear. In this study, we focused on a model animal, *Caenorhabditis elegans,* which is able to integrate sensory inputs and choose behavior using only 302 neurons. The worms display positive chemotaxis toward a number of odorants such as benzaldehyde (Bz) and diacetyl (DAc). First, we developed an assay to investigate whether the worms choose staying in the vicinity of the source of the Bz or leaving for the remote source of the DAc. Then, we performed screening using 1498 mutant strains and found that a metabotropic glutamate receptor, MGL-1 in the interneurons AIY, is required for the behavior. Next, we performed calcium imaging to visualize the neuronal activities during sensory integration. Although the DAc or Bz alone evokes simple activation or inhibition of the AIY, respectively, we found that the calcium level of AIY was decreased transiently and activated soon by simultaneous stimulation of olfactory neurons sensing the DAc and Bz, suggesting that the AIY neurons integrates the multiple odor inputs. Additionally, we found that the timing of the integration was disturbed in the *mgl-1* mutant. From the observation and genetic approaches, we are considering that the summation of sensory inputs in a single interneuron, which is regulated by the MGL-1, controls the behavioral choice. (COI: No)

2P-242

Two-photon imaging of neuronal activity in motor cortex of non-human primate during reaching tasks

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Two-photon imaging has revealed neuronal activities underlying a variety of brain functions in rodents and other small animals. Here we extend this technique to record the neuronal activity in motor cortex of marmosets during reaching tasks**.** Because marmosets have posed a challenge due to limited success in training on the tasks, we first developed the protocol to train marmosets to perform upper-limb reaching tasks. The marmosets learned to control a manipulandum to move a cursor to a target on a screen after 2–5 months of training sessions. We succeeded in two-photon calcium imaging of layer 2/3 neurons in the motor cortex during this motor task performance and detection of task-relevant activity from multiple neurons, dendrites and axonal boutons. In a two-target reaching task, some neurons showed movement direction-selective activity over the training days. In a short-term force-field adaptation task, some neurons dynamically changed their activity when the force field is on. In addition to two-photon calcium imaging, optogenetics in behaving marmosets may become a fundamental technique for analysing neuronal circuits underlying action and cognition. We are now validating optogenetic tools to be readily applied to manipulate neuronal activity in the marmoset cortex. In this meeting we will discuss the functional impact of the optical stimulation to modulate the neuronal activity and the behaviour. (COI: No)

2P-243

Calcium imaging data from premotor area predict features of upcoming movement

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The rostral forelimb area of the motor cortex is regarded as a motor planning region. Neural activities in this area may encode upcoming movements. As conventional multi-electrode array recordings are biased to more active neurons and provide limited spatial relationship of recorded neurons, we tested the possibility of using 2-photon calcium imaging data to predict features of upcoming movement. We collected GCaMP6f calcium signals from rostral forelimb area in layer 2/3 of the motor cortex while mice performed a two-dimensional lever reaching task. We applied a deep convolutional neural network architecture to learn spatial characteristics of the calcium imaging data and predict features of upcoming movements, namely the location of the maximum reach of the forelimb. We found that the prediction accuracy was higher than the probability of pure chance. Thus, our study demonstrated that imaging data containing spatial relationship of active neuronal clusters from motor planning region provide significant information related to movement target. (COI: No)

2P-244

In vivo Ca^{2+} imaging of mouse brain by two-photon excitation spinning-disk confocal microscopy

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Two-photon excitation laser scanning microscopy (TPLSM) has been usually used for visualizing neural activities in animal brains, since near-infrared laser lights for excitation offer a superior penetration depth and is less-invasive for living specimens. Previously, we had developed novel TPLSM by utilizing a spinning-disk confocal scanning unit (CSU-MP φ100, Yokogawa Electric) to achieve high speed tomographic imaging (TPLSM-SD; Otomo, *et al.,* **Anal. Sci.** 2015). In this study, TPLSM-SD with Yb-based laser (λ: 1040 nm, power: 4 W, rep. rate: 10 MHz, pulse width: 300 fs, femtoTrain, Spectra-Physics) or Ti:Sa laser (920 nm, 1.2 W, 80 MHz, 100 fs, Mai Tai DeepSee, Spectra-Physics) was applied for *in vivo* imaging in mouse brains. As a result, we successfully demonstrated cross-sectional imaging of individual neurons expressing EYFP in Thy1-EYFP-H mice at ~400-μm depth from the surface. In GLT1-GCaMP7tg mice (kindly given by Dr. Hirase in RIKEN CBS, Monai, *et al.,* **Nat. Commun.** 2016) , we succeeded in *in vivo* volumetric imaging of Ca^{2+} signals (80-100 μm depth at 10-μm intervals) from spontaneous activities of astrocytes and neurons, at 1 stack/s temporal resolution. At present, we are trying to observe neuronal responses in the layer 2/3 of the auditory cortex with high temporal resolution. Thus, TPLSM-SD system might complete in a shorter time to visualize finer cortical structures as well as neural activities with a higher temporal resolution. (COI: Properly Declared)

2P-245

Uptake and Release of Mn Ions from Neuron as a Basis of Mn MRI

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As nerve cells uptake Mn ions through Ca channel depending on nerve activity, and Mn ions induce the increase of T1 signal of MRI, Mn-MRI is used to monitor the brain activity in vivo. Then, we studied the rate of Mn uptake and its relese from nerve cells using cultured rat Hippocampal neurons, Ca ions inside the cells were monitored using Fluo4 which induces fluorescence by binding with Ca ions, The fluorescence of fluo4 is reduced by Mn ions which bind very strongly with Fluo4. When nerve cells were activated by glutamate Mn ions enter into nerve cells slowly. When Fluo4 was charged after Mn ions uptake, glutamate activation induced Ca entry into the cells followed by reduction of fluorescence due to release of Mn ions. This result indicates that Mn ions inside the cells were uptaken to the vesicles inside the cells and Mn ions were released from the vesicles by the Ca induced manner. When Mn ions charged cells were treated several timed with with glutamate, Mn ions inside the cells disappeared, and the flurescence of Fluo4 was not reduced by glutamate activation, Therefore, Mn ions inside the cells were released by nerve activation. The present study suggests that mouse should be activated only when Mn ions were charged. We found that mouse brain is strongly activated by restraint stress by Mn-MRI method using Bruker 9.4T MRI machine with cryoprobe. (COI: No)

2P-246

Two-photon laser ablation cut sole neural processes without severe damage on surrounding astrocytes

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Information processing in brains is achieved as network functions of neurons and glial cells. In particular, several reports have shown that astrocytes might modulate flows of information. To shut off specific flows of information, we have recently improved two-photon laser ablation (TPLA) to cut single neural processes within deep regions of living mouse brains. In addition, traumatic brain injuries have been reported to increase intracellular $[Ca^{2+}]$ in astrocytes (astrocytic $[Ca^{2+}]_i$) via IICR pathway (Kanemaru, *et al.*, 2013). Here, we examined effects of TPLA targeted neural processes on surrounding astrocytes by *in vivo* Ca^{2+} imaging. First, we mated YFP-H mice with G-CaMP7-G7NG817 mice (kindly given from Dr. Hirase in RIKEN, Monai *et al.*, 2016) to visualize neurons and astrocytic $[Ca^{2+}]_i$ simultaneously. TPLA targeted to a single neural process increased astrocytic $[Ca^{2+}]_i$, nearby, that eliminated within 10 minutes. In contrast, TPLA directly applied to astrocytes induced their morphological distortions and astrocytic $[Ca^{2+}]_i$ increases persisting over 10 minutes. Therefore, TPLA targeted to a single neural process might not induce severe damages on surrounding astrocytes. In addition, these different astrocytic $[Ca^{2+}]_i$ increases suggested that different TPLA might trigger corresponding Ca^{2+} signaling pathways. Such *in vivo* manipulations and analyses would give us insights on modulation of network functions. (COI: No)

2P-247

Topical pH change in the brain by visual stimulation revealed by CCD pH image sensor

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Our brain functions (e.g. memory, learning, and perception) are regulated by precise control of neurotransmitters in neural circuit. Recent studies have shown that proton functions as neurotransmitters (Du et al, 2014), and localized pH fluctuations occur in the brain (Magnotta et al, 2012). Previously, ion sensitive field effect transistor (IS-FET) has been applied for neurophysiological measurements (Bergveld et al, 1970), and a charge transfer type pH image sensor was realized to visualize two dimensional chemical phenomena (Hizawa et al, 2005, Martinoia et al, 2001). However, pH change in the brain have not been elucidated by neural circuit level. In this study, a new type ion imaging sensor was inserted into the visual cortex to visualize pH condition, and visual stimulation was applied to stimulate neuron in the visual cortex. Interestingly, we found that different topical pH changes for each direction of the stimulation. Recent study has reported that protons are released from nerve ending as a neurotransmitter, extracellular pH can directly change neural activity. Thus, the topical pH change might be caused by protons released from visual stimulated neurons. Furthermore, pH decrease in psychological diseases including schizophrenia and bipolar disorder (Hagihara et al, 2017). Thus, pH imaging in the brain of neural disorder's model using this pH sensor might provide new insight into elucidation of neural diseases. (COI: No)

2P-248

Differential characteristics of D1 and D2-type medium spiny neuron via cortico-striatal stimulation

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Striatum is major subcortical region in the forebrain which play an essential role in higher brain functions including voluntary movement and reward learning. These behavioral properties are thought to be mediated by excitatory input from cerebral cortex to striatum, which in turn modulates the basal ganglia output. The striatal inhibitory projection neuron divided to the dopamine D1 receptor (D1)-type medium spiny neuron (MSN) and the dopamine D2 receptor (D2)-type MSN. These two classes of MSNs could account for the direct and indirect pathways, respectively. In the present study, we evaluated the transient response of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) of D1- and D2-type MSN via the brief repetitive stimulation with various frequencies from the cortical excitatory input. To evaluate $[Ca^{2+}]i$ in striatal cells, we prepared acute striatal slice from transgenic mice expressing yellow fluorescent protein in D1 and D2-type MSN, respectively. In both MSN, the rate of $[Ca^{2+}]i$ transients become larger as the frequency range of stimulation increased. In addition, the D1-type MSN showed significant increase $[Ca^{2+}]i$ transient rates in the stimulation of beta frequency range compared with that of D2-type MSN. Our findings suggest that the difference in the specific frequency response between D1 and D2-type MSN may involve in the modulation of motor mechanism. (COI: No)

2P-249

Error signals in the red nucleus drive adaptation in reaching

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We have recently shown that motor cortices (primary motor cortex and premotor cortex) and parietal areas (areas 5 and 7) encode information on end-point errors in reaching, and that post-movement microstimulation to these regions causes trial-by-trial increases in errors. Based on these results we suggested that these areas provide error signals that drive trial-by-trial adaptation in reaching movement (Inoue et al., 2016; Inoue & Kitazawa, 2018). The red nucleus (RN) receives inputs from motor cortices and area 5 and sends output to the climbing fibers of the cerebellar cortex via the inferior olivary nucleus. We thus aimed at testing whether the error signals in RN are causally related to adaptation in reaching. To this end, we examined neuronal activities of RN while two monkeys made rapid reaching movements toward a visual target. Approximately half of the RN neurons encoded information on target positions before the onset of movement and/or visual errors after the end of movement. These results were similar to those of complex spikes of Purkinje cells (Kitazawa et al. 1998). We then delivered electrical microstimulation after the touch by using the same electrode. Repetitive pairing of reaching movements with microstimulation produced a gradual and significant increase of the endpoint error opposite to the preferred direction of visual errors. These results suggest that the RN provide error signals that drive trial-by-trial adaptation in reaching movement. (COI: No)

2P-250

Withdrawn

2P-251

LTD is regulated by drebrin isoforms conversion likely due to the difference in the isoform dynamics

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Drebrin increases F-actin stability by elongating its helical crossover. It localizes in dendritic spines and regulates spine shapes. Drebrin E is expressed in the developing brain and is replaced by drebrin A after maturation. To elucidate the role of drebrin isoform conversion, we developed drebrin A knockout (DAKO) mice, in which drebrin E is expressed throughout development, and adulthood and drebrin E and A double knockout (DXKO) mice and analyzed the LTD in these mice using slice electrophysiological techniques. While low frequency stimulation (LFS) induced NMDAR-dependent LTD in the developing hippocampus in wild-type mice, it did not induce LTD in DXKO mice. On the other hand, LFS induced mGluR-dependent LTD in both developing and adult brains of DAKO mice. These indicate that drebrin expression is critical for NMDAR-dependent LTD induction, and the drebrin conversion regulates mGluR-dependent LTD. Then we hypothesized that the drebrin conversion regulates mGluR-dependent LTD by changing F-actin stability. We analyzed the dynamics of drebrin E and drebrin A in dendritic spines using cultured hippocampal neurons by fluorescence recovery after photobleaching analysis. Drebrin A had a larger stable fraction than drebrin E, and F-actin depolymerization reduced the stable drebrin A fraction. Thus, *in-vivo* preferential binding of drebrin A to F-actin likely causes the higher stable fraction, although *in-vitro* F-actin-binding ability of drebrins E and A are comparable. (COI: Properly Declared)

2P-253

A strategy of NMDA receptor-dependent oscillation in the visual cortex of rats

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Periodic fluctuation of electrical signals generated in the brain must include important codes that convert signals into information. However, many issues related to the oscillatory phenomena are unclear. Not only individual neurons but also neuron networks can generate membrane potential oscillation. As one of experimental model for the study of oscillation, caffeine-induced oscillation was proposed, in which periodic fluctuations of postsynaptic potentials are generated following caffeine treatment of rat neocortical slices. Optical recording using voltage-sensitive dye enables us to detect spatio-temporal wave propagation. By using this method, it was found that this type of oscillation is composed of oscillator-activities and traveling waves. The main oscillator-activities were NMDA receptor-dependent, and the origin of the oscillation was fixed in the secondary visual cortex. An interesting finding was that repetitive delivering of the NMDA receptor-dependent signals made new routes emerged as shortcut pathways between the visual cortex and the retrosplenial cortex. Changes of the route reduced the signal traveling time by about 40ms. These results propose a new concept of use-dependent network changes, in which traveling neural signals may try to find suitable paths of propagation during repetitive signal traveling. Spatial refinement with the opening of a shortcut and subsequent reductions in signal traveling time may be an important strategy. (COI: No)

2P-254

Retrieval-Induced Forgetting in Young Mice

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Retrieval-induced forgetting (RIF) is a phenomenon that retrieval practice on a subset of target items leads to forget the other, non-target items. We have previously reported that RIF occurs in adult and aged mice in a modified spontaneous recognition test. However, it is not known whether RIF occurs during a developmental stage. Here, we examined the RIF occurrence in 4-weeks old young male and female C57BL/6J mice. The test consisted of three phases; sample, retrieval-practice (Rp) and test. The intervals between the sample and Rp phase and between Rp and test phases were 1 h. Mice were assigned to three experimental conditions (Rp+, Rp-, Nrp). In the sample phase of the Rp+ condition, mice explored a field in which two objects (A, B) were placed. In the Rp phase, one of the two objects was replaced to an object identical to the other (A, A). In the test phase, one of the objects used in the retrieval phase was replaced again to a novel object (A, X). In the Rp- condition, after the Rp phase, mice were explored two objects different from those in Rp phase, but one of which was identical to the one in the sample phase (B, Y). In the Rp phase of the Nrp condition, two objects (C, D), which were not used in sample and test phase, were placed in the field. The discrimination index in the Rp- condition was significantly lower than that in the Rp+ condition in both male and female mice. These results indicate that RIF also occurs in young mice. (COI: No)

2P-255

The mitochondrial system of hippocampal adult-born neurons in the Tg2576 mouse model

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A large number of studies indicate that mitochondria are involved in neuroplasticity and their dysfunctions participate in neurodegenerative diseases, particularly in Alzheimer's disease (AD). In parallel, alterations of hippocampal adult neurogenesis have been described in AD patients and in several mouse models of AD. The present study was designed to decipher whether altered mitochondrial system in adult-born neurons of Tg2576 mice could participate to impaired hippocampal adult neurogenesis in these mice. We examined for the first time, the mitochondrial network of adult-born hippocampal neurons in Tg2576 mice. We used a retroviral vector pCMMP-IRES2eGFP-WPRE expressing the enhanced green fluorescent protein (GFP) to birth-date and follow developing adult-born hippocampal neurons in Tg2576 and non-Tg mice. Immunodetection of GFP and mitochondria (OXPHOS antibody) resulted in confocal images that allowed to quantify dendritic spines and mitochondrial content of adult-born neurons in these mice. Our results showed that new neurons of Tg2576 mice display lower mitochondrial content both in their soma and dendritic compartments, which might be either due to a decreased number and/or size of the mitochondria, compared to non-Tg mice. These findings suggest that mitochondria may be pertinent and innovative targets for enhancing or rescuing neurogenesis-dependent hippocampal plasticity, thus opening new avenues for early therapeutic intervention to fight AD. (COI: No)

2P-256

Effects of PDIA3 on Neurogenesis in the Dentate Gyrus of Normal and Ischemic Gerbils

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Purpose: In the present study, we investigated the effects of PDIA3 on cell proliferation and neuroblast differentiation in the dentate gyrus of gerbils under two different conditions: normal and ischemic damage.

Method: On day 24 of Tat-PDIA3 treatment, transient forebrain ischemia was conducted by occlusion of both common carotid arteries for 5 min. Animals were euthanized 2 h post last Tat-PDIA3 treatment and were processed to conduct immunohistochemical staining for Ki67 and doublecortin (DCX).

Results: Ki67 positive nuclei and DCX immunoreactive neuroblasts were significantly higher in the PDIA3-treated group compared to the untreated control group. In ischemic control and PDIA3-treated groups, the number of Ki67 positive nuclei and DCX immunoreactive neuroblasts were significantly higher as compared to those in the normal control and PDIA3-treated group, respectively. Transient forebrain ischemia increased the expression of phosphorylated cAMP response element binding protein (pCREB) in the dentate gyrus, but the administration of PDIA3 significantly increased pCREB positive nuclei in the normal gerbils, but not in the ischemic gerbils.

Conclusion: These results suggest that Tat-PDIA3 enhances cell proliferation and neuroblast differentiation in the dentate gyrus in normal, but not in ischemic gerbils, by increasing pCREB expression. (COI: Properly Declared)

2P-257

Different mechanism of actions of testosterone and estradiol on cognitive impairment in male rats

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We had previously reported that orchidectomy (ODX)-induced androgen deficiency impaired spatial learning and memory in male rats. However, it is questioned how testosterone (T) acts, via androgen or estrogen pathway, because T can be converted to 17β-estradiol (E_2), and estrogen receptors are also expressed in male brain. Here, we determined the mechanism of action of dihydrotestosterone (DHT) and E_2on transcript expression of genes associated with cognition in rats. ODX rats were daily treated with distilled water (DW; 1 ml p.o.), DHT (1mg/kg BW, s.c.) or E_2(80μg/kg BW, s.c.) for 2 months. After 2 months, rats were accessed spatial learning and memory ability by Morris Water Maze test. The mRNA expression levels of genes associated with synaptic plasticity (SP), neurofibrillary tangle (NFTs) and amyloid plaques (AP) in hippocampus were determined using qrt-RTPCR. Both treatments with DHT and E_2prevented the ODX-induced spatial learning and memory impairment although DHT had a greater effect than E_2. Likewise, DHT and E_2could prevent the ODX-induced formation of NFTs by lowering *Tau3*and *Tau4*mRNA levels and could maintain the SP by increasing *Syn*, *GluN1*and *Bdnf* mRNA levels. Noting that DHT had a greater efficacy on NFTs, while it was on SP for E_2. Besides, DHT could also decrease AP by reducing *App* mRNA expression.These indicate that either T or E_2can be used to prevent cognitive impairment in males, however, the different mechanisms of action need to be considered. (COI: No)

2P-258

Modulation of dentate granule cell activity during fear memory extinction in freely moving mice

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Granule cells (GCs) in the hippocampal dentate gyrus are capable of processing pattern separation of episodic memories. Nevertheless, little is known about how this is achieved in behaving animals.

To tackle this issue, we expressed the calcium sensor GCaMP6f in GCs of adult C57BL/6 mice by means of stereotaxic adeno-associated viral vector injection. We recorded fluorescent signals representing Ca^{2+} transients in freely moving animals by using a miniature microscope during a procedure of cued fear conditioning, retrieval and extinction.

After conditioning, cue exposure triggered freezing behavior, confirming the successful retrieval of cued fear memory. Along with extinction of the fear memory, cue-triggered freezing behavior decreased significantly in conditioned mice. Our imaging data revealed a significant increase in the number of active GCs and in the activity-dependent induction of Fos immediate early gene during cued fear extinction. Furthermore, GCs of conditioned mice exhibited a transient increase in firing frequency and a higher cue selectivity during extinction sessions. Finally, chemogenetic inhibition of GCs not only decreased cue-induced freezing, but also undermined the further extinction of the fear memory when mice were exposed to the second extinction session.

Overall, our results suggest that transient increase in the activity of dentate GCs during the course of memory extinction is necessary for the extinction process to proceed efficiently. (COI: No)

2P-259

Impairment of memory and hippocampal synaptic plasticity induced by high-fat diet in animal model

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The metabolic syndrome is a common disorder that causes the increasing prevalence of obesity. High fat diet (HFD) is a common cause of obesity which is accompanied by not only peripheral but also brain insulin resistance that increases the risk of memory impairment. The main part of the brain involved with memory is the hippocampus. Hippocampal synaptic plasticity including the long-term potentiation (LTP) plays an important role in the formation of new memories. Previous studies have shown that brain insulin resistance may be manifested as altered cognitive deficits in memory. However, there is no much information on whether HFD affects hippocampal synaptic plasticity. In this study, 8-week old, male C57BL6N mice were randomly assigned a high fat diet (60% energy from fat) or a standard diet (SD) for 12 weeks. First, HFD group gained significant body weight compared with the SD group. Second, we also found that HFD impaired memory in the novel object recognition (NOR) behavioral test. Furthermore, HFD caused detrimental effects on hippocampal synaptic plasticity including LTP impairment by using electrophysiology. The results suggested that the memory impairment and hippocampal synaptic plasticity deficits were induced by HFD in animal model. (COI: No)

2P-260

Overexpression of K+ Cl- cotransporter promotes activity dependent synaptic plasticity and learning

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KCC2 is a neuron-specific K+-Cl- cotransporter which has been known to play an important role in regulating intracellular chloride concentration of the neurons. Besides its Cl- transport function, the role of KCC2 on spine has been reported in the development and function of glutamatergic synapses. However, the role of KCC2 in synaptic plasticity of adult CNS remains incompletely understood. Here, we examined the effects of KCC2 overexpression for synaptic plasticity in the motor cortex and on motor learning in vivo. We created a genetically modified mouse which is overexpression of KCC2 downstream of the calcium-calmodulin-dependent protein kinase II promoter (KCC2/over). KCC2/over mice more greatly improved motor learning compared with WT mice. Using transcranial two-photon imaging, we investigated synaptic remodeling during learning, especially new spine formation. Spine formation rate of KCC2/over mice, but not the WT, after the first training significantly increased compared with that before training. A more spines generated at the 1st day of training in KCC2/over mice consequently led to a more survival of new spines in number at the 2nd day than WT mice. And, the ratio of newly formed spines of KCC2/over mice at 1st day of training co-relates with an improvement of motor performance at the 2nd day. Those finding suggested that overexpression of KCC2 enhanced the number of new spine formation for activity-dependent, and might be lead to improvement of motor learning. (COI: No)

2P-261

Investigating the effects of muscle wasting on Alzheimer's disease

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With aging, there are progressive functional declines in multiple organ systemse. One of the major physiological problems observed in aged people is skeletal muscle loss. This age-related muscle loss results in muscle weakness, which in turn may reduce physical activity and quality of life in the elderly and also influences the progression of several diseases, especially Alzheimer's disease (AD). AD is an age-related neurodegenerative disorder, characterized clinically by progressive cognitive deficits. A previous study has suggested that loss of muscle mass and strength could be linked to the risk of developing AD. In addition, unintended weight loss often happens in AD patients and may reflect dementia severity. However, the causal relationship of muscle atrophy and cognitive deficits in AD remains unknown. Here, we found that amyloid precursor protein (APP) and presenilin 1 (PS1) double-transgenic (APP/PS1) mice in older age exhibited lower body weight and lean tissue mass than sex- and age-matched wildtype (WT) mice. In addition, muscle atrophy and the extent of memory decline was strongly correlated in APP/PS1 mice. We also detected that myostatin levels were elevated in the tibialis anterior and gastrocnemius of 12 month-old APP/PS1 mice and delineated the cellular and molecular mechanism of muscle atrophy was through by ubiquitin-proteasome system. These results demonstrate that increased myostatin could be a mediator to trigger muscle atrophy and cognitive deficits. (COI: No)

2P-262

HSYA improves cognitive function in MCAO rats via recovering synaptic plasticity in the hippocampus

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Hydroxysafflor yellow A (HSYA) is the major active chemical component of the safflower plant flower, which is widely used in Chinese medicine for cerebrovascular and cardiovascular disease treatment. Recent studies have demonstrated that HSYA exerts neuroprotective effect on cerebral ischemia, such as neuronal anti-apoptosis, antioxidant activity and oxygen free radical-scavenging. However, whether and how HSYA has a protective effect on cognitive impairment induced by cerebral ischemia reperfusion remains elusive. In the present study, by using the middle cerebral artery occlusion (MCAO) model, we found that 8 mg/kg and 16 mg/kg HSYA administration by common carotid artery injection improved impaired cognitive function in Morris water maze and passive avoidance tasks, but not 4 mg/kg HSYA treatment, suggesting that HSYA treatment in a certain concentration can improve cognitive impairment in MCAO rats. Furthermore, we found that 8 mg/kg HSYA treatment rescued the impaired Long-term potentiation (LTP) in hippocampus of MCAO rats. Taken together, these results for the first time demonstrate that HSYA has the capacity to protect cognitive function and synaptic plasticity against cerebral ischemia-reperfusion injury, and provide a new insight that HSYA may be a promising alternative for recovery of cognitive dsyfunction after brain ischemic injury. (COI: Properly Declared)

2P-263

The response to whisker stimulation in the visual cortex of monocular deprived mice *in vivo*

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Sensory inputs are essential to detect the external environment, but a part of them is disturbed in the patients of blindness. Traditionally, the concept of cross-modal plasticity has been raised, which an impaired sensory input is compensated by the other sensory systems. The study using positron emission tomography showed that the blind people use their visual area while reading Braille. Whisker-dependent activation of visual cortex was demonstrated in the eye enucleated mice. However, the functional changes of lost sensory cortical area *in vivo* have not been shown yet. In this research, we try to unravel the effect of early visual deprivation on the activation of the visual area with whisker stimulation. We first visualized the axonal projection from S1 (primary somatosensory cortex) to V2 (extrastriate cortex) by injecting Cholera Toxin Subunit B (Recombinant), Alexa Fluor™ 488 Conjugate, a retrograde neuroanatomical tracer. We hypothesized the activation triggered by whisker stimulation in S1 can be transmitted and processed in V2 as well. To verify our hypothesis, we combined *in vivo* two-photon imaging with the AAV injection to label the neuron in V2 with Ca^{2+} indicators and stimulated whisker to see its response in V2. Furthermore, we assessed synaptic pruning by microglia, which might be a driving force to modify the neurocircuit. This study will be an important clue to understand the compensating ability of the cortex for the future therapeutic target. (COI: No)

2P-264

Metabotropic glutamate receptor 5 (mGluR5) has a critical role in behavioral flexibility

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Behavioral flexibility is the ability to adjust behavior according to changing circumstances. This cognitive function is disrupted in many different neuropsychiatric and neurodegenerative disorders, however the underlying mechanism is still under investigation. Different study groups have reported that mice with genetic ablation or pharmacological inhibition of mGluR5 showed impairments in fear extinction and spatial reversal learning, indicating that mGluR5 signaling is involved in the regulation of behavioral flexibility. While most of these behavioral studies exposed the testing mice in high stress conditions using electric foot-shocks and forced water swimming, mGluR5 has also been reported to be implicated in the stress response. Therefore, evaluating the direct role of mGluR5 in low stress condition is important, to clearly distinguish between stress-induced cognitive rigidity and the mGluR5 gene effect. We have evaluated behaviors of WT and mGluR5 KO mice in a low stress rodent touchscreen operant platform using the appetitive conditioning. Groups of adult male mice were evaluated in a series of touchscreen tasks including two-choice visual discrimination-reversal, extinction, ratio-schedules and the effort-related choice. As a result, mGluR5 KO mice showed impairments in reversal and extinction, which was driven by a perseverative responding behavior. Altogether, our results indicate that mGluR5 signaling has a critical role in behavioral flexibility. (COI: No)

2P-265

Increase of sleep spindle density induced by rTMS for major depression

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Background: Cortical pyramidal cell is reciprocally connected to both thalamocortical neuron (Th-Cx) and reticular nucleus of the thalamus (RE). Th-Cx is delta oscillator and RE is spindle oscillator. We previously reported that high frequency repetitive transcranial magnetic stimulation (rTMS) to the left DLPFC induced localized power enhancement of the slow wave activity at around the stimulation site. In this study, we investigated whether high frequency rTMS could enhance spindle oscillation.**Methods:** Twelve patients with major depression underwent 10 daily rTMS sessions over two weeks. The frequency was 20Hz and the stimulation site was left DLPFC. Polysomnographic data were recorded 4 times (adaptation, baseline, post 5 sessions and post 10 sessions). Sleep spindles were visually identified by a single rater who was completely blind to sleep EEG data profile. **Results:** Sleep spindle density at F3 electrode increased significantly at post 5 rTMS sessions (4.80 /min) as compared to the baseline level (3.37 /min) (t=3.70, df=11, p=0.003). Also, total amount of sleep spindle was significantly increased (t=3.33, df=11, p=0.007) from the baseline (828.4 /night) to post 5 rTMS sessions (1284.3 /night).**Conclusion:** A series of high frequency rTMS sessions to the left DLPFC increased the spindle density at the stimulation site. High frequency rTMS may induce facilitative effect on RE through cortical pyramidal cell, resulting in enhancement of sleep spindle activity. (COI: No)

2P-266

Speed representation in the hippocampus and entorhinal cortex

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Place cells in the hippocampus and grid cells in the medial entorhinal cortex (MEC) are functionally specialized cells that represent an animal's current location. To update spatial representation that reflects the ongoing self-motion, place cells and grid cells must use information regarding the current direction and speed of the animal's movement. Speed is encoded by context-invariant, speed-responsive "speed cells" in the MEC and hippocampus. However, speed cells in different cell layers and sub-regions of the hippocampal-entorhinal circuit have not yet been fully characterized quantitatively. Using data recorded simultaneously from the hippocampus and MEC (Mizuseki et al., 2014, F1000Res 3:98), we compared speed representation across sub-regions and layers in the entorhinal-hippocampal loop. Consistent with previous reports (Kropff et al., 2015, Nature 523, 419-424; Ye et al., 2018, PNAS 115, E1627-E1636), we found that a significant fraction of speed-responsive cells in the hippocampus and MEC were putative interneurons. Reportedly, many speed cells in the MEC encoded speed prospectively, whereas those in the hippocampus retrospectively (Kropff et al., 2015). Further, we found that the CA1, CA3 and each layer in the MEC had a distinct degree of prospective and retrospective representations of the running speed. Therefore, our results suggest that each anatomical station in the hippocampal-entorhinal circuitry handles speed-related information in a distinct manner. (COI: No)

2P-267

Withdrawn

2P-268

Hippocampal, amygdala neuronal, and sympathetic nerve activities in odor-cue fear conditioned rats

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The hippocampus and amygdala are critically involved in formation of recall fear memories. Fear memory recall is associated with patterned changes in sympathetic nerve activity (SNA) and cardiovascular function. There is a lack of direct evidence on hippocampal neuronal, amygdala neuronal, and sympathetic nerve activities in response to fear memory recall. In the present study, we attempted to measure the time course of changes in hippocampal CA1 neuronal (HpCA1NA), central amygdala neuronal (CeANA), renal sympathetic (RSNA), and lumbar sympathetic nerve (LSNA) activities during fear memory recall in odor-cue fear conditioned rats. Male Wistar rats were chronically implanted with electrodes for measurement of HpCA1NA, CeANA, RSNA, LSNA activities, electroencephalogram (EEG), electromyogram (EMG), and with a catheter for measurement of arterial pressure (AP). For fear conditioned rats , trials consisted of an odor conditioned stimulus (CS; anisole) and a fear-producing shock unconditioned stimulus (US; 5 mA, 1 sec). Contextual and anisole-cue conditioned stimuli caused increases in freezing behavior, LSNA, and AP, and a decrease in heart rate. Insignificant changes were detected in RSNA and HpCA1NA. These data suggest that the amygdala is involved in fear memory recall triggered by odor conditioned stimuli, as well as differential changes in SNA and cardiovascular function. (COI: No)

2P-269

Two groups of SPNs in cholinergic modulation of corticostriatal plasticity in dorsomedial striatum

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The cholinergic interneurons (CINs) of the striatum are crucial for behavioral flexibility. CINs of the dorsomedial striatum (DMS) play a role in strategy switching. However, how CINs modulate the neural circuitry underlying strategy switching is unclear. The glutamatergic afferents from the cerebral cortex to the striatum display activity-dependent plasticity in the corticostriatal synapses, and may be involved in certain types of learning. One hypothesis is that strategy switching may be realized by a modulatory effect of CINs oncorticostriatal plasticity. Here, we investigated the effect of CINs on activity-dependent plasticity in the corticostriatal synapses. To optogenetically inactivate CINs, AAV encoding halorhodopsin (NpHR) was injected into DMS of ChAT-cre mice. AAV injected mice expressed NpHR in CINs. We recorded EPSPs induced by electrical stimulation of corpus callosum using ex vivo slice whole-cell recording from spiny projection neurons (SPNs) – the output neurons of the striatum. Activity dependent synaptic plasticity was induced by high-frequency stimulation under the Mg-free conditions. This conditioning stimuli conbined with optogenetically inactivation of CINs during HFS induced the long-term potentiation in some SPNs. However, other group of SPNs showed long-term depression This result might indicate that CIN activity modulate corticostriatal plasticity in different manner between direct and indirect SPNs. (COI: No)

2P-270

Contribution of Thyrotropin-Releasing Hormone to Cerebellar Long-Term Depression and Motor Learning

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Thyrotropin-releasing hormone (TRH) regulates various physiological activities through activation of receptors expressed in the central nervous system, including the cerebellum. A synthetic TRH analogue, taltirelin, has been shown to ameliorate ataxic behavior in hereditary ataxic mice, and is used for treatment of patients with spinocerebellar degeneration. However, the function of endogenous TRH in the cerebellum and the mechanisms by which the TRH analogue mitigate cerebellar ataxia remain to be clarified. To address this, we studied TRH knockout (KO) mice. In the Rotarod test, but the learning speed of KO mice in subsequent trials was significantly slower than that of WT littermates, suggesting impaired motor learning. Since long-term depression (LTD) at parallel fiber-Purkinje cell synapses is thought to be cellular basis for cerebellar motor learning, we examined LTD at these synapses using the patch clamp technique. We found a complete absence of LTD in KO mice, which was rescued by bath-application of TRH or 8-bromoguanosine cyclic 3',5'-monophosphate, a membrane-permeable cyclic guanosine monophosphate (cGMP) analog. Notably, intraperitoneal injection of TRH in KO mice rescued the motor learning deficit. Thus, TRH is critically involved in cerebellar LTD, possibly by production of cGMP in Purkinje cells, and, consequently, in motor learning, through which taltirelin may exert its therapeutic action in mutant mice and patients with spinocerebellar degeneration. (COI: No)

2P-271

Sharp-wave ripples facilitate memory consolidation via activation of cAMP

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Sharp-wave ripples (SPWr) are important for memory consolidation. We hypothesize that fast oscillations (200 Hz) and burst firing of pyramidal neurons caused by SPWr facilitate memory consolidation by providing an influx of Ca2+ required for activation of the cyclic adenosine monophosphate (cAMP) pathway. We contextual fear conditioned (CFC) rats and afterwards they were allowed to sleep and were either left undisturbed (naïve control group) or their hippocampal activity during sleep was transiently suppressed immediately upon detection of SPWr to interrupt them (SPWr (-) group) or 250 ms later (delayed stimulation control). Upon completion of the post-learning session, animals were anesthetized and sacrificed by decapitation. Their dorsal and medium hippocampi were dissected into CA1, CA3 and DG regions. Using Western-blot analysis we measured activation of PKA and Epac content. We also tested whether pharmacologically activating the cAMP signaling pathway through intra-hippocampal injections of Sp-cAMPs in the absence of SPWr could rescue the contextual fear memory. Our results show a significant decrease in the pPKA/PKA ratio in the dorsal CA1 only in SPWr suppressed animals. Further, pharmacologically activating the cAMP singling pathway reverted SPWr suppression induced memory impairments. This is the first evidence that SPWr provide a natural trigger of cAMP signaling pathway for memory consolidation in sleep. (COI: No)

2P-272

Real-time dynamism of hippocampal CA1 firings after the 4 different episodic stimuli

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The hippocampal CA1 is necessary to maintain experienced episode. To monitor the temporal dynamics, we recorded multiple-unit firing of CA1 neurons in freely moving male rats before, during, and after episodic events. Rats were experienced either restraint stress, or first encounter with a female, a male, or an object for 10 min. Although CA1 neurons mostly showed sporadic firings in their home cage, multiple neurons exhibited high frequent spontaneous firings (super burst) during and soon after episodes. Episodes with female or restraint frequently induced the super bursts, while episode with male or object induced the events inconsistently. Minutes after initiation of episode, multiple neurons repeatedly exhibited ripple-like synchronized firings with less-firing silent periods. Repeated ripple-like events showed a wide diversity of the shape, duration, or frequency, but the number of events depended on experienced episode.

To further examine synaptic plasticity, we made acute brain slices 30 min after episodes. Compared with inexperienced controls, episodes with female, male, and restraint significantly increased the amplitude of miniature EPSCs and IPSCs, while episode with novel object increased the amplitude of miniature IPSCs only. Kernel density analysis further exhibited episode-specific diversity at the excitatory/inhibitory synapses.

Here we found experience-specific spontaneous firing patterns and synaptic plasticity in the pyramidal layer of CA1 neurons. (COI: No)

2P-273

Understanding the mechanism of odor-specific memory formation in *Caenorhabditis elegans*

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Purpose: Accurate assessment of the surrounding environment is important for survival, and animals ranging from *C. elegans* to mice rely on their sense of smell to gain such information. Odors are detected through the combinatorial activation of odorant receptors, which comprise the largest protein families in many organisms. In mice, an important strategy for odor discrimination is the one-cell-one-receptor rule, a highly regulated process that allows transcription from one receptor gene allele while the rest, as many as a few thousand alleles, stays repressed. On the other hand, the nematode *C. elegans* possess only a few odor sensory neurons, with each neuron expressing multiple odorant receptors. How such set up allows for sufficient odor discrimination and survival is not known.

Methods: To study of how odor discrimination is achieved in the simple nervous system of *C. elegans*, we used the odor memory phenomenon in various genetic backgrounds to see whether worms can form distinct memories to odors detected by the same sensory neurons.

Results and Conclusion: Previously we have identified additional odorants that are detected by AWC sensory neuron. Using the expanded library of odors, our preliminary results show that even within the odorants detected by the same neuron, worms show different levels of discrimination. We are currently conducting a candidate mutant screen to identify factors involved in this phenomenon. (COI: No)

2P-274

Withdrawn

2P-276 (Y-19)

Characterization of a novel and potent neuronal Kv7/M opener SCR2682 for anti-epilepsy

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Voltage-gated Kv7/M-channels play an essential role in control of membrane potential and neuronal excitability. Activation of neuronal Kv7-current represents an attractive therapeutic strategy for treatment of hyperexcitability-related neuropsychiatric disorders such as epilepsy, pain and schizophrenia. In this study, we synthesized and characterized a novel series of 2,6-dimethyl-4-(piperidin-yl)phenyl)-amide derivatives that exhibit selective and potent activation of neuronal Kv7 channels. Whole-cell patch clamp recordings of HEK293 cells expressing Kv7.2/7.3 channels show that a representative compound SCR2682 selectively activates the channel current in dose-dependent manner with an EC_{50} of 9.8 ± 0.4 nM, which is about 100-fold potent than antiepileptic drug retigabine approved by FDA for partial epilepsy. SCR2682 shifts the voltage-dependent activation of Kv7.2/7.3 current towards more negative membrane potential about -37 mV ($V_{1/2}$). SCR2682 also activates native M current of rat hippocampal neurons, causing a marked hyperpolarization and potent inhibition of evoked action potentials. In rat epileptic models, intraperitoneal and intragastric administrations of SCR2682 results in a dose-dependent inhibition of seizures. Taken together, our findings demonstrate that a novel SCR2682 selectively and potently activates neuronal Kv7 channels and reverses epileptic activity in rats. Thus, SCR2682 may warrant further evaluation for clinical development of antiepileptic therapy. (COI: No)

2P-277 (AP-1)

Chronic stress causes excessive aggression by altering synaptic actin dynamics in the mPFC

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Behavioral and psychological symptoms of dementia (BPSD) are an integral part of dementia syndrome. In particular, BPSD such as chronic stress induced excessive aggression is known to be more stressful to caregivers than the cognitive and functional problems of the patients with dementia. Therefore, the effective treatment for excessive aggressive behavior is required. There is evidence that functional circuits in the medial prefrontal cortex (mPFC) regulate social cognitive functions including aggressive behaviors. Also, social isolation, one form of chronic stress environment, can lead to the development of excessive aggression. However, the underlying cellular and molecular mechanisms of the mPFC neural network involved in chronic stress environment induced aggression is largely unknown.

To clarify the molecular mechanism of mPFC neuronal network with excessive aggression, we examined aggressive behavior in rat model of chronic social isolation focusing on mPFC synaptic plasticity. We further investigated the relationship between synaptic actin dynamics and AMPARs delivery in spines of mPFC of chronic stressed animals. Here, we show that chronic stress environment changes spines in the mPFC by reducing actin dynamics, leading to the decrease of synaptic AMPA receptor delivery and altered social cognition and aggressive behavior. Our study provides molecular and cellular mechanisms underlying the influence of chronic stress environment on social cognition and aggression. (COI: Properly Declared)

2P-278

ASD-like Behaviors and Synaptic Defects Inherit to Subsequent Generations in VPA-Induced Rat Model

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder mainly characterized by impaired sociability and often accompanied with affective comorbidities. Recent studies indicated that the etiology of ASD, a male-predominant disease, is based on genetic and environmental factors. Prenatal exposure to valproate (VPA) is a well-established environmentally-induced ASD model. We have previously found that the VPA-induced offspring exhibit ASD-like behaviors which strongly linked to its synaptic pathophysiology within amygdala. However, whether VPA-induced autistic traits pass to the subsequent generations is still unclear. Thus, we aim to investigate the heritability of VPA-induced ASD model. Among the study, the second generation (F2) was produced by mating the first generation (F1) male offspring with naïve females. We compared the sociability, anxiety-like behavior and synaptic efficacy in F1 and F2 VPA-induced offspring. Our results showed that both F1 and F2 generations displayed social deficit and anxiety-like behavior in three-chambered social test and elevated plus maze, respectively. Moreover, in the lateral nucleus of the amygdala of both generations, the basal synaptic response performed normally, yet the paired-pulse ratio decreased significantly during electrophysiological recordings. To sum up, our results suggested that VPA, an environmental toxin of ASD, also has impact on their descendants, which supported epigenetics, a novel hypothesis for ASD etiology. (COI: No)

2P-279

Withdrawn

2P-280

Genome-wide screening of genes involved in tau aggregation by CRIPSR/Cas9 system

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Aggregation of microtubule-associated protein tau is a common pathological character in tauopathy, which represents a wide range of neurodegenerative disorders including Alzheimer disease, Pick disease and frontotemporal dementia (FTD). Several point mutations in *MAPT* gene that encodes tau protein have been identified in familial forms of FTD, suggesting that tau aggregation plays an important role in the pathogenesis of tauopathies. Tau is highly soluble and natively unfolded. However, once tau undergoes conformational changes to form oligomer, aggregated tau starts to show broad range of pathological features: formation of amyloid fibrils, propagation between cells and impairing the cellular function. However, a detailed molecular mechanism of trigger or maintenance of tau aggregation has not been well characterized. To elucidate this mechanism in detail, we carried out genome-wide screening of tau aggregation using CRISPR/Cas9 system and tau fibril specific fluorophore PBB3. We first established Cas9/tau expressing HEK293A cells with whole genome targeting gRNA lentiviral library. Because PBB3 specifically interacts with tau fibrils, changes in tau aggregation in cells can be detected by alterations of fluorescent intensity. We then collected cells with abnormal PBB3 intensity using FACS. Genes targeted by CRISPR/Cas9 system in these cells are analyzed by deep sequencing. We will present our progress of the screening and validation experiments. (COI: No)

2P-281

Berberine attenuated the cytotoxicity induced by t-BHP via inhibiting oxidative stress and mitophagy

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Neurodegenerative diseases, including Parkinson's disease(PD), and Alzheimer's disease(AD), which all involved with oxidative damage and mitochondrial dysfunction in pathogenesis. Oxidative stress (OS) results in a dysfunction of mitochondria and initiation of the cell death cascade. Berberine (BBR), a traditional Chinese medicine, which have been reported that has anti-oxidative stress effect, but the mechanism on regulating mitophagy under oxidative stress remains unclear. The present study was undertaken to evaluate the beneficial effects of BBR on the tert-butyl hydroperoxide (t-BHP)-induced cytotoxicity, apoptosis, oxidative stress in cells. Our results demonstrated that BBR effectively inhibited t-BHP-stimulated cytotoxicity, and cell apoptosis and suppressed reactive oxygen species (ROS) overproduction, cytochrome c release and recovered the ratio of Bcl-2/Bax. Additionally, BBR recovered the normal level of mitochondria membrane potential ($\triangle\Psi m$) and ATP production. Furthermore, BBR reduced LC3, SQTM1/p62 expression and maintained mitochondria and lysosome normal function, which was involved with restored the upstream signaling pathway AKT and mTOR phosphorylation levels. These findings suggested that BBR protects cells from t-BHP-induced cytotoxicity and apoptosis through inhibiting oxidative stress and mitophagy via PI3K/AKT/mTOR signaling pathways. In conclusion, BBR may be a potential therapeutic strategy for the treatment of neurodegenerative diseases. (COI: No)

2P-282

Chloroquine promotes the recovery of SCI by inhibiting inflammation and ER stress

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Spinal cord injury (SCI) is a severe nervous system disease that may lead to lifelong disability. Studies have shown that autophagy plays a key role in various diseases; however, the mechanisms regulating crosstalk between autophagy, inflammation and endoplasmic reticulum (ER) stress during SCI recovery remain unclear. This study was designed to investigate the mechanism by which chloroquine (CQ) inhibits autophagyassociated inflammation and ER-stress in rats during acute SCI. We evaluated the locomotor function, level of autophagy, and levels of inflammatory cytokines and ER-stress associated proteins and examined the degradation of the key regulator of inflammation I-κBα through autophagy by analyzing the colocalization of I- κBα, p62 and LC3-II. In addition, overexpression of the p62, ATF4 silencing plasmids was used to verify the important roles for autophagic degradation and ER-stress. In this study, locomotor function is improved, and autophagy and inflammation are significantly inhibited by CQ treatment in the model rats. In addition, CQ significantly inhibits the degradation of ubiquitinated I-κBα and blocks the nuclear translocation of NF-κB p65 and expression of inflammatory factors. Moreover, the ATF4 signaling pathway is required for ER-stress induced activation of autophagy. These findings reveal a novel mechanism underlying the beneficial effects of CQ on the recovery of SCI, particularly the mechanisms regulating crosstalk between autophagy, inflammation and ER-stress. (COI: No)

2P-283

GLYX-13 alleviates chronic stress-induced depression-like behavior through its actions in midbrain

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Purpose
Major depressive disorder affecting more than 100 million people worldwide every year is a heterogeneous illness. To date, current pharmacotherapies require prolonged administration from several weeks to months for an appreciable response. GLYX-13 is a rapid-acting and long-lasting antidepressant. However, it is still unclear that whether GLYX-13 alleviates chronic stress-elicited depression-like behavior through its actions in midbrain periaqueductal gray (PAG).
Methods
Depression-like behavior in the rats were induced by footshock stress paradigm. Forced swim test (FST) and sucrose preference test (SPT) were used to study the depression-like behavior. Miniature excitatory postsynaptic currents (mEPSC) were recorded to elucidate the neuronal activity.
Results
Rats receiving footshock stress procedure exhibited an increase in immobile time during the FST and a reduction in sucrose consumption. Reduced amplitude and frequency of mEPSCs in the PAG were found in rats receiving footshock stress. Intravenous GLYX-13 injection decreased immobile time during the FST and increased sucrose consumption in rats receiving footshock stress. Intravenous GLYX-13 injection increased both amplitude and frequency of mEPSCs in the PAG obtained from rats receiving footshock stress.
Conclusions
GLYX-13 alleviates chronic stress-induced depression-like behavior including despair behavior and anhedonia through its actions in the midbrain periaqueductal gray. (COI: No)

2P-284

Effects of optogenetic inhibition of 5-HT neurons in the dorsal raphe nucleus on respiratory control

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5-HT neurons in the dorsal raphe nucleus (DRN) affect vigilance state switching and those in the median raphe nucleus (MRN) influence anxiety-related respiratory control. It has been reported that anxiety depends on the balance of 5-HT neural activity between the DRN and the MRN. In the present study, we investigated whether 5-HT neurons in the DRN affect respiratory control. Genetically modified mice (Tph2-ArchT), in which ArchT is expressed in central 5-HT neurons selectively, were used. In the *in vitro* experiments, activity of the DRN neurons was recorded extracellularly in the slice preparation from the mice. In the *in vivo* experiments, the mice were anesthetized and an optical fiber was inserted above the DRN. More than 1 wk after insertion of the optical fiber, green light illumination was carried out, while the mice were placed in a whole body plethysmograph in the dark and respiratory curves in free-moving mice were recorded. Neuronal firings *in vitro*, and respiratory variables in vivo were compared with/without ArchT expression in 5-HT neurons. Green light illumination decreased firing frequency of DRN neurons in vitro and tended to induce respiratory facilitation *in vivo*. These results suggest that ArchT stimulation inhibits 5-HT neuronal activity in the DRN of the mice, which is related to respiratory control. The outcomes in the Tph2-ArchT did not contradict our previous results in Tph2-ChR2 mice, in which channelrhodopsin-2 was expressed in central 5-HT neurons. (COI: No)

2P-285

Astrocytic Ca^{2+} signals via IP_3 receptor type2 mediate reactive astrocytes after status epilepticus

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Introduction: Using pilocarpine-induced status epilepticus (SE) model, we have already reported that reactive microglia appeared first, which followed by reactive astrocytes ("epileptogenic astrocyte") contributing to epilepstogenesis after SE. However, the molecular mechanisms underlying activation of astrocytes after SE are still unknown. In this study, we investigated astrocytic Ca^{2+} signals and determined its pathophysiological relevance to astrogliosis after SE.

Methodology: Pilocarpine was administrated to induce SE in 8-week-old male mice and IP_3 receptor type2 (IP_3R2) knockout mice. Microglial activation and astrogliosis were assessed by immunohistochemistry. For functional analysis, Ca^{2+} was imaged using Fluo-4 in hippocampal slices.

Results: We detected a significant increase in the area of Iba1-positive microglia at 1 day after SE, which was followed by the increase in the area of GFAP-positive astrocytes (epileptogenic astrocytes) in the hippocampal CA1 area at 4 weeks after SE in WT mice. Such astrocytes displayed significantly larger and more sustained Ca^{2+} signals, which were mediated by IP_3R2. In IP_3R2KO mice, the SE-induced increase in the area of GFAP-positive astrocytes was significantly reduced without affective the area of Iba1-positive microglia.

Conclusions: These results suggest that IP_3R2-mediated astrocytic Ca^{2+} signaling should play a central role in induction of epileptogenic astrocytes induced by initially activated microglia after SE. (COI: Properly Declared)

2P-286

CSD is accompanied by mitochondrial oxidaization wave revealed with Flaboprotein autofluorescence

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Migraine is a severe and disabling condition and a public health problem which impacts on not only the patient and its family but society. Cortical spreading depression (CSD), a wave of cellular depolarization that propagates slowly across the brain surface followed by suppression of brain activity, has been presumed to be the physiological substrate of the migraine aura. Previous reports showed chronic intermittent administration of nitroglycerin (NTG) to mice resulted in a chronic migraine model animal. In this study using the autofluorescence of mitochondrial flavoproteins and conventional recording electrodes, we imaged cortical activity in the awake animals either in normal control mice or the NTG mice. After high concentration potassium chloride induced excitation, CSD was observed followed with an intrinsic fluorescent signal changes in mitochondrial flavoproteins which slowly propagates on the cerebral hemisphere in the normal mice. In the NTG mice, oxidative mitochondrial activities were easily expressed on the cerebral hemisphere not only by high-potassium excitation, but by some environmental stimulating trigger such as light strikes on the eye. During CSD expression, those NTG mice showed little activity to become listless. We hypothesized CSD is an oxygen and energy consuming phenomenon, that results in the disabling condition in migraine. (COI: Properly Declared)

2P-287

Impaired olfactory identification in patients with cerebrovascular disease

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The importance of olfactory assessments for patients with neurological diseases has recently attracted attention. Although cerebrovascular disorder is one of the most frequent disease in Japan, there are a few reports on olfaction in patients with stroke. Using T&T olfactometry and Open Essence, we examined olfaction in detail in patients with cerebrovascular disease. The subjects were 50 patients hospitalized in our hospital for neurorehabilitation after stroke. Twenty-eight female and 22 male patients had sufficient cognitive function for olfactory testing. None of them complained of olfactory loss. A significant discrepancy between the perception and identification thresholds in T&T olfactometry and only one answer of "no odor detected" in Open Essence were obtained. Image study revealed that odor identification was not impaired in patients with a putamen-thalamus lesion. Odorants B, D, H, I, G and K in Open Essence were correlated to the total scores. Patients with stroke showed impaired odor identification with intact odor perception. For odor identification, the direct pathway from the piriform cortex to the orbitofrontal cortex is considered to be essential. Correlation analysis suggested that six odorants are sufficient for olfactory assessment. (COI: No)

2P-288

Physiological characteristics of rhythmic masticatory muscle activity during sleep in children

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[Purpose] Sleep bruxism (SB) is a sleep related movement disorder characterized by the frequent occurrence of rhythmic masticatory muscle activity (RMMA). Prevalence of SB is highest during childhood and decreases with age. This study aims to investigate physiological characteristics of RMMA in children in comparison to adults. [Methods] Overnight polysomnography was performed for ten children (M/F : 6/4, 10.1 ± 2.5 years old) and seven young adults (M/F : 3/4, 23.1 ± 1.6 years old) without any physical/neurological problems. Both children and adults were diagnosed as having SB based on the assessment of RMMA episodes using masseter EMG and video records. The occurrence of RMMA in association with sleep stages, arousals, body movements were assessed. [Results] Children and adults exhibited RMMA at 6.0 ± 1.4 times/hr and 6.5 ± 2.4 times/hr, respectively. In both group, RMMA occurred in light non-rapid eye movement sleep (children : 70.1 ± 10.3%, adults : 69.0 ± 15.3%) in association with arousals (children : 94.4 ± 3.7%, adults : 93.4 ± 3.4%). However, RMMA occurred more frequently with body movements in children (51.0 ± 14.5%) than in adults (25.1 ± 20.1%) ($p<0.05$), while it occurred less frequently with leg movements in children (23.8 ± 8.4%) than in adults (45.5 ± 16.8%) ($p<0.05$). [Conclusion] The occurrence of RMMA in children with SB can be associated with the higher hierarchy of arousal response compared to that in adults with SB. (COI: No)

2P-289

Masseter muscle activity during REM sleep in young adults with sleep bruxism

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[Introduction] In sleep bruxism (SB) patients, rhythmic and non-rhythmic masticatory muscle activity occasionally occur during rapid eye movement (REM) sleep. [Purpose] This study aimed to clarify the characteristics of masseter muscle activity during REM sleep in young SB subjects. [Methods] Full-night polysomnographic recording was done in 25 young healthy subjects. Sleep variables and rhythmic masticatory muscle activity (RMMA) were scored. They were classified into 10 subjects (CTL group; M: 6, F: 4, age: 23.2 years) with RMMA less than 2 times per hour of sleep and 15 subjects (SB group; M: 8, F: 7, age: 23.2 years) with RMMA more than 4. Masseter muscle activity during REM sleep was assessed by the percentage of 3-sec mini-epochs with a rise of any EMG activity and by the percentage of 30-sec epoch without atonia and arousals (e.g., %RWA). [Results] Sleep macrostructure (e.g., the percentage of sleep stages) did not differ between the two groups. RMMA occurred more frequency during NREM and REM sleep in SB than in CTL group. The percentage of mini-epochs with masseter muscle activity was significantly higher in the SB group than CTL group (SB: 3.60±2.37%, CTL: 1.63±0.91%, $p<0.05$), while the percentage of 30-sec epoch without masseter atonia did not differ between two groups. [Conclusion] During REM sleep, SB group can have an increased activity in jaw motor system rather than a loss of motor inhibition. (COI: No)

2P-290

Role of cortico-brainstem circuits in poststroke rehabilitation-induced functional recovery

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Cortico-brainstem tracts is one of the putative substrates for reorganization of the descending system in poststroke recovery. We investigated the rehab-induced reorganization of the cortico-rubral tract (CRT) and the cortico-reticular tract (CReT) and its contribution to recovery. Internal capsule hemorrhage (ICH) rats were received forced-impaired limb use (FLU) as rehab for days 1-8 (D1-8) after ICH. Anterograde tracing revealed that ICH-FLU group rats showed significant increase of the sprouting of the CRT compared to ICH group at D12 and D51. In contrast, the sprouting of the CReT was not different between groups. Then, we injected retrograde lentivirus NeuRet-TRE-EGFP.eTeNT or NeuRet-MSCV-Cre into the red nucleus or the reticular formation, and then anterograde adeno-associated virus AAVdj-CaMKII-rtTAV16 or AAVdj-Flex-DIO-hM4D-mCherry into the rat's motor cortex. Selective CRT blockade by doxycycline administration after FLU at D13-20 caused apparent impairment of the recovered forelimb function in ICH-FLU group. However, this selective CRT blockage was gradually disappeared. Interestingly, additional CReT blockade by clozapine N-oxide administration at D21-28 under CRT blockade caused impairment of the forelimb function again. In contrast, only CReT blockade at D13-20 did not affect the FLU-induced recovery. These data suggest that the CRT plays a primal role in rehab-induced recovery after capsular stroke, but the CReT also plays alternative role on demand. (COI: No)

2P-291

The effect of orally-administered baclofen on spinocerebellar ataxia type 3 (SCA3) model mice

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Spinocerebellar ataxia type 3 (SCA3: also known as Machado-Joseph disease) is an inherited progressive neurodegenerative disease caused by abnormal CAG repeat expansion (polyglutamine tract) in the gene of ataxin-3. SCA3 exhibits cerebellar atrophy and a wide variety of motor dysfunction, and it is the most frequent type of hereditary spinocerebellar diseases in Japan. So far, there is no effective treatment for SCA3. Our previous study demonstrated that application of low-dose baclofen, an agonist of the GABAb receptor, to the cerebellum alleviates motor dysfunction in SCA1 model mice. In the present study, we examined whether orally-administered low-dose baclofen could improve motor performance in SCA3 model mice. Low-dose baclofen had no effect on motor behavior of WT mice, as measured by rotarod test. In contrast, baclofen improved motor coordination of ataxic SCA3 model mice. Interestingly, considering the pharmacokinetics of baclofen in the mouse brain, additional experiments indicated that the improvement of rotarod performance in SCA3 mice might require motor training (or activity) concomitant with the sufficient concentration of baclofen in the brain. This activity-dependent effect of baclofen on motor coordination might be a potential therapeutic approach for SCA3. (COI: No)

2P-292

Withdrawn

2P-293

Transgeneration of environmental chemicals-primed rat hyperactivity

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Attention deficit hyperactivity disorder (ADHD) is characterized by behavioral and cognitive symptoms such as hyperactivity, inattention, disorganization, and impulsivity [The etiology is considered to be multifactorial. Interaction of genetic and environmental risk factors would be affected in expression of the disorder.

The animal model for hyperactivity disorders was produced by Shaywitz et al. (1976), who demonstrated that rat pups treated with 6-hydroxydopamine at 5 days of age developed increased motor activity, leading to cognitive difficulties in shuttle-box learning between 2-4 weeks of age. According to their protocol, we previously demonstrated that neonatal exposure to environmental disruptors caused hyperactivity in the juvenile and/or adulthood.

Rat models of ADHD was created by neonatal rotenone lesions; rotenone (3mg/kg) was orally exposed to Wistar male pups at 5-day old. Their spontaneous motor activity was higher 1.3 fold than that of control rats at 11 weeks of age. At 26 weeks of age, the treated rats were mated with Wistar female rats. We examined the two strains of such mating and found the spontaneous motor activity of the offspring were much higher 1.5~2.0 fold than those of both control offspring and the parents.

Thus, in this study we show the rat hyperactivity caused by neonatal rotenone lesions was transmitted, indicating the de novo multifactorial effects and not soft inheritance of environmental chemicals. (COI: No)

2P-294

Social isolation during developmental critical window affects inhibitory neuronal circuits in mPFC

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Social isolation during developmental critical windows could be highly detrimental to proper functioning of mature prefrontal cortex (PFC). We previously reported that in mice, social isolation for 2 weeks after weaning induces prefrontal cortex dysfunction. To investigate what underlie the dysfunction, we focused on 2 types of Layer-5 pyramidal cells in the mouse mPFC: prominent h-current (PH) cells and nonprominent h-current (non-PH) cells. We showed that a 2-week social isolation after weaning leads to a specific deterioration in action potential properties and reduction in excitatory synaptic inputs only on PH cells. However, the specific inhibitory circuits of mPFC that involve social experience-dependent maturation are poorly understood. Here we show that juvenile social isolation in mice leads to increased inhibitory synaptic inputs on PH cells, with whole-cell patch clamp recording in the brain slices. We also found that social isolation from P21 to P35 lowered the spike threshold of fast spiking (FS) interneuron. So it is plausible that the effect of social isolation on inhibitory inputs onto PH cells arise from increased excitability of FS interneuron. We conclude that juvenile social experience plays crucial roles in the functional circuit development between a subtype of Layer-5 pyramidal cells and FS interneuron in the mPFC. (COI: No)

2P-295

The 40Hz-ASR may be a good predictor of conscious outcome in patients with severe head injury

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The 40Hz auditory steady-state response (40Hz-ASR) was examined in 27 patients who had swith severe head injury. All of them were deeply comatose- and evaluated as being from three to eight on the Glasgow coma scale. In this prospective study, the usefulness as a predictor of consciousness outcome was examined. The auditory brainstem response (ABR) was recorded simultaneously, and the results were compared to clarify the characteristics of, and pathophysiological differences in, of these responses. The change inof patients' consciousness level was noted successively and the condition at discharge was evaluated as the final outcome. The patients who lost waves III and V of the ABR had a poor outcome, and, in general, showed no 40Hz-ASR. The patients who exhibited wave V had low mortality. However, if they did not exhibitshow any 40Hz-ASR during their stationary stage, the consciousness disturbance was persisted and some of them became vegetative. The patients who exhibitedshowed good or fair 40Hz-ASR in the acute or subacute stage recovered from the coma. In conclusion, as the ABR correlated with the vital outcome and the 40Hz-ASR correlated with the consciousness outcomeone, they might be utilized as the indices of the life risk and consciousness recovery, respectively. Moreover, as the 40Hz-ASR tends to change prior toin advance of an alteration of the consciousness level, it may be useful tool for early prognostic evaluation of comatose patients. (COI: No)

2P-296

Deep brain stimulation for depression in rats: correction of left/right hemispheric imbalance

Yukitoshi Sakaguchi; Yoshio Sakurai (*Graduate School of Brain Science, Doshisha University, Japan*)

Hemispheric brain asymmetries are related to stress coping in both humans and rodents, and imbalanced neural activity between the left and right medial prefrontal cortexes (mPFCs) is observed in depression disorders. Electric and magnetic brain stimulations are sometimes effective to cure depression symptoms. We therefore hypothesized that the imbalanced activity of the mPFCs as well as depressionlike behaviors can be induced by chronic stress in rats, and that deep brain stimulation (DBS) can treat such behavior by correcting the asymmetrical activity of the brain regions. Our results indeed show that chronic stress exposure by social isolation (SI) causes depressionlike behavior and left/right mPFC activity changes. SI suppressed the activity of both the prelimbic and the infralimbic cortex; however, the extent of the suppression in these regions was oppositely asymmetric. Two weeks of DBS recovered the depressionlike behavior and corrected the imbalanced brain activity. In addition, original weight differences between the left and right adrenal glands (AGs) were decreased by SI and recovered by DBS. Furthermore, the index that integrates the asymmetry scores of both the mPFC and the AGs correlated better with the extent of depressionlike behavior than either score alone. (COI: Properly Declared)

2P-297

Experience and cell type–dependent induction of MeCP2 in the visual thalamus

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An increase or decrease in expression of the transcription factor MeCP2 leads to syndromic autism spectrum disorders. This indicates that the precise regulation of MeCP2 expression during development is critical for mental health. However, it remains unclear how MeCP2 expression is regulated during circuit formation and maturation. Particularly, how MeCP2 expression is induced upon sensory experience in excitatory and inhibitory neurons is not well known.
In this study, we investigated MeCP2 expression levels during the visual sensitive period of the thalamic visual relay center dorsal lateral geniculate nucleus (dLGN). We found that the MeCP2 protein levels in the dLGN gradually increased in glutamatergic but not in GABAergic neurons during development. Interestingly, dark rearing during visual sensitive period decreased MeCP2 expression selectively in the dLGN glutamatergic neurons, suggesting that the MeCP2 expression is induced in a visual experience- and cell type-dependent manner.
(COI: No)

2P-298

Function of the primate medial frontal cortex in the control of mood and affect: an rTMS study

Shinya Nakamura; Ken-Ichiro Tsutsui (*Laboratory of Systems Neuroscience, Graduate School of Life Sciences, Tohoku University, Japan*)

The medial frontal cortex (MFC) including the anterior cingulate cortex (ACC) has long been considered to be involved in the control of mood and affect. Particularly, human imaging studies have repeatedly reported that patients with mood disorders show abnormal functioning of the ACC located anterior and ventral to the genu of corpus callosum (pregenual and subgenual). In this study, we causally examined how these parts of the MFC are involved in the control of mood and affective state in monkeys by using repetitive transcranial magnetic stimulation (rTMS) as a tool of manipulating local neural activity. Low-frequency (1 Hz) rTMS (LF-rTMS), which is considered to have an inhibitory effect on the stimulated regions, was delivered for 20 min (total 1200 pulses). Following the LF-rTMS targeting the rostroventral MFC, we observed significant decrease in the spontaneous behavioral activity, motivation and sociability, and significant increase in the evening plasma cortisol level. In contrast, such changes were not observed by the LF-rTMS to the dorsal or posterior part of the MFC. Furthermore, the administration of ketamine, which has been recently considered to have a rapid and robust antidepressant effect, normalized the abnormal state induced by the rTMS intervention. These results indicate that the rostroventral MFC is critically important for the control of mood and affective state in monkeys, and the inhibition of its activity can be regarded as a model of depression. (COI: No)

2P-299

Withdrawn

2P-300

Widespread Hyperalgesia and Autonomic Dysregulation in a Rat Model of Chronic Back Pain

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Purpose Chronic back pain induced by complete Freund Adjuvant (CFA) injected in back muscles was shown to decrease sympathetic reactivity. The aim of the present study was to further characterize this chronic back pain model using behavioral pain tests and to clarify the mechanisms of decreased sympathetic reactivity.
Methods Chronic back pain was induced by a CFA injection in left T2 paraspinal muscles, while controls received saline. In Exp1, mechanical sensitivity was assessed by paw withdrawal threshold over a two-month period. In Exp 2, pain sensitivity was assessed using the Formalin test. In Exp 3, renal sympathetic nerve responses induced by hind paw stimulation were examined to test the potential sympathetic output decrease in CFA rats. In addition, intravenous phenylephrine administration was used to test potential peripheral receptor desensitization in CFA rats.
Results CFA rats showed widespread mechanical hyperalgesia over 2 months ($p<0.001$) and increased pain behaviors during the Formalin test ($p<0.05$), compared with controls. In spite of decreased sympathetic reactivity, however, no significant change was observed for renal sympathetic nerve responses to stimulation or adrenergic receptor sensitivity (both $p>0.05$).
Conclusions This study provides a model of chronic pain in which widespread hyperalgesia and pain hypersensitivity are observable over at least 2 months. However, the mechanisms of decreased sympathetic reactivity still remain to be clarified. (COI: No)

2P-301

TRPA1 mediates the uterine PGE2-induced cross-organ reflex sensitization in anesthetized rats

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Cross-organ sensitization between the uterus and the urethra may underlie the high concurrence of obstetrical/gynecological and chronic pelvic pain. However, the neural basis involved are still unclear. We tested the hypothesis that the prostaglandin E2 (PGE2) activates transient receptor potential ankyrin 1 (TRPA1) induces cross-organ sensitization on the pelvic-urethra reflex activity. In female SD rats, instilling PGE2 into the uterus sensitized the pelvic-urethra reflex activity that was reversed by an intrauterine pretreatment with HC-030031, a TRPA1-selective antagonist. Intrathecal injection of Co-101244, an NMDA NR2B-selective antagonist abolished the cross-organ reflex sensitization caused by PGE2 instillation. Our results demonstrated that TRPA1 contributes to the PGE2-dependent uterus-urethra crosstalk via phosphorylating NR2B subunit of spinal NMDA receptor. (COI: No)

2P-302

Inhibitory effects of Sake lees (Sake Kasu) on stress-induced hyperalgesia in the rats

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[Introduction] Sake lees are byproducts generated in the production of Japanese Rice Wine (Sake). Evidence indicated that intakes of Sake lees could be beneficial for our health. In this study, we tested the effects of Sake Lees on nocifensive behavior in the hindpaw under psychophysical stress conditions. [Methods] Male rats were subjected to repeated forced swim stress treatments (FST) for 3 days, and immobility time (IT) was measured to quantify the depression-like behaviors. Saline, Sake Lees Extract (SKE), Sake Lees without ethanol (SKX) was intraperitoneally injected 30 min after each FST. On Day 4, 5% formalin was injected into the hindpaw to assess nocifensive behaviors for 45 min, and Fos immunohistochemical study was conducted in the lumbar spinal dorsal horn (DH). FST increased IT at Day 3 compared to Day 1. [Results]Daily administration of SKE and SKX significantly decreased IT and nocifensive behaviors in the hindpaw compared to saline, indicating that SKE and SKX had inhibitory effects on depression and stress-induced hyperalgesia. Further, SKE and SKX reduced Fos expression in DH, especially, laminae I-II and V in FS rats. [Conclusions] These findings indicated that psychophysical stress had facilitatory effects on pain that could be due to the increases in neural activity in the spinal cord. Further, repeated Sake Lees administration can inhibit stress-induced hyperalgesia in the hindpaw with the reduction of nociceptive neural activities in the DH. (COI: No)

2P-303

Renin-angiotensin system and angiotensin II receptors in rat geniculate ganglion

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<Purpose> To clarify the role of the renin-angiotensin system (RAS) in the trigeminal somatosensory system, we investigated gene expression of RAS components and angiotensin II receptors in the trigeminal ganglion in rats. Angiotensin II, the end-product in the RAS, is well known to control blood pressure and fluid/electrolyte balance through activation of angiotensin II receptors. Recently, angiotensin II has been known to modulate neuronal excitability, neurite elongation and neuronal migration through activation of the receptors in neuronal cells. We hypothesized that if the receptors are expressed in neural pathway, angiotensin II may regulate formation and function of neural circuitry via the receptors. <Methods> Trigeminal ganglia were obtained from rats under anesthesia. Total RNA was extracted from trigeminal ganglia and cDNA was synthesized from the RNA template by reverse transcription. The gene expression levels were determined by real-time PCR. <Results & Conclusions> Expression of angiotensinogen, renin, angiotensin-converting enzyme, angiotensin II type-1a, -1b and type-2 receptor mRNAs were evident in the trigeminal ganglion. This result suggests that angiotensin II produced in the local RAS may regulate trigeminal neural circuitry via the receptors expressed in the trigeminal ganglion. (COI: No)

2P-304

Inhibitory effect of bee venom on the reserpine-induced pain and depression-like behavior in mice

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We designed this study to investigate the effect of bee venom treatment on the reserpine-induced pain and depression in mice. To induce an animal model, reserpine was administered subcutaneously with a dose of 1 mg/kg once a day for 3 consecutive days. Bee venom at doses of 0.008, 0.016 or 0.08 mg/kg was subcutaneously applied into the bilateral knee area once daily at 3 days after reserpine injection for 5 days. Von Frey filament was applied to assess pain response and immobility time of forced swimming test was measured. In a separate set of experiment, traveled distance of mice in the open field box was recorded and analyzed. Reserpine treatment successfully induced pain and depression-like behaviors. Repeated bee venom treatment significantly ameliorated reserpine-induced mechanical allodynia, immobility time responses and moved distance. In conclusion, results of this study suggest that the bee venom stimulation with appropriate dose may be a good candidate to treat as non-pharmacological alternative medicine for pain and depression patients. (COI: No)

2P-305

Distribution of HCN4 positive cell in mouse spinal dorsal horn

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We have generated double transgenic mouse in which the expression level of pacemaker channel HCN4 (hyperpolarization-activated, cyclic nucleotide-sensitive channel subtype 4) can be reversibly switched off by the application of doxycycline (DOX). In this mouse, the expression locus of HCN4 can be also visualized by chemical luminescence by firefly luciferase, and we found robust signal in the dorsal horn of spinal cord. We therefore carried out immunohistochemistry with an anti-HCN4 antibody; the HCN4-immunoreactivity existed in the dorsal horn, which was not detectable when the expression of HCN4 was completely inhibited by DOX. The HCN4-immunoreactive neurons were concentrated in laminae II inner (IIi) and III, and most of them were located ventrally to the protein kinase C gamma (PKCg)-immunoreactive plexus (a maker of lamia IIi), while a few of them were colocalized with (PKCg)-immunoreactivity.

It has been reported that HCN4-immunoreactivity was found in the parvalbumin expressing, inhibitory interneurons in the laminae IIi and III of the dorsal horn. In order to test the consistency between our results and the previous reports, genetic marking of inhibitory interneurons as well as HCN4 may be necessary in the future study. (COI: No)

2P-306

Response properties of premotor heat-sensitive neurons in awake behaving monkeys

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To evaluate whether the ventral premotor cortex (PMv) neurons receive nociceptive inputs and modulate the execution of various body movements, two Japanese monkeys (*Macaca fuscata*) were trained to detect small temperature changes applied to the facial skin, and single neuronal activities were recorded from the PMv during heat detection performance.

Fifty-seven neurons altered their activities during application of heat stimuli to the facial skin and also responded to non-noxious tactile stimuli to the face. T1 neurons increased their spike activity during an initial temperature (T1) change but not during a second temperature (T2) change (n=12). T2 neurons increased their activities during the T2 but not a T1 stimulus (n=35). T1/T2 neurons were activated during both T1 and T2 stimuli (n=10). T1 and T1/T2 neurons increased their activity during T1 stimuli in response to the T1 stimulus intensity, and T2 and T1/T2 neurons also increased their firing frequency during T2 stimuli following an increase in T1 stimulus intensity.

The present study demonstrated that many PMv neurons in awake behaving monkeys respond to tactile stimulation of the face and/or to other parts of the body, and some of them also respond to heat stimulation of the face and light stimulation. The present findings suggest that three types of heat-sensitive PMv neurons are differentially involved in modulation of complicated motor performances in awake behaving monkeys. (COI: No)

2P-307 (Y-20)

Molecular mechanism of dopamine-induced itch in mice

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An itch is an extremely unpleasant skin sensation that elicits the desire or reflex to scratch. Recently, it was reported that some Parkinson's disease patients treated with L-DOPA suffered from an itch feeling. Notably, dopamine D1-like receptors mediate itch, and compound 48/80-induced scratching was suppressed by D1-like receptor antagonist. Here, we showed the ability of endogenous dopamine to produce an itch. TPRV1 can act as a molecular target of endogenous dopamine-induced itch.

Dopamine induced scratching in a dose-dependent compared with vehicle. Cheek model mice displayed increased itching without pain after with dopamine and SKF38393. Dopamine compared with vehicle and ergocryptine, while ergocryptine never induced itching or pain. Dopamine and SKF38393 induced itching decreased in D5 receptor knockdown mice compared with scrambled control mice and D1 receptor knockdown mice. The response of D1 receptor knockdown mice was similar to that scrambled. Dopamine was induced transient Ca^{2+} responses, it was the inward current and the discharge rate of the single fibers is reduced by the capsazepine. The capsazepine reduced dopamine- and SKF38393-induced itching. We confirmed the co-localization of the D5 receptor, GRP, and TRPV1 in DRG neurons.

These results show that TRPV1 activation via the D5 receptor mediates the dopamine-induced itch response. Thus, TRPV1 may serve as a new therapeutics target, including for the itching side effect of L-DOPA in the PD patients. (COI: Properly Declared)

2P-308

Negative modulation of TRPV1 by alpha 2 adrenergic receptor agonist, Dexmedetomidine

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Dexmedetomidine, an α2 adrenergic receptor agonist and novel sedative drug with minimal respiratory suppression, shows anti-nociceptive activity in various pain models by poorly understood mechanisms. Because α2 adrenergic receptor is up-regulated and co-localized with TRPV1 polymodal nociceptive receptor in neuropathic pain animal models, the analgesic activity might be mediated through inhibition of TRPV1. This study aims to test whether TRPV1 and α2 adrenergic receptor are co-expressed and dexmedetomidine would modulate TRPV1 activity in mice dorsal root ganglion (DRG) neurons. To estimate modulation of TRPV1 activity by dexmedetomidine, we measured the capsaicin-induced increase of intracellular calcium concentration with and without dexmedetomidine pretreatment in mice primary cultured dorsal root ganglion (DRG) neurons, by fura-2 based intracellular calcium ratiometry. Concentration of capsaicin applied was 400 nM, 3 times that of EC50 concentration. As result, dexmedetomidine (2, 10, 50 μM) significantly reduced capsaicin responses ($P<0.01$), in dose-dependent manner. RT-PCR analysis revealed expression of TRPV1 and all three subtypes of α2 adrenergic receptor in mice DRG neurons. In summary, these results suggested that the inhibition of TRPV1 by dexmedetomidine might be a plausible mechanism that contributes to the anti-nociceptive action of the drug. (COI: No)

2P-309

Direct Mechanical stimulation evoked Gd^{3+}-sensitive inward current in trigeminal ganglion neurons

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Purpose: Pulpitis results in the rise of tissue pressure in the dental pulp, which affects C neurons mechanically, since the head tissue surrounding the dental pulp (dentin/enamel) provides a low compliance enviroment in the pulp. However, detailed properties for mechanosensitivity of the trigeminal ganglion (TG) neurons remained to be clarified. We classified TG neurons into 3 types of diameter, and measured biophysical and pharmacological properties of mechanical stimulation-induced ionic currents. Materials and Methods: TG neurons were isolated from neonatal Wistar rats (5-8 days old) under pentobarbital sodium anesthesia (50 mg/kg). Dissociated TG cells were cultured with 48 hours at 37℃ (95% air and 5% CO2). We classified TG cells into large, medium and small-sized, and identified these cells showing voltage-dependent inward currents as neurons by the whole-cell patch-clamp recording. Results: We recorded voltage-dependent inward/outward current from all sized TG neurons and then recorded mechanical stimulation-induced currents which were consisted by 2 components; a transient current with fast activation and inactivation kinetics (first component) and a lasting current with slow activation and inactivatation kinetics (second component). Strech-activated channels (SACs) antagonist (1 μM Gd^{3+}) significantly inhibited the inward current amplitudes induced by mechanical stimulation. The results suggested that SACs involves in the mechanical sensitivity of TG neurons. (COI: No)

2P-310

ASIC 3 contributes to mechanical hypersensitivity in the rat model of cold exposed osteoarthritis

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In the present study, we investigated the involvement of peripheral acid-sensing ion channel 3 (ASIC3) in mechanical hypersensitivity in the rat model of ambient cold exposed osteoarthritis (OA).

OA was induced in male SD rats by intra-articular injection of monosodium iodoacetate (MIA). Mechanical hypersensitivity was evaluated by measuring weight bearing forces of affected limb (WBFs) before and after exposure to cold (4 °C, 6 h) or room temperature (RT). The pH of synovial fluids was evaluated at post injection day 2. The articular tissues were examined using immunofluorescence assay. Relative mRNA expression of ASIC3 in the synovial tissue were determined by quantitative real-time RT-PCR. The effects of a selective ASIC3 inhibitor (APETx2) were tested.

Rats exposed to ambient cold showed significant decrease in WBF ($p<0.05$), compared to arthritic rats housed in RT. The average pH of synovial fluids was significantly lower ($p<0.05$), compared to those of naïve and rats housed in RT. The mRNA expression of ASIC3 in synovial membrane was up-regulated approximately 2.4-fold when exposed to ambient cold. Intra-articular injections of APETx2 recovered the WBFs in the arthritic group.

We provide the evidences that ambient cold exposure enhances the decrease of pH in the synovial fluid and the severity of synovitis in the knee joints of MIA-induced arthritic rats. Consequently, it may contribute to mechanical hypersensitivity through the up-regulation of peripheral ASIC3. (COI: No)

2P-311

Increased transport of spinal l-lactate from astrocytes causes mechanical hyperlageisa via PKA

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Spinal astrocytes are activated in neuropathic pain. These reactive astrocytes are suggested to play an important role in the maintenance of neuropathic pain. We recently reported that the selective activation of spinal dorsal horn astrocytes causes mechanical hyperalgesia through the excessive L-lactate supply to neurons via monocarboxylate transporters (MCTs). In this study, we investigated the cellular mechanisms of spinal L-lactate-induced mechanical hyperalgesia. We examined the effect of MCTs knockdown on intrathecally administered L-lactate-induced mechanical hyperalgesia using siRNA to each MCTs. Knockdown of MCT2, but not MCT1 or MCT4, attenuated the L-lactate-induced mechanical hyperalgesia. Since MCT2 is mainly expressed in neurons, the L-lactate transported into neurons may produce mechanical hyperalgesia. Next, we examined the intracellular signaling of L-lactate-induced mechanical hyperalgesia. Intrathecal treatment with L-lactate increases the expression of both phosphorylated protein kinase A (PKA) and cAMP response element binding protein (CREB), which were attenuated by the pretreatment with MCT inhibitor. The L-lactate-induced mechanical hyperalgesia was also attenuated by the pretreatment of PKA inhibitor. These results suggest that the L-lactate supplied from astrocytes to neurons sensitizes the nociceptive transmission in the spinal cord through the phosphorylation of PKA and CREB. (COI: No)

2P-312

Neuronal representation in the S1 cortex during formalin-induced spontaneous pain in mice

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The objective assessment of spontaneous pain is a longstanding challenging issue in the fields of pain and neuroscience. In this study, we directly examined neuronal activity patterns in the primary somatosensory cortex (S1) and behavioral responses simultaneously under formalin induced spontaneous pain condition in mice. Using *in vivo* two-photon Ca^{2+} imaging, we recorded and analyzed neuronal activities at the cellular and population level from the S1 of awake and head-fixed mice during formalin induced pain. We found that S1 neuronal activity increased in the 1st early phase and 2nd late phase after a formalin treatment, and decreased to the resting state levels during the interphase, which were correlated with behavioral responses. These changes were highly dependent on the concentration of formalin. This result suggests that S1 neuronal activities would be valuable for the quantification of spontaneous pain. (COI: No)

2P-313

Effects of Cinnamic Acid on Chemotherapy-Induced Peripheral Neuropathy

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Single injection of oxaliplatin (Oxa, 6 mg/kg, i.p), a platinum based chemotherapeutic agent, can induce cold and mechanical allodynia (chemotherapy-induced peripheral neuropathy, CIPN), which could interrupt the treatment schedule. In our previous article, we reported that Cinnamomi cortex (CC) could reduce cold and mechanical hypersensitivity induced by oxa injection. Based on this result, we conducted further research to divulge which chemical substances of CC play a major role in this analgesic effect. By using High Performance Liquid Chromatography (HPLC), we found that Cinnamic acid (CA) and Cinnamaldehyde were the main components of CC. Thus, we firstly focused on CA and conducted behavioral tests with three different doses of CA (10, 20, and 40 mg/kg, i.p) in rats. Among these doses, 20 mg/kg was shown to possess the strongest analgesic effect both on cold and mechanical allodynia induced by Oxa. This analgesic effect appeared one hour after injection. In order to confirm the analgesic effect of CA, we conducted *in vivo* electrophysiology experiments in the rat spinal cord. Single Oxa injection significantly increased the frequency of spinal neuronal activities compared to vehicle on both the cold (acetone drop) and mechanical (press, pinch, and brush) stimuli. CA (20 mg/kg, i.p) effectively attenuated this increased frequency induced by Oxa. These results suggest that CA may be used an effective as a therapeutic option for CIPN. (COI: No)

2P-314

Effect of Bee Venom Derived Phospholipase A2 on Nerve Injury-Induced Neuropathic Pain

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Bee venom (BV), which is widely used in traditional Korean medicine to relieve pain, has an analgesic effect on several neuropathic pain (NP) models. However, which component of BV attenuates symptoms of NP is poorly studied. In our previous study, we showed that intraperitoneal injection of bee venom derived phospholipase A2 (bvPLA2), which is a major component of BV, could attenuate oxaliplatin induced cold and mechanical allodynia through the noradrenergic system, via activation of the α_2-adrenergic receptors.

This study was designed to further investigate the effect of bvPLA2 on the nerve-injury induced NP in rats. To induce NP, spinal nerve ligation was performed in Sprague Dawley rats. Moreover, we checked whether mechanical and cold allodynia developed after surgery through repetitive behavior assessment. Mechanical allodynia occurred within 4 days after surgery and cold allodynia occurred within 7 days after surgery. Both allodynia remained at least 3 weeks after surgery. From 14 days after the surgery, bvPLA2 (0.2 mg/kg, i.p.) was administrated for five consecutive days. We observed that repetitive treatment of bvPLA2 relieve mechanical and cold allodynia. Furthermore, we also investigated the involvement of the noradrenergic receptor activation in the analgesic effect of bvPLA2. (COI: No)

2P-315

EP_4 receptor-mediated augmentation of I_h currents in Abeta DRG neurons underlies neuropathic pain

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Injury or disease of the somatosensory system causes neuropathic pain. We have analyzed the mechanism of neuropathic pain in rats with the left L5 spinal nerve transection under the humane care. Ten days after surgery, these rats acquired tactile and thermal hyperalgesia. The current-clamped enzymatically isolated L5 dorsal root ganglion neurons of all sizes in the ipsilateral side exhibited significantly higher excitability than those in the contralateral side. Under the voltage clamp, however, only neurons with a diameter of 40-50 μm in the ipsilateral side showed significantly larger density of an inward current density at <-80 mV (I_h current) with a rightward shifted activation curve compared with those in contralateral side. Ivabradine, an I_h current inhibitor inhibited I_h currents in these neurons at both sides in a similar concentration-dependent manner with an IC_{50} of ~3 μM. Moreover, oral administration of ivabradine significantly alleviated the mechanical and thermal hyperalgesia in the ipsilateral side. An inhibitor of adenylyl cyclase or an inhibitor of EP_4 receptors (CJ-023423) inhibited ipsilateral but not contralateral I_h currents in these neurons. Furthermore, intrathecal administration of CJ-023423 significantly attenuated neuropathic pain in the ipsilateral side. Thus, ivabradine and/or CJ-023423 may be a lead compound for the development of novel therapeutics against neuropathic pain. (COI: Properly Declared)

2P-316

Effects of Venlafaxine on Oxaliplatin and Paclitaxel Induced Neuropathic Pain in Mice

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Chemotherapeutic agents, such as oxaliplatin and paclitaxel, are reported to induce severe peripheral neuropathy, characterized by cold and mechanical allodynia. Venlafaxine belongs to antidepressant drugs that cause inhibition of serotonin and noradrenaline reuptake in CNS, and the antinociceptive effects of venlafaxine have been demonstrated in different pain models such as nerve injury induced neuropathic pain. Here, we investigated whether acute treatment of venlafaxine has antinociceptive effects on oxaliplatin and paclitaxel induced neuropathic pain in mice. Both single administration of oxaliplatin (6mg/kg, i.p.) and multiple administrations of paclitaxel on four alternate days (days 0, 2, 4, 6, accumulative dose of 8 mg/kg, i.p.) induced significant cold and mechanical allodynia. The behavioral signs of mechanical and cold allodynia were evaluated by von Frey hair test and acetone test on the hind paw, respectively. Single intraperitoneal administration of 40 and 60 mg/kg of venlafaxine significantly reduced the allodynia induced by oxaliplatin and paclitaxel, whereas 10 mg/kg did not. Furthermore, this relieving effect of venlafaxine on oxaliplatin and paclitaxel induced cold allodynia was not blocked by the pretreatment of three consecutive administration of DL-p-chlorophenylalanine (PCPA, 150 mg/kg, i.p). These results indicate that venlafaxine treatment strongly alleviates chemotherapy induced cold allodynia in mice, but not via the serotonergic system. (COI: No)

2P-317

Plastic changes in cortical excitatory responses in the model rat with infraorbital nerve ligation

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Neuropathic pain is known as one of the major postoperative problems in oral and maxillofacial region for patients. Although recent studies have revealed the molecular mechanisms regarding chronic pain, the effectiveness of the treatment method has not been provided so far. Here we examined whether plastic changes in the cerebral cortex is induced by trigeminal nerve ligation and investigated effects of minocycline on the cortical plastic changes. We performed the partial infraorbital nerve ligation (PNL) in Wistar male rats. We confirmed mechanical nocifensive behavior to stimulation with von-Frey filaments. Then, we performed optical imaging in the cerebral cortex to record the response to electrical stimulation of the mentum, maxillary and mandibular molar dental pulp using a voltage-sensitive dye RH1691. The withdrawal threshold to mechanical stimuli was decreased 1 day after PNL. Cerebral cortical responses to mentum, maxillary and mandibular molar dental pulp stimulation increased 1 day after PNL, suggesting that PNL model rats presented allodynia. And the enhanced responses lasted for at least 14 days after PNL. By the administration of minocycline before PNL, the PNL induced hyper excitation in the cerebral cortex was suppressed 3 days after PNL. These results suggest that somatosensory and insular cortical excitation is facilitated by PNL, and minocycline treatment counteracts the hyper excitation. (COI: No)

2P-318

Perineural expression of TNF-α contributes to long-term mechanical allodynia in CRPS model mice

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Complex regional pain syndrome (CRPS) is an intractable chronic pain. The cause of CRPS is mostly unknown, so the elucidation of the pathophysiological mechanism and the clinical application of new therapeutic drugs for CRPS are necessary. In this study, we developed a CRPS model mouse, and then evaluated the therapeutic efficacy of TNF-α neutralizing antibody (Ab). Male ddY mice were used to create a general neuropathic pain model with sciatic nerve partial ligation (PSNL). To develop the CRPS model, the injured limbs were fixed with casts for 2 weeks immediately after the PSNL treatment. After that, TNF-α Ab was injected around the ligated nerve 3 times weekly. In the CRPS group, swelling and color change appeared in the injured limbs, and a marked decrease in mechanical pain threshold was observed up to 6 weeks.The pain threshold gradually increased after administration of TNF-α Ab and almost completely recovered after 6 weeks. In the ipsilateral nerves of CRPS model mice, various inflammation-related factors were highly expressed. These inflammatory reactions were also suppressed after administration of TNF-α Ab. In contrast, in PSNL-treated group without casts, no swelling or change in color tone was observed in injured limbs, and reduction in pain threshold was temporary. These results suggest that perineural expression of TNF-α contributes to long-term mechanical pain hypersensitivity in CRPS model mice. TNF-α Ab may be useful therapeutically for CRPS. (COI: No)

2P-319

Acute nociceptive stimuli induce the activity of serotonin and noradrenalin neurons in awake mice

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Nociception is an important type of perception that has major influence on daily human life. There are some descending pathways related to pain management and modulation, which are collectively known as the descending antinociceptive system (DAS). Noradrenalin (NA) in the locus coeruleus (LC) and serotonin (5-HT) in the rostral ventromedial medulla (RVM) are components of the DAS. Although most 5-HT neurons in the dorsal raphe (DR) have ascending projections rather than descending, they project to the thalamus which modulates nociception. Both the DAS and the DR are believed to be involved in pain-emotion symptoms. In this study, we utilized a fiber photometry system to specifically examine the activity of LC NA neurons and RVM/DR 5-HT neurons using DβH-tTAmice or TPH2-tTA mice and site-specific infection of AAV carrying a TetO GCaMP6 gene. After confirmation of specific expression of GCaMP6 in the target populations, changes in green fluorescent signal intensity were recorded in awake mice upon exposure to acute nociceptive stimulation consisting of a pinch and application of heat (55°C) to the tail. Both stimuli resulted in rapid and transient (<15 sec) increases in the activity of LC NA neurons and RVM/DR 5-HT neurons while the control stimuli did not induce any changes. The present results clearly indicate that acute nociceptive stimuli increase the activity of LC NA neurons and RVM/DR 5HT neurons and suggest a possible therapeutic target for pain treatment. (COI: No)

2P-320

Effects of naftopidil in substantia gelatinosa neurons of the rat spinal dorsal horn

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Naftopidil is used clinically for the treatment of voiding disorders in benign prostatic hyperplasia. Previous *in vivo* studies in which naftopidil was applied intrathecally abolished rhythmic bladder contraction, suggesting that naftopidil might inhibit a voiding reflex through interaction with spinal dorsal horn neurons. In this study, we aimed to clarify the mechanism of action of naftopidil on spinal dorsal horn neurons by using patch-clamp recording of rat spinal cord slices. In about 30% of neurons, naftopidil increased the frequency of miniature inhibitory postsynaptic currents (mIPSCs) tested. However, naftopidil did not change the amplitude of mIPSCs. These naftopidil activities were reversible and concentration-dependent manner. Naftopidil enhanced the amplitude of both GABAergic and glycinergic evoked-IPSCs (eIPSCs) that were elicited by focal stimuli in the presence of either the non-NMDA receptor antagonist CNQX, or the NMDA receptor antagonist APV. These data suggest that naftopidil enhances the release of GABA and/or glycine by activating inhibitory interneuron terminals in the spinal dorsal horn via an unclear site(s) other than an alpha-1 adrenoceptor and thereby modulates sensory transmission in SG. (COI: No)

2P-321

Profiles of excitatory projection from the insular cortex to trigeminal spinal subnucleus caudalis

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The trigeminal spinal subnucleus caudalis (Sp5C) receives orofacial noxious information from the orofacial region, and sends the information to the higher central nervous system. The insular cortex (IC) plays a principal role in processing nociception, and direct descending projections from IC to Sp5C have been reported. However, little information is available in terms of the these descending projection profiles. Here, we examined how IC projections modulate the activities of Sp5C neurons. Whole-cell patch-clamp recording was performed using VGAT-Venus transgenic rats that received AAV-ChR2-mcherry injection into IC and Cholera toxin subunit B (CTB) injection into the parabrachial nucleus. We investigated the feature of synaptic transmission from IC to glutamatergic and GABA/glycinergic Sp5C neurons by an optogenetic technique in combination with pharmacological manipulation of synaptic transmission. Selective stimulation of IC axons in Sp5C induced EPSCs both in excitatory and inhibitory Sp5C neurons, whose amplitudes were comparable. The optogenetically induced EPSCs were diminished by tetrodotoxin, but were rescued by 4-aminopyridine. Besides, we recorded unitary IPSCs in the connections from Venus(+) to Venus(-) neurons and found the high failure rate of unitary IPSCs, which were insensitive to bicuculline but sensitive to strychnine. These results suggest that IC projections induce excitatory rather than inhibitory effects on excitatory projection neurons in the Sp5C. (COI: No)

2P-322

Dexmedetomidine inhibits voltage-gated sodium channels in trigeminal ganglion neurons

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Dexmedetomidine, an α2-adrenoceptor agonist, is widely used as a sedative and analgesic agent in a number of clinical applications. However, little is known about the mechanism by which it exerts its analgesic effects on the trigeminal system.

Two types of voltage-gated sodium channels, $Na_v1.7$ and $Na_v1.8$, as well as α2-adrenoceptors, are expressed in primary sensory neurons of the trigeminal ganglion (TG). Using whole-cell patch-clamp recordings, we investigated the effects of dexmedetomidine on voltage-gated sodium channel currents (I_{Na}) via α2-adrenoceptors in dissociated, small-sized TG neurons. Dexmedetomidine caused a concentration-dependent inhibition of I_{Na} in small-sized TG neurons. I_{Na} inhibition by dexmedetomidine was blocked by yohimbine, a competitive α2-adrenoceptor antagonist. Dexmedetomidine-induced inhibition of I_{Na} was mediated by G protein-coupled receptors (GPCRs) as this effect was blocked by intracellular perfusion with the G protein inhibitor GDPβ-S. Our results suggest that the I_{Na} inhibition in small-sized TG neurons, mediated by the activation of Gi/o protein-coupled α2-adrenoceptors, might contribute to the analgesic effects of dexmedetomidine in the trigeminal system. Therefore, these new findings highlight a potential novel target for analgesic drugs in the orofacial region. (COI: No)

2P-323

In vivo Ca^{2+} imaging of somatosensory cortex in postoperative and inflammatory pain models of mice

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Postoperative pain is a major problem with increased surgical operation. Accumulated evidence suggests that plastic changes in the primary somatosensory cortex (S1) are essential for the formation of chronic pain. However, little is known about how individual S1 neurons fire differently during the formation of pain. In this research, we used two pain models of mice to study the activity pattern of S1 neurons: one which had the incision of hind paw as a postoperative pain model, the other which received the injection of Complete Freund's adjuvant into the paw as an inflammatory pain model. Behavioral responses to mechanical/thermal stimuli were estimated. The paw withdrawal threshold significantly decreased in the injured paw of both models. Then, we used *in vivo* two-photon calcium imaging to monitor the S1 activity during the formation of pain. The activity synchronization among GCaMP6f-expressing pyramidal neurons increased in the acute phase of both models,and the period was more extended in inflammatory model. Furthermore, the amplitude of Ca^{2+} traces in S1 was tend to increase in inflammatory model, but not in postoperative model. This result indicates that the intensity of pain in inflammatory model is higher than in postoperative model.This research will be able to clarify the mechanism of transition from acute to chronic pain. In future, it is necessary to examine not only the spontaneous activity but also the changes in S1 activity during hind paw stimulation. (COI: No)

2P-324

Alteration of spinal sensory processing from the LUT in rats with streptozotocin-induced diabetes

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Purpose:

Bladder dysfunction is often induced in diabetes. Previous studies reported that peripheral tissues damage including vesical afferent fibers results in bladder dysfunction following streptozotocin (STZ)-induced diabetes. However, the mechanism of spinal sensory circuit from the lower urinary tract (LUT) in the naïve and diabetes conditions remains uncertain. The aim of this study was to examine how spinal sensory processing from LUT is changed following STZ-induced diabetes.

Methods:

Sprague-Dawley rats were used, and diabetes model was made by intraperitoneal injection of STZ. Neuronal firings of the spinal dorsal horn were simultaneously recorded by using in vivo extracellular recordings under continuous infusion of saline into bladder.

Results:

We were classified with dorsal horn neurons into two types based on their firing patterns. Type 1 neurons that elicited firings at the peak of intravesical pressure during voiding, and type 2 neurons characterized by a burst of action potentials that correlated to a rise in intravesical pressure for micturition. In STZ model rats, the population of type 1 neurons was decreased in STZ model rats.

Conclusions:

Our results indicate that sensory processing from LUT was separately carried to the different two types of dorsal horn neurons. A loss of this spinal neuronal function may induce voiding dysfunction in diabetes. (COI: No)

2P-325

Effects of ethanol on nociceptive synaptic transmission in the rat spinal dorsal horn

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Recent studies have shown that ethanol produces a widespread modulation of neuronal activity in the CNS. It is not fully understood, however, how ethanol changes nociceptive transmission. We investigated acute effects of ethanol on synaptic transmission in the substantia gelatinosa (SG, lamina II of the spinal dorsal horn) and mechanical responses in the spinal dorsal horn. In SG neurons, bath-application of ethanol at low concentration (10 mM) did not change the frequency and amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs). At medium to high concentrations (20-100 mM), however, ethanol elicited a barrage of large amplitude sIPSCs. In the presence of TTX, such enhancement of IPSCs were not detected. In addition, ethanol (20-100 mM) increased the frequency of spontaneous discharge of vesicular GABA transporter (VGAT)-Venus labelled neurons, and suppressed the mechanical nociceptive response in wide-dynamic-range neurons in the spinal dorsal horn. The present results suggest that ethanol may reduce nociceptive information transfer in the spinal dorsal horn by enhancement of inhibitory synaptic transmission. (COI: No)

2P-326

Dexmedetomoidine suppresses rat nodose ganglion tetrodotoxin-resistant voltage-gated sodium current

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[Purpose] We examined whether dexmedetomidine, an alpha-2 adrenergic agonist and applied clinically as a sedative and an analgesic, suppresses tetrodotoxin-resistant voltage-gated sodium current (I_{NaR}) in neurons isolated from nodose ganglia (NG), which contains vagus-mediated visceral afferent neurons.

[Methods] Wistar rat (10 to 12-day-old, 21 to 29 g, n = 18) was deeply anesthetized with isoflurane and after respiration ceased the NG was dissociated and the NG neurons were isolated. Recording of the I_{NaR} was performed in 2 to 6 hours after the preparation by using the whole cell voltage-clamp technique. Following control I_{NaR} recordings obtained in an external solution containing 1 μM tetrodotoxin, we applied dexmedetomidine (10, 30, 100 or 300 μM) to the recorded neuron. The fitting of the dose-response curve was examined by using the Hill equation (4-parameter logistic): $f(x) = Imin + [(Imax – Imin)/\{1 + (C50/[x])h\}]$. Here, Imin refers to the baseline response, Imax refers to the maximum response obtainable with drug x, C50 is the concentration at half-maximal response (inhibitory or excitatory), and h is the Hill slope.

[Results] dexmedetomidine decreased the peak I_{NaR} amplitude in dose-dependent manner. The concentration at half-maximal response was 128 μM, and the Hill slope was 1.98

[Conclusion] results suggest that dexmedetomidine modifies vagus-mediated visceral sensation through its suppressive effect on I_{NaR} kinetics in the NG neurons. (COI: No)

2P-327

Expression of c-Fos and the cardiovascular response evoked by an odor fear stressor in the rat

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It is known that neurons in the hypothalamus and midbrain are involved in stress response derived from the defensive reaction. The defensive reaction is consisted by "fight or flight" and "freezing" responses. Although it is thought that neurons in the midbrain periaqueductal grey are involved in the cardiovascular response during "freezing" reaction evoked by fear sensation, it is still not unclear about the brain mechanism. In the present study, we investigated the cardiovascular response and distribution of c-Fos expression in the hypothalamus and the midbrain during an odor fear stress in conscious Wistar rats. Two-Methylthiazoline (2-MT) was used as the odor stressor and was delivered into an odor pod within the home cage. Stimulation by 2-MT caused pressor response and bradycardia in the rat. Dense c-Fos immuno-reactive neurons were observed in the dorsomedial hypothalamic area (DMH) and the ventrolateral(vl)/dorsolateral(dl) periaqueductal grey of the midbrain (PAG). These results suggest that, neurons in the DMH and the dlPAG are involved in the pressor response and that neurons in the vlPAG are involved in the bradycardia during the odor stress of 2-MT. (COI: No)

2P-328

Does listening to Mozart's or Bach's music have any effects on autonomic nervous activity?

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It has been suggested that listening to Mozart's and Bach's music has health benefits, and such music is being marketed commercially for wellness even today. Specifically, multiple music sources have stated that this music has a regulatory effect on autonomic nervous activities (ANA). However, few scientific studies show critical or logical evidence demonstrating the effects of listening to such music on health. We aimed to evaluate the effect of Mozart's K448 and Bach's BWV1049 on heart rate (HR), blood pressure (BP), and ANA in young adults. Thirteen subjects participated in 3 tasks consisting of 3 experimental conditions. Including 10 min of rest and test-to-load on ANA, the conditions were listening to K448 and BWV1049, or silence as control, for 8 min in randomized order. Electrocardiography was continuously performed from the start to end of each data collection point. There was no significant change in HR, BP, or high frequency (HF) during K448, BWV1049, or silence. However, the low frequency (LF)/ HF ratio (LF/HF) was lower in women after listening to BWV1049 ($p=.005$) and in men after listening to K448 ($p=.033$). Additionally, in the participants who had no special music education, change in LF/HF was lower after listening to K448 ($p=.001$). In conclusion, change in LF/ HF differed between women and men. Our findings suggest that listening to K448 or BWV1049 may have an effect on ANA by changing LF/ HF, though the pattern of change in LF/ HF differed between gender. (COI: No)

2P-329

Effects of GABA agonist injection into the ventrolateral medulla on oropharyngeal swallowing

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The swallowing central pattern generator includes two groups of neurons located within the medulla: the dorsal swallowing group (DSG) in the nucleus tractus solitarius and the ventral swallowing group (VSG) in the reticular formation (RF) in the vicinity of nucleus ambiguous (NA). DSG is involved in rhythm generation during repetitive swallowing. Although VSG seems to be involved in swallowing pattern generation, roles of VSG has not been fully elucidated. Thus, we evaluate the effects of injection of a GABA receptor agonist, isoguvacine, into the VSG on activity patterns of vagus (VNA) and phrenic nerves (PNA) during fictive respiration and swallowing in a perfused brainstem preparation of rat.

9 juvenile SD rats were used in this study. Isoguvacine was injected into the RF just dorsal to the NA.To elicit fictive sequential swallowing, the superior laryngeal nerve (SLN) was electrically stimulated (20Hz, 10s duration) with stimulus 20μA - 160μA intensities. Neither the PNA or the VNA during fictive respiration were significantly altered by the isoguvacine. However, the latency of SLN-evoked swallowing increased after isoguvacine injection at lower stimulus frequency intensity ($p < 0.05$). In addition we observed that sequential swallowing was interrupted by PNA breakthrough during the 10s SLN-stimulus period at lower stimulus frequency ($p < 0.05$). We conclude that VSG may mediate part of the SLN sensory input and significantly determines the sensory threshold for swallowing. (COI: Properly Declared)

2P-330

Coordinated involvement of the amygdala and claustrum for blood pressure control during exercise

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[Purpose] Behavioral performance increases proportionally with the arousal level, but over-arousal with excessive emotion inversely reduces performance. However, the neuronal mechanisms linking emotional arousal and behavioral performance remain obscure. Here, we investigated how the claustrum (CL) and the central nucleus of the amygdala (CeA), known as brain areas involved in arousal and emotional processing, respectively, coordinately control cardiovascular regulation during exercise.

[Methods] First, we examined whether the CL and CeA are activated during treadmill exercise using c-Fos immunostaining. Subsequently, we investigated the arterial-pressure responses to electrical stimulation, as well as the anatomical connections and functional interaction between the CL and CeA.

[Results] The CeA and the posterior CL (pCL), but not the anterior CL, showed exercise-intensity dependent c-Fos activation. Microstimulation of the pCL induced a depressor response, in contrast to the CeA stimulation which showed a pressor response. We observed anatomical bidirectional connections between them. Interestingly, simultaneous stimulation of the pCL and CeA resulted in a greater pressor response than that induced by CeA stimulation alone.

[Conclusion] These results suggest that the CeA and pCL are not independent and may be functionally connected to each other. This functional linkage might be involved in coordinated tuning of cardiovascular regulation during exercise with emotional arousal. (COI: No)

2P-331

Hormonal secretion from the thyroid gland is promoted by mechanical stimulation of the pharynx

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Recently, we showed that low-current electrical stimulation of the superior laryngeal nerve (SLN) promotes hormonal secretion from the rat thyroid gland. Since SLN innervates the pharynx, we hypothesized that the mechanical stimulation to the pharynx can facilitate thyroid function. The purpose of this study is to clarify whether and how the mechanical stimulation of the pharynx increases thyroxine (T4) and calcitonin (CT) secretion from the thyroid gland. Male SD rats were anesthetized with urethane and artificially ventilated. To measure T4 and CT secretion rates, the thyroid venous blood were sampled and analyzed by ELISA. A mechanical stimulation of the pharynx were conducted using balloon which inserted from mouth, moving from the oral cavity to the pharyngeal cavity. As a results, T4 and CT secretion rates were increased twofold during pharyngeal stimulation, and these responses were abolished after cutting SLNs. The SLN afferent activity were increased during pharyngeal stimulation. The sympathetic efferent activity of thyroid branch from cervical sympathetic ganglia was not affected by that stimulation. However, the parasympathetic efferent activity of thyroid branch from SLN was increased during pharyngeal stimulation. We concluded that a mechanical stimulation to pharynx induced reflex increase in T4 and CT secretion from thyroid gland via SLN afferent and thyroid parasympathetic efferent nerve. (COI: No)

2P-332

Exercise improve stress-induced high blood pressure and abnormal gene expression in the amygdala

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[Purposes] It has been reported that chronic restraint stress (CRS) induces high blood pressure, whereas exercise has anti-stress and anti-hypertensive effects. However, these mechanisms remain unknown. Since the amygdala (AMY) is known to be involved in stress-induced physiological responses including BP regulation, we tested whether stress-induced hypertension and anti-hypertensive effects of daily exercise are both mediated by changes in gene expression patterns in the AMY. [Methods] Eighteen male Wistar rats were allocated into control (Co, n=6), CRS (St, n=6), and CRS + voluntary exercise (SE, n=6). CRS was maintained through immobilization for 3 weeks, 5 times per week, for 1 hour per day. The arterial pressure (AP) of Co, St, and SE groups was measured before and after intervention. We also investigated whether exercise modified gene expression after CRS by a microarray experiment. [Results] Mean AP was significantly higher in St compared with two other groups whereas it was not different between Co and SE. 201 genes in the AMY of St were identified as significantly altered compared to Co, however 169 genes of those in SE showed no changes from Co, suggesting that abnormal expression in 80% of stress-sensitive genes were normalized by daily exercise. [Conclusions] The results suggest that altered gene expression in the AMY might be involved in the mechanism of stress-induced high BP and preventive effects of exercise. (COI: Properly Declared)

2P-333

Ethanol injection differently activated autonomic nerve activity in anesthetized rats

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The previous study reported that ethanol injection inhibited gastrointestinal motilities through the afferent vagal pathway in rats (Naunyn-Schmiedeberg's Arch Pharmacol 365: 17–21, 2002). However, it is unknown whether ethanol directly affects the afferents of gastrointestinal vagal nerves. Thus, using electrophysiological recording, we examined effects of ethanol injection into the gastrointestinal organs on afferent vagal nerve activities and efferent sympathetic branches to the kidney and brown adipose tissue in rats anesthetized with the mixture of urethane and α-chloralose.

Intragastric injection of ethanol (15%or 40%, 1mL) increases afferent gastric vagal nerve activity in a dose-dependent manner, and intra-duodenal injection of 40% ethanol also stimulated afferent celiac nerve activity. Similarly, ethanol injection into the portal vein also dose-dependently activated afferent vagal nerve signal of the liver. As well as afferent vagal responses, intragastric injection of 40% ethanol activated efferent sympathetic outflow to the kidney but not the brown adipose tissue. In addition, both intragastric and intra-duodenal injection of ethanol significantly lowered blood pressure and heart rate. Thus, these data suggest that in alcohol drinking, ethanol directly may affect the gastrointestinal organs and stimulate afferent vagal nerve signals to inform the brain and regulate efferent renal sympathetic nerves and cardiovascular function. (COI: Properly Declared)

2P-334

Estradiol-dependent gene expression profile in the amygdala of ovariectomized SHRs

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Pre-menopausal women exhibit a lower arterial pressure (AP), a lower sympathetic outflow and a greater baroreceptor reflex than age-matched men. However, AP and subsequent cardiovascular diseases dramatically increase in post-menopausal women, but the molecular mechanisms are still not clear. Amygdala is a major nucleus of the limbic system involved in the control of cardiovascular, behavior and hormonal response to stress or fear. Since amygdala exhibits differences in behavioral response to stress after menopause, we hypothesized that a different neuronal function in this brain area, characterized by a differential gene expression profile after menopause, could contribute to the difference in cardiovascular homeostasis.

Young Female SHRs were ovariectomized (OV) and half of them received an estradiol treatment (OV+E2) for one month. Estradiol substitution significantly decreased AP in OV rats. Gene expression profiling of the amygdala of OV rats shows that estradiol significantly down-regulated the expression of 1118 genes and up-regulated the expression of 468 genes. Interestingly, pathways analysis revealed that the down-regulated genes especially categorized in "neuro-active ligand-receptor interaction", "serotoninergic synapse", "cholinergic synapse" and "dopaminergic synapse" pathways as well as "amphetamine, cocaine and alcohol addiction" pathways. Whether these genes mediate the estrogen-dependent regulation of blood pressure remains to be determined. (COI: No)

2P-335

Discharge activities of diaphragm motor units during inspiratory load

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Various studies on the Henneman's "size principle" have been revealed. However, previous studies were limited to the electrophysiological experiments using spinal reflex and the results obtained by morphological studies. It was difficult to examine the "size principle" during natural movements. Therefore, we analyzed the discharge activities of a single diaphragm motor units (MUs) when increasing the diaphragm muscle output by gradually increasing the inspiratory load under spontaneous breathing. The experiment was approved by the Animal Research Committee of Ibaraki Prefectural University of Health Sciences. The inspiratory load was applied to the four-step loads. The duration of each loads were about 5 minutes, and a period of 5 minutes were provided between each load. The phrenic nerve activities were recorded in the cervical and the thoracic regions. Two needle electrodes were inserted into the diaphragm and a pair of single diaphragm MUs was recorded. Then, the conduction velocity (CV) of the obtained the pairs of MUs and the onset of discharge were compared. We analyzed the CV of 25 single diaphragm MUs and compared the corresponding 10 pairs. The slowest CV was at 24.1 m/s, the earliest CV was 89.8 m/s. It was found that MUs with the faster onset time has the lower CV, and MUs with the later onset time has the faster CV. Our results show the recruitment of the diaphragm MUs during inspiratory loads follows the "size principle". (COI: No)

2P-336

A role of TRPA1 in oxygen detection

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TRPA1 (transient receptor potential cation channel, subfamily A, member1), expressed in the trigeminal and vagus nerves, detects irritants and hypoxia in the inspired gasses (Nat Chem Biol 7:701, 2011; Sci Rep 3:3100, 2013; Phys Rep 4:e13098, 2016). Here we examined role of TRPA1 in hypoxia-induced physiologic responses. Four lines of experiments were performed using TRPA1 knockout (KO) mice and wild type (WT) littermates. First, avoidance to low (15%) oxygen environment was significantly less in KO mice than in WT mice. Second, ventilatory response to hypoxia was measured with body plethysmography. KO mice showed attenuated responses to mild hypoxia (15% O2) but not severe hypoxia (10% O2). Third, wakeup response to ramp hypoxia (from 20% to 10% O2 in 40 sec) was measured using EEG electrodes indwelling mice. While WT mice woke at 13-14% O2 within 30 sec, KO mice did not wake until O2 reached 10%. Fourth, we examined possible effect of long term (8-9 days) mild hypoxia (15% O2) on the heart and lungs. KO mice showed exaggerated right ventricular hypertension, cardiac hypertrophy, and lung arteriolar thickening. These data clearly show that TRPA1 is indispensable for mild hypoxia-induced multiple physiologic responses. We propose that TRPA1 in the sensory nerves along the airway plays a protective role against hypoxia presumably before hypoxemia activates classical hypoxia sensor, carotid body. (COI: No)

2P-337

Descending inhibition on spinal seizure-like activity in the phrenic nerve output

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In the brainstem-spinal cord preparation, spinal seizure-like burst activity induced by pharmacological treatments such as blockade of $GABA_A$ergic and/or glycinergic inhibitory synaptic transmissions appears in various spinal ventral roots including the fourth cervical ventral root (C4) but not in the phrenic nerve. After a transverse section at the C1 level, the inspiratory activity disappeared from the C4 and phrenic nerve, whereas seizure-like activity became to appear in both nerve recordings. We hypothesized that putative inhibitory descending pathways other than $GABA_A$ergic and/or glycinergic systems (from the medulla to the spinal cord) work to avoid disturbance of the regular respiratory-related diaphragm contraction by seizure-like activity. To reveal neuronal mechanisms of the descending pathways, we tested several kinds of antagonists; e.g. GABAB, μ-opiate, adenosine, serotonin, noradrenergic and cannabinoid receptor antagonists. The experiments have been performed in the brainstem-spinal cord preparation from 0- to 1-day-old rats. In most experiments, the left phrenic nerve activity and right C4 activity were recorded simultaneously. The seizure-like activity was induced by application of 10 μM bicuculline and 10 μM strychnine. We found that only cannabinoid receptor antagonist, AM251 was effective to induce seizure-like activity in the phrenic nerve. We suggest that cannabinoid receptors are involved in the descending inhibitory system. (COI: No)

2P-338

Measurement of paraventricular nucleus neuronal and sympathetic nerve activities in conscious rats

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The hypothalamic paraventricular nucleus (PVN) is involved in regulation of sympathetic nerve activity (SNA). However, to our knowledge, there has been no attempt to measure PVN neuronal activity (PVNNA) and SNA simultaneously in freely moving animals. Thus, the functional relationship between PVNNA and SNA remains unclear. In the present study, we developed a method for simultaneous and continuous measurement of PVNNA and SNA in freely moving rats to examine their relationship. Male Wistar rats were chronically implanted with multiple electrodes (100-μm stainless steel wire) for measurement of hypothalamic paraventricular nucleus neuronal activity, as well as electrodes for measurement of renal SNA (RSNA), electroencephalogram, and electromyogram. Electrocardiogram electrodes with a catheter were used for measurement of arterial pressure (AP). PVNNA and RSNA were measured continuously during the rats' daily activity and in response to air jet stress. Accordingly, we succeeded in measuring PVNNA and RSNA simultaneously and continuously in freely moving rats. We found that rapid eye movement sleep increased PVNNA and AP, while RSNA and heart rate (HR) decreased. Air jet stress increased PVNNA, RSNA, and MAP, and HR increased simultaneously in a sustained fashion. Thus, we demonstrated that PVNNA is involved in regulation of AP during daily activity and in response to external emotional stress as well. However, PANNA and RSNA did not always respond in a unidirectional manner. (COI: No)

2P-339

Projection from the midbrain to the rostroventral medulla and the cardiovascular response to stress

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It has been shown that the sympathetic vasomotor pathway of the stress-induced cardiovascular response is mediated via neurons in the rostroventral medulla (RVM) indirectly from the dorsomedial hypothalamic where is a possible autonomic center for stress response. However, it is still unknown where the sympathetic vasomotor response is relayed. In the present study, we investigated the direct projection to the RVM and distribution of c-Fos expression during an acute psychologicalstress (social defeat stress or restraint stress) in conscious rats. At 2~7 days before the stress exposure, the animal was microinjected a retrograde tracer (CTB or Fluoro-gold) into the unilateral RVM. After the exposure to the stress, c-Fos protein and the tracer were stained and observed the brain sections from the pons to the hypothalamus. Each stress evoked pressor response and tachycardia. The c-Fos positive- and the tracer containing-neurons were distributed at the caudal part of dorsal raphe in the midbrain (just ventral to the midbrain aqueduct). These results suggest, at least in part, neurons caudal dorsal raphe in the midbrainmay mediate the pressor response evoked by the acute stress. (COI: No)

2P-340

Gut hormone signal alters lick microstructure and taste reactivity to sweet stimulation in mice

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Taste stimulation into the oral cavity elicits innate ingestive and aversive taste reactions such as tongue protrusion, lateral tongue protrusion, paw licking as ingestive ones and gaping, forelimb flailing, chin rubbing as aversion ones. Sweet taste usually elicits continuous licks and ingestive taste reactions when it is infused into the oral cavity in rodents. Metabolic challenge and psychological status such as food preload and/or fullness alter the behavioral responses to a sweet taste substance. Here, we examined whether postingestive (visceral) signals altered sweet taste palatability/reactivity using peripheral administration of a gut hormone, peptide YY (PYY). Nocturnal-fed mice received 5–day training to lick a 0.3 M sucrose solution during the light phase. Lick microstructure analysis revealed significant reduction of burst licking duration when they received PYY (25 nmol/kg) administration. In addition, taste reactivity test revealed that the PYY administration significantly decreased ingestive taste reactions. Marginal increase of aversive taste reactions were also found. Taken together, peripheral PYY administration decreased taste palatability of the sucrose solution and hedonic motivation to consume the sugar. Our present study suggests that gut hormone signaling after tastant ingestion plays a role in decline of sweet palatability of food/fluid during a meal. (COI: Properly Declared)

2P-341

Hyposalivation and impaired parasympathetic vasodilation in parotid glands with diabetes mellitus

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[**Purpose**]Activation of parasympathetic nerves elicited by sensory input in the orofacial area induces an increase in blood flow (BF) along with salivation suggesting that salivary secretion depends on the increase in glandular BF. Therefore, disturbances in parasympathetic nerves due to diabetic neuropathy is considered an important etiological factor of hyposalivation in diabetes mellitus (DM). Herein, we examined the relationship between increase in parasympathetic glandular BF and salivation. [**Methods**] The lingual nerves (LN) of type 2 DM and non-DM rats were stimulated. Hemodynamics in salivary glands and salivation were examined via laser speckle imaging flow meter and saliva collection, respectively. [**Results**] Salivation and BF increase in the three glands were noted in the DM and non-DM rats following LN stimulation; however, these changes were significantly lower in the parotid glands (PG) of the DM rats when compared to those in non-DM rats. Although intravenous administration of acetylcholine increased BF in the PG, the response was significantly lower in DM rats when compared with the non-DM rats. The mRNA expression of M3 mAChR in the PG of the DM rats were lower than that of the non-DM rats. [**Conclusions**] Type 2 DM appears to impair parasympathetic vasodilation and salivation in the PG. Hence, disturbances in the cholinergic vasodilator pathway may contribute to the underlying mechanisms involved in disorders of glandular parasympathetic vasodilation. (COI: No)

2P-342

Acute myocardial infarction activates hypothalamic vasopressin and oxytocin neurons

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Myocardial infarction is a leading cause of death worldwide. Those who survive often suffer chronic heart failure, which increases circulating vasopressin (antidiuretic hormone) levels. Vasopressin is secreted by magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei. However, it is not known whether myocardial infarction acutely activates vasopressin neurons, or oxytocin neurons, which are also found in the supraoptic and paraventricular nuclei. Therefore, we used Fos protein to determine whether vasopressin and oxytocin neurons and their afferent inputs are activated following myocardial infarction. A higher proportion of vasopressin-positive and oxytocin-positive neurons were Fos-positive in the supraoptic and paraventricular nuclei 90 min after myocardial infarction than after sham-operation. Similarly, there were more Fos-positive tyrosine hydroxylase-positive (noradrenergic) neurons in the nucleus tractus solitarius and rostral ventrolateral medulla 90 min after myocardial infarction than after sham-operation. Furthermore, there were more Fos-positive neurons in the circumventricular organs, the area postrema and subfornical organ, 90 min after myocardial infarction than after sham-operation. Hence, vasopressin and oxytocin neurons are activated after acute myocardial infarction as are their main afferent inputs. (COI: No)

2P-343

Phospholipase C-related inactive protein type-1 deficiency alters propofol-induced EEG activity

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Intravenous anesthetic propofol mainly exert their anesthetic actions via the $GABA_A$-R. Phospholipase C-related inactive protein type-1 (PRIP-1) plays important roles in the regulation of $GABA_A$-R activity. PRIP-1 deficiency attenuates *in vivo* anesthetic action of propofol. In this study, we examined the effects of propofol on EEG in WT and PRIP-1 knockout (*PRIP-1 KO*) mice. The power spectral density of EEG signals was compared between genotypes before and after propofol injection. PRIP-1 deficiency induced increases in EEG absolute powers but did not markedly change the relative spectral powers during waking and sleep states. *PRIP-1 KO* mice showed delayed induction of and rapid emergence from propofol anesthesia. Propofol induced increases in low-frequency EEG activity in WT but not in *PRIP-1 KO* mice, indicating that PRIP-1 deficiency blocked propofol-induced EEG slowing and loss of consciousness. Our findings suggest that PRIP-1 is involved in the anesthetic EEG dynamics produced by propofol. (COI: No)

2P-344

A microsensing system for the *in vivo* real-time detection of local drug kinetics and dynamics

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Drugs are effective tools to study physiological phenomena in live animals. The underlying mechanisms will be markedly clarified if local behaviors of systemically administered drugs and their actions *in vivo* could be simultaneously measured. However, conventional methods require considerable sample quantities and have poor sampling rates. Additionally, they cannot address how drug kinetics correlates with target function over time. Here, we developed a system with two different sensors. One is needle-typeof boron-doped diamond microsensorwith tip diameter ~40 μm, and the other is a glass microelectrode. We first tested the bumetanide, an Na^+,K^+,$2Cl^-$-cotransporter blocker, which serves a diuretic but can induce deafness. In the guinea-pig cochlea, changes of bumetanide concentration and the extracellular potential underlying hearing were simultaneously measured in real time. We further examined an antiepileptic drug lamotrigine, which inhibits Na^+channel, in the rat brain, and tracked its kinetics and at the same time the local field potentials mirroring neuronal activity. The action of the anticancer reagent doxorubicinwas also monitored *in vivo*. This microsensing system will contribute to advances in life science. (COI: No)

2P-345

Treatment of Alzheimer's disease by a disease-modifying small molecule

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Alzheimer's disease (AD) is one of the most common neurodegenerative disorders around the world. Current drug treatments can only improve the symptoms but cannot cure the disease. Nevertheless, several approaches aiming at suppressing disease progression have advanced to the stage of clinical trials. We found recently that GSK-J4, a histone demethylase inhibitor with the ability to cross blood brain barrier, could induce BDNF production. Therefore, it exerts the beneficial effect on AD. In this study, we aimed to investigate whether GSK-J4 could act as a potential therapeutic agent for AD. 9-month old 3xTg female AD mice were intraperitoneally injected with GSK-J4 for 30 days. Behavioral assays demonstrated that SLAD treatment was beneficial in enhancing the performance of the animals in both Morris water maze and novel object recognition tests. Histological analysis revealed no significant reduction in the numbers of amyloid plaques in both the hippocampus and cortex of GSK-J4-treated mice. However, the magnitude of long-term potentiation of hippocampal CA3-CA1 pathway increased by 30% after GSK-J4 treatment, which was accompanied by upregulated expressions of both PSD-95, BDNF in the hippocampus detected by immunohistochemistry. Taken together, our data support that GSK-J4 could be a promising new small molecule in treating AD.

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2P-346

Andrographolide relieved pain generated by post-operative pain model in rat

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Context: Andrographolide (Andro) is anti-inflammatory. It reduced allodynia in a sciatic nerve injury model. This effect only show after 3 days post operation (P.O.), and was correlated to anti-inflammation in the CNS component. Objective: To test Andro's ability and active time frame to reduce pain sensation in an open wound model. Material and methods: SD rats received an open wound injury and : 1. treated with saline (Saline-group); 2. treated with Andro via direct injection to the paw (Andro-inject); 3. andro blended PLGA embedded under the open wound (Andro-tablet). Mechanical allodynia was assessed by von Frey tests at 1, 3, 5 hours and 1- 5, days P.O.. Paw samples were collected at 5 days P.O., and stained for HE for gross morphology, and various markers of inflammation reactions. Results: The thresholds for inducing allodynia increased and the response percentage reduced in the Andro-inject group when compared to the Saline-group, as soon as 3 hours P.O. and the effect lasted until 3-4 days P.O. No changes in gross morphology is found for the Andro-inject group. The threshold for inducing pain behavior and response frequency for Andro-tablet was not significantly different to the saline treated group. The tissue showed some extent of edema. Conclusion: Andro significantly reduced mechanical allodynia when compared to the saline treated group, both in short and longer time frame. (COI: No)

2P-347

Withdrawn

2P-348

Mouse strain-dependent BBB (blood-brain barrier) permeability of AAV-PHP.B

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Adeno-associated virus (AAV) vector is a powerful tool for transgene expression in the central nervous system (CNS) of mammals. In 2016, Deverman et al. reported a capsid variant of AAV serotype 9 (AAV-PHP.B) highly permeable to the blood-brain barrier in C57BL/6 mice, which enabled us to express transgenes throughout the CNS simply by the intravenous injection of AAV-PHP.B. Meanwhile, a different group found failure of the BBB penetration when they used a different mouse strain (BALB/c), and suggested that BBB transmission of AAV-PHP.B might be limited to a C57BL/6 strain. Here we further characterized the AAV-PHP.B in terms of the BBB permeability in different mouse strains. The BBB permeability was evaluated by GFP fluorescence intensity of the whole brains 2 weeks after intravenous injection of AAV-PHP.B expressing GFP. We found that the F1 hybrid obtained by crossing C57BL/6 and BALB/c became entirely impermeable to AAV-PHP.B. On the other hand, AAV-PHP.B showed great BBB permeability in different mouse strains such as DBA/2, SJL/J and FVB/N. Intriguingly, ICR mice, a closed colony, showed variable BBB permeability. These results indicate that great BBB permeability of AAV-PHP.B is not restricted to C57BL/6 strain, and rather, the impermeability is likely limited to BALB/c and the filial generation. Comparison of the BBB structure between BALB/c and other strains may provide a key insight for the mechanism that defines the BBB transmission efficacy of AAV-PHP.B. (COI: No)

2P-349

A coagulation factor IX peptide regulates endothelial barrier function in brain

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Recently, coagulation factor IX (FIX) and its activation peptide (F9-AP) have been reported to affect endothelial cells by inducing spreading and suppressing endothelial permeability. In this study, the effects of F9-AP on cell was investigated in vivo with traumatic brain injury (TBI) model rats in which, dysfunction of the blood brain barrier (BBB) with increasing vascular permeability promotes the progression of neuropathy. TBI model rats were generated by controlled cortical impact (CCI). After CCI, rats were intravenously injected with 350 μg/kg of F9-AP or PBS as a control, every day for a month. Human umbilical vein endothelial cells (HUVEC) were treated with F9-AP, and assessed the form of cell adhesion and its function in vitro.

The treatment significantly reduced brain edema by 34% and extravascular leakage of Evans blue by 91%. CCI caused recessus of 333.4±147.1 and 58.9±22.0 mm^3 in control and F9-AP-treated rats, respectively ($P<0.01$). Nissl staining showed that neural cells adjacent to recessus induced by CCI were lost in control rats, but saved in F9-AP-treated rats. In the neural examination, the treated rats performed significantly better than control rats. The cell area of HUVEC increased 16% after treatment with F9-AP. Transmission electron micrography revealed that HUVEC treated with F9-AP had long intercellular adhesions that formed a complex interface. F9-AP maintained vascular barrier function and improve prognosis in TBI model rats. (COI: No)

2P-350

Fatty acid-responding neurons in mouse glossopharyngeal nerve

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In the last decade, GPR40, GPR120 and CD36 have been reported as fatty acid sensors in rodents' taste systems. We previously found fibers showing a maximal response to oleic acids (F-type) in ~18% of mouse chorda tympani (CT) nerve. Furthermore, a half or more of fibers showing a maximal response to sucrose (S-type) or monopotassium glutamate (M-type) showed responses to fatty acids. GPR120 was revealed to be involved in the responses in these fiber types. In the present study, we performed single fiber recordings from the glossopharyngeal (GL) nerve in GPR120-KO and wild type (WT) mice to examine fatty-acid-responding neuron types and potential involvement of CD36. Among recorded GL fibers, percentage of F-type fiber was ~9% and ~2 % in WT and GPR120-KO mice respectively. More than 60% of S-type or M-type fibers showed responses to fatty acids in both mice strain. The proportion of F type and the other fibers showing response to fatty acid is 2: 3 for the CT and 2: 7 for the GL of WT mouse. In GPR120-KO mice, although fatty-acid-responding S- and M-type fibers of the GL were significantly inhibited by an inhibitor of CD36, those of the CT were not inhibited. These results suggest that the proportion of nerve fibers for fatty acid preference in the GL is much more than those in the CT. CD36 may be involved in the information pathways. (COI: No)

2P-351

The role of HCN4-positive cells in the gastrointestinal development and motility of zebrafish

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Background/Aims: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels help control the rhythmic activity of pacemaker neurons during brain development. However, little is known about the role and cell type specificity of the HCN channels in the enteric nervous system (ENS). Here, by crossing gSAlGFF249A transgenic fish expressing the Gal4 transcriptional activator gene and UAS:GFP transgenic animals, we detected the HCN4-positive cells within the zebrafish gut. We characterized the HCN4-positive cells and investigated their role in the gut motility. **Methods:** To identify the nature of the HCN4-positive cells, anti-GFP, anti-HuC/D and anti-5HT antibodies were used in immunohistochemical staining. Additionally, we generated transgenic zebrafish expressing the light-sensitive channelrhodopsin-wide receiver (ChRWR) gene in the HCN4-positive cells. Gut motility was assessed by using Cell Motion Imaging System SI8000 (SONY) in vivo. **Results:** Our results showed that the HCN4-positive cells are serotonergic neurons which increase in number at the early stage of the ENS development. The light stimulation of HCN4-positive enteric neuron evoked retrograde propagating contractions, while anterograde propagating contractions were not influenced. **Conclusions:** Using the zebrafish system, we found that HCN4-positive enteric neurons specifically control retrograde peristalsis at the early stage of the ENS development. (COI: No)

2P-352

NHEJ and BER are Concurrently Engaged by APE1 in Oxidative DNA Damage Repair in Rat Cortical Neurons

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Background: BER is commonly thought as a major pathway of oxidative DNA damage repair in neurons, which are particularly prone to such damage because of their highly active metabolism and elevated reactive oxygen species production. Many studies have suggested that neurons are deficient in other DNA repair pathways, including NER and HR. However, earlier studies reported that neurons could efficiently repair glutamate- and menadione-induced oxidative DNA damage, which produce DSBs. We hypothesized that BER and NHEJ work together to repair oxidative DNA damage in neurons.

Methods: Immunostaining and confocal microscopy were employed to examine the co-localization of APE1, γH2AX, and *p*53BP1 and to determine the APE1 and occurrence of DSBs and repair. The neutral comet assay was applied to examine and quantitate formation of DSB.

Results: We showed that γH2AX and *p*53BP1 were upregulated and co-localized with APE1 in the nuclei of cultured neurons subjected to oxidative insults. The foci of *p*53BP1 immunostaining were efficiently abolished, while those of γH2AX foci persisted, following suppression of APE1 activity. Comet assays demonstrated that the inhibition of APE1 decreased DSB formation.

Conclusions: Our results indicate that APE1 can also engage the NHEJ mechanism in the repair of oxidative damage in neurons. Our findings provide an explanation for the ability of neurons to repair their nuclear DNA efficiently despite their high oxidative burden. (COI: No)

2P-353

Remote control of neuronal function using X-ray

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Understanding brain function requires non-invasive control of activities in well-defined neuronal populations during ongoing natural behavior. Although several wireless optogenetic approaches using implanted LED devices or upconverting phosphors have been demonstrated, remote control of a large neuronal population deep in the brain of freely behaving animals is still challenging. Scintillation materials emit visible luminescence upon X-ray radiation. Given that X-ray well penetrates biological tissues, scintillation luminescence could be useful for transcranial actuation of opsin-expressing neurons. In this study, we show the feasibility of a novel wireless optogenetic approach using inorganic scintillators. We screened opsin-scintillator combinations which could effectively depolarize or hyperpolarize cellular membrane potentials in vitro. With either yellow- or blue-emitting scintillator crystals and their best partner opsins, we successfully actuated specific neuronal populations in ventral tegmental area to drive behavioral changes of freely moving, X-irradiated mice. This technology allows the use of X-ray for remotely alter neural processing in the deep brain of living animals and opens up opportunities for new treatments of neurological disorders. (COI: No)

2P-354

Development of lentiviral vectors for glutamatergic-selective gene expression in cultured neurons

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Targeting gene expression to a particular subset of neurons helps study the cellular function of the nervous system. Although neuron-specific promoters, such as the synapsin I promoter and the α-CaMKII promoter, are known to exhibit selectivity for excitatory glutamatergic neurons *in vivo*, the cell type-specificity of these promoters has not been thoroughly tested in culture preparations. Here, by using hippocampal culture preparation from the *VGAT-Venus* transgenic mice, we examined the ability of five putative promoter sequences of glutamatergic-selective markers including synapsin I, α-CaMKII, the vesicular glutamate transporter 1 (VGLUT1), Dock10 and Prox1. Among these, a genomic fragment containing a 2.1 kb segment upstream of the translation start site (TSS) of the *VGLUT1* implemented in a lentiviral vector with the Tet-Off inducible system achieved the highest preferential gene expression in glutamatergic neurons. Analysis of various lengths of the VGLUT1 promoter regions identified a segment between –2.1 kb and –1.4 kb from the TSS as a responsible element for the glutamatergic selectivity. Consistently, expression of channelrhodopsin under this promoter sequence allowed for selective light-evoked activation of excitatory neurons. Thus, the lentiviral system carrying the VGLUT1 promoter fragment can be used to effectively target exogenous gene expression to excitatory glutamatergic neurons in cultures. (COI: No)

2P-355

Effects of Cigarette Smoking on the motor nerve conduction study parameters among young adults

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Introduction: Cigarette smoking is recognized as a risk factor for many diseases such as hypertension, ischemic heart diseases and cancer.The aim of this study was to determine the effect of smoking on the nerve conduction study of the median nerve (motor part).

Methodology: This study was descriptive institutional based case control study carried out at AL-Neelain University Faculty of Medicine during 2017. Thirty one smokers volunteered to participate and 34 healthy non-smokers,The data were collected using a self-administered questionnaire and the nerve conduction study was measured using power lab serial number G607. Nerve conduction velocity, amplitude, proximal latency and distal latencies were recorded.

The data was analyzed using (SPSS) Version 22 and graph pad prism 7.

Results: The result revealed that healthy smokers had lower amplitude of nerve conduction study compared to healthy non-smokers (3.1±.08 and 4.9±1.50 respectively, P value <0.001). There was no statistically significant difference in the nerve conduction velocity of smokers and non-smokers (P value 0.07).Also, there were no statistical significant differences in the proximal and distal latencies between smokers and non-smokers (P value more than 0.05 for both).

Conclusion: Cigarette smoking attenuates the amplitude of the motor part of median nerve. The changes induced by smoking were not related to the duration of smoking and the number of smoked cigarettes per day. (COI: No)

2P-356

Dysregulated microRNA expression profiles in extracellular vesicles of schizophrenia

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Schizophrenia is a severe psychiatric disorder with unknown the detailed molecular mechanism. At present, the diagnosis of schizophrenia is ineffective that often lead to the delay or misdiagnosis and subsequently causes poor treatment outcome. Exosomal miRNAs have been reported to play important roles and purposed as potential biomarkers for neurodegenerative diseases. Here, we compared the miRNAs profiling from plasma-derived exosomes from drug-naive schizophrenia patients with healthy controls. The isolated exosomes revealed cup-shaped double-membrane vesicles with the average size less than 150 nm and were positive for exosome marker proteins, flotilin1, TSG101, CD63 and CD81. From principal components analysis of NanoString results, the first two principal components explained 43.7% of the total variance and the calculated average of miRNA-expression profiles between schizophrenia patient and healthy control groups showed a moderate separation according to PC1. We found that 26 and 11 miRNAs were significantly up-regulated and down-regulated, respectively. KEGG pathway analysis from predicted target genes of dysregulated miRNAs revealed pathways that involved with neurological functions such as dopaminergic synapse, MAPK signaling pathway, axon guidance and hippo signaling pathway. Collectively, our results demonstrate dysregulated exosomal miRNAs and their potential as molecular biomarkers for schizophrenia. (COI: No)

2P-357

Oral capsaicin sensitivity and preference for spicy food in Japanese medical students

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When spicy compounds in food, such as capsaicin, are received in the oral cavity, the information is conveyed through the trigeminal nerve to the CNS for its perception and recognition. However, little is known about the relationship between the oral sensitivity and the preference. In the present study, we examined the correlations of oral capsaicin threshold with recognition thresholds for four basic tastes (*i.e.*, sweet, salty, sour, and bitter) and preference for spicy food and seasonings in our medical students. All the thresholds were measured at the anterior part of the tongue by the filter-paper disc method. 1) The thresholds for capsaicin in 103 subjects with a median age of 21-year-old showed a bimodal distribution. 2) The data also showed weak positive correlations between the thresholds for capsaicin and sour taste (tartaric acid), and those for capsaicin and bitter taste (quinine), respectively. 3) In the questionnaire survey, the subjects with low thresholds for capsaicin tended to have negative images and dislike some spicy food and seasonings such as kimchi and cayenne pepper. Our results imply that high sensitivity to oral spicy, sour and bitter compounds affects spicy food preference in the Japanese. (COI: No)

2P-358

Hypnotic and anti-inflammatory actions of bromovalerylurea

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Bromovarelylurea (BU), an old hypnotic/sedative, is rarely used nowadays. We have focused on its anti-inflammatory effects on macrophages and microglia. BU markedly suppressed pro-inflammatory gene expression by LPS-treated microglia and macrophages. BU suppressed ATP production of primary cultured glial cells. BU (50mg/kg) has therapeutic effects on animal models of some inflammatory disorders. However, there is no available literature describing its hypnotic actions. Therefore, we investigated the effects of BU using EEG recordings. BU was subcutaneously administered at zeitgeber time 3 to male Wistar rats (3 months-old) at doses 50, 125 and 250 mg/kg. The 24h-EEG recordings showed that BU increased non-REM and total sleeping time at doses of 125 and 250mg/kg. At all the doses examined, BU markedly reduced REM durations during light periods. A patch clamp examination on slices from murine somatosensory cortex (L2/3) suggested that BU modulates GABAA receptor as a hypnotic . However, a GABAA antagonist, bicuculline did not affect the inhibitory effect of BU on LPS-induced nitiric oxide release by BV2 cells. These findings suggest that anti-inflammatory doses and hypnotic doses are quite different and sleep pattern of BU is similar to those of barbiturate or benzodiazepines, since those also suppress REM sleep.Collectively, BU may have two distinct actions as a hypnotic and an anti-inflammatory agent based on independent mechanisms. (COI: No)

2P-359

Memantine selectively ameliorates gait impairment to hyperalgesia in MPTP-injected mice

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Parkinson's disease (PD), characterized by both motor and non-motor dysfunctions is the second most common neurodegenerative disease. Combined with the evidence for striatal glutamatergic overactivity in animal models of Parkinsonism, NMDA receptor antagonists have been suggested to prevent dopaminergic neuron death and ameliorate PD symptoms. Whether NMDAR antagonists selectively prevent PD symptoms in experimental PD models and its mechanisms were not disclosed. In our automated gate analysis (CatWalk system), MPTP treatment decreased average speed and cadence with decreased stand index, swing speed, and stride length. It also increased stance and swing duration. All the impairment of gait behavior was ameliorated by the treatment of memantine, an extrasynaptic NMDAR (eNMDARs) antagonist. In an agreement, MPTP treatment decreased the number of TH-positive cells in substantia nigra pars compacta (SNpc) compared with the control group, whereas additional memantine treatment significantly attenuated the decrease in the dopamine cell numbers. Interestingly, however, memantine did not affect MPTP-induced hyperalgesia in mice. Our results demonstrated that the eNMDAR antagonist, memantine, ameliorates gait deficits and progressive loss of dopaminergic neurons in MPTP induced PD model mice. Ongoing experiments assess the role of eNMDAR-mediated currents of SNPc dopaminergic neurons in MPTP injected mice.

Keywords: Catwalk, gait, MPTP, memantine, Parkinson's disease. (COI: No)

2P-360

Physiologic process before rhythmic jaw movements after ketamine injections in guinea pigs

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[Purpose] Rhythmic jaw movements (RJMs) occurred spontaneously under ketamine anesthesia. The changes of physiological states associated with the occurrence of spontaneous RJMs remain unknown. This study aimed to investigate the processes in cortical, cardiac and respiratory activities in the occurrence of RJMs after ketamine injections in guinea pigs. [Methods] Seven male Hartley guinea pigs were prepared for the polygraphic recordings of electroencephalogram (EEG), electrocardiogram, electromyograms of the jaw muscles simultaneously with jaw movements and nasal expiratory airflow under ketamine-xylazine anesthesia. Injection of supplemental ketamine (12.5 mg/kg, i.v.) was repeated after RJMs occurred. Cortical activity, heart rate and respiratory rate were quantified and the time-course changes after ketamine injections before the onset of RJMs were analyzed. [Results] RJMs occurred approximately 20 to 30 min after ketamine injections. After the injection of ketamine, delta and theta bands of cortical activity significantly increased and gradually decreased towards the onset of RJMs. Heart and respiratory rates decreased after injections and gradually increased before the onset of RJMs. These variables at the onset of RJMs did not differ from those before the injection of ketamine. [Conclusion] Spontaneous RJMs occurring after ketamine injections were preceded by a stereotypical change in cortical, cardiac and respiratory activities in guinea pigs. (COI: No)

2P-361

Mitochondrial disease diagnosis by urinary tRNA modification analysis

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Mitochondrial disease is characterised by mitochondrial dysfunction which result in various organopathy. Recent study revealed that defection of taurine modification in tRNA account for mitochondrial dysfunction, especially Mitochondrial Encephalomyopathy, Lactic Acidosis and Strokelike episodes (MELAS).

We found RNA nucleoside including taurine modification are discharged from cell and they are not reabsorbed by the kidney.

At first we analysed tRNA modification in several disease model. By using LC/MS/MS , we found taurine modification was significantly decreased in MELAS cell model supernatant and urine from mouse model.

In the same way, to confirm whether urinary taurine modification can be a biomarker for mitochondrial disease, we analysed urine from MELAS pationts and healthy controls.

Here we suggest novel noninvasive diagnosis for mitochondrial disease. (COI: No)

2P-362

Age-related changes in hemodynamics and their mechanisms in the orofacial area

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Cardiovascular diseases are related with aging of blood vessels. Although the effects of aging on hemodynamics in the orofacial area are still unclear,parasympathetic vasodilation may be important for the orofacial hemodynamics with age because of rapid and wide-spread blood flow increases. However, there are no reports of relationship between aging and parasympathetic vasodilation in the orofacial area. We examined this relationship investigating the blood flows of the masseter muscle (MBF) and the lower lip(LBF) evoked by trigeminal afferentsin young and old anesthetized rats. In young rats, electrical stimulation of central cut end of the lingual nerve (LN) caused significant increases in theMBF and LBF accompanied by systemic arterial blood pressure increase, but in the masseter, LN stimulationinduced a biphasic change consisting vasodilation and vasoconstriction. This vasoconstriction was reduced by pretreatment with guanethidine (antihypertensivedrug). Vasodilations of the masseter and lower lip evoked by LN stimulation significantly decreased in old rats than in young rats. Vasoconstriction of the masseter significantly increasedwith age. Our results indicate that parasympathetic vasodilations are inhibited by aging in the orofacial area, and suggest that these inhibitory effects may be mediated by suppression of vasodilation and enhancement of vasoconstriction withage. (COI: No)

2P-363

Proteomic analysis of the transport system in a connective tissue of the mammalian cochlea

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The cochlea of the inner ear is filled with a unique extracellular solution that exhibits 150 mM [K^+] and a highly positive potential of +80 mV. This called 'endolymph' also shows stable osmolarity of 320 mOsm and pH of 7.5. These properties are essential for hearing and likely to be maintained by transport of not only ions but also bioactive substances across lateral wall of the cochlea. The lateral wall is made up of two different components, an epithelial-like tissue, stria vascularis, and a connective tissue, spiral ligament. A number of experiments have identified different ion channels and transporters in the stria vacularis and such apparatus play key roles in maintenance of the endolymph. Nevertheless, molecular architecture of the transport system in the ligament remains to be understood. Therefore, in the present study we examined protein expression of this tissue with liquid chromatography(LC)-MS/MS in rats. In the first step, we perfused a biotin-containing solution to the surface of the ligament to selectively label the membrane proteins. Thereafter we extracted the proteins and purified the biotinylated fractions with affinity beads. LC-MS/MS analysis of the samples detected 474 membrane proteins, which contain five ion channels and 19 transporters. The protein library described here may be useful to elucidate not only overall architecture of the transport system in the lateral wall but also pathological processes of hearing disorders. (COI: No)

2P-364

Rodent posterior parietal cortex controls ipsilateral as well contralateral movement

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It is well known that the posterior parietal cortex (PPC) and frontal motor cortices in primates preferentially control voluntary movements of contralateral limbs. The PPC of rats has been defined based on patterns of thalamic and cortical connectivity. The anatomical characteristics of this area suggest that it may be homologous to the PPC of primates. However, its functional roles in voluntary forelimb movements have not been well understood, particularly in the lateralization of motor limb representation, that is, the limb-specific activity representations for right- and left-forelimb movements. We examined functional spike activity of the PPC and two motor cortices: the primary motor cortex (M1) and the secondary motor cortex (M2), when head-fixed rats performed right or left unilateral movements. Unlike primates, PPC neurons in rodents were found to preferentially represent ipsilateral forelimb movements, in contrast to the contralateral preference of M1 and M2 neurons. Consistent with these observations, optogenetic photoactivation of PPC and motor cortices, respectively, evoked ipsilaterally and contralaterally biased forelimb movements. Finally, we examined the effects of optogenetic manipulation on task performance. PPC or M1 photoinactivation shifted the behavioral limb preference contralaterally or ipsilaterally, respectively. These paradoxical observations suggest that the PPC plays evolutionarily different roles in forelimb control between primates and rodents. (COI: Properly Declared)

2P-365

Development of a Low-cost, Comprehensive Recording System for Circadian Rhythm Behavior

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One of the widely accepted circadian rhythm behavioral experiment is measuring the wheel-running activity (WRA) of rodents. However, the price for commercially available WRA recording system is not easily affordable for researchers due to high-cost implementation of sensors for wheel rotation. Here, we developed a cost-effective and comprehensive system for circadian rhythm recording by measuring the house-keeping activities (HKA). We have monitored animal's HKA as electrical signal by simply connecting animal housing cage with a standard analog/digital converter: input to the metal lid and ground to the metal grid floor. We show that acquired electrical signals are combined activities of eating, drinking and natural locomotor behaviors which are well-known indicators of circadian rhythm. Post-processing of measured electrical signals enabled us to draw actogram, which verifies HKA to be reliable circadian rhythm indicator. To provide easy access of HKA recording system for researchers, we have developed software, Circa Analysis. This software provides functions for easy extraction of scalable "touch activity" from raw data files by automating seven steps of post-processing and drawing actograms with highly intuitive user-interface and various options. We anticipate our system will benefit many researchers who would like to study circadian rhythm. (COI: No)

2P-366(Y-21)

Molecule REST interacts with brain 5-HT system in tilapia fish during social stress

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Social stress-induced alteration to the brain serotonin (5-HT) system play a key part in the pathogenesis of depression. Repressor element-1 silencing transcription factor (REST) is a transcriptional factor, which is involved in neuroprotection by inhibiting apoptosis and enhancing cell survival. In our study, we aimed to study REST function in 5-HT system during social stress in Nile tilapia fish as a social stress model which shows 5-HT system dysfunction. First, we examined *rest* gene expression in the brain of control and socially defeated groups and localized *rest* positive cells in the brain using real time PCR and in situ hybridization. *Rest* mRNA expression was significantly higher in the midbrain of defeated group and *rest* widely expressed throughout the midbrain in which majority of 5-HT neurons locate. Besides, we examined *rest* positive cell type using neuronal-/glial-cell markers and studied co-localization of *rest* with 5-HT cells in control and defeated groups. Double fluorescent in situ hybridization and immunocytochemistry study identified neuron-specific expression of *rest* and co-localized *rest* positive cells with 5-HT in dorsal and ventral parts of periventricular pretectal nucleus (PPd and PPv), paraventricular organ neucleus (PVO) and dorsal and medial raphe nucleus (DR and MR). Also, *rest*/5-HT co-localization pattern was nearly similar in both groups. Our results suggest REST may regulate 5-HT system through modulating cell viability during social stress. (COI: No)

2P-367(Y-22)

Altered electrical responsiveness of CA1 pyramidal neurons in a *valproic acid rat model* of autism

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Autism spectrum disorder (ASD) is a neurobehavioral disorder which could be due to the imbalance of excitatory and inhibitory neurotransmission in certain part of the brain including hippocampus. However, the cellular mechanisms of possible changes are not fully understood. The aim of the present study is to get a better understanding of possible alterations in the neuronal active properties associated with autism in an autistic-like model of rat induced by prenatal exposure to valproic acid (VPA). The evoked firing characteristics of CA1 pyramidal neurons in young adult offspring rats prenatally treated with VPA (VPA, 500mg/kg, on gestation day 12.5) were examined using whole-cell patch-clamp recording under current clamp condition and the results were compared with control groups. Prenatal treatment with VPA induced a significant decrease in the peak amplitude of evoked action potentials in autistic offspring. In addition, hippocampal pyramidal neurons of the offspring of autistic male rats showed a significantly decrease in rise and decay time constants of elicited action potential when compared to control group. Furthermore, maximum rise slope as well as maximum decay slope and area under curve of action potential were significantly decreased following ASD induction. Findings indicate that induction of autism were associated with changes in evoked neuronal activity**.** (COI: No)

2P-368(Y-23)

Lumbrokinase improves neurological deficit by preventing endoplasmic reticulum stress

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Ischemic stroke result from reduction of cerebral blood flow and it is a major cause of neurological dysfunction and neuronal death that lead to disability. Therefore, reducing survivor's disability is important research direction in clinical. Lumbrokinase, extract from lumbricus rubellus, is a group of bioactive proteolytic enzymes include plasminogen activator and plasmin. Lumbrokinase has not causing hemorrhage since the thrombolytic activity of lumbrokinase only in the presence of fibrin. In clinical, lumbrokinase has been used to improve perfusion in patient with stable angina or stroke. In this study, we used C57BL/6 mice to clarify the neuroprotective effect of lumbrokinase on ischemic stroke and its underlying mechanism. Lumbokinase was administered intraperitonealy 20 minutes after focal cerebral ischemia-reperfusion (I-R) injury. We found that post-ischemic treatment with 1 mg/kg lumbrokinase significantly attenuated infarct volume, and improved the neurological function in mice subjected to focal cerebral I-R injury. Post-ischemic treatment with lumbrokinase also inhibited the enhancement of the I-R induced endoplasmic reticulum stress, apoptosis, autophagy, NLRP3 inflammasome formation. These data suggest that post-ischemic treatment with lumbrokinase could protect against ischemic stroke by decreasing endoplasmic reticulum stress, thereby reducing apoptosis, autophagy, and reducing inflammasome formation and inflammation. (COI: No)

2P-369(Y-24)

Oxytocin effects on nicotine aversion and anxiety in nicotine-exposed early adolescent rats

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Nicotine exposure in early adolescence can lead to behavioral and psychological problems. Although oxytocin is suggested to use as a treatment for nicotine addiction due to reciprocally affecting vulnerability to drug use, its role is still unclear. Here, we assessed the effect of oxytocin on initial nicotine aversion and anxiety in nicotine-exposed rats during an early adolescent period. There were no significant differences in body weight changes among all conditioned rats. Nicotine rats showed decreased aversive responses to initial nicotine exposure compared to saline rats regardless of oxytocin exposure. When nicotine concentration was switched from low to high dose, oxytocin exposes in nicotine rats was decreased nicotine aversion compared to saline rats. There were significantly increased anxious behaviors in oxytocin rats regardless of nicotine exposure, whereas oxytocin did not affect locomotor behavior. Moreover, oxytocin rats and nicotine rats showed a significant correlation between the increase of anxiogenic behavior and the decrease of nicotine consumption in initial nicotine exposure, whereas, there was a tendency toward the opposite correlation in nicotine rats with oxytocin exposure. Taken together, we suggest that oxytocin in repeated nicotine exposure rats during early adolescent induces the decrease in aversive nicotine responses, and oxytocin increases in anxiety regardless of exposure to nicotine. (*Supported by NRF-2016R1D1A1B03934263*) (COI: No)

2P-370(Y-25)

Mesenchymal stem cell conditioned medium therapy modulates neuroinflammatory symptoms

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Neuroinflammation plays a crucial role in expression of symptoms of numerous autoimmune and neurodegenerative diseases such as pain during rheumatoid arthritis. Overproduction of pro-inflammatory cytokines have been strongly implicated in the generation of pathological pain states, particularly at central nervous system sites and induction of spinal neuroinflammatory symptoms. The wide ranges of research to define new therapeutic approaches, including neuroimmune-modulators like stem cells are in progress. Mesenchymal stem cells conditioned medium (MSC-CM) has anti-inflammatory factors which can regulate the immune responses. The aim of this study is to investigate the effect of administration of MSC-CM on behavioral, cellular and molecular aspects of adjuvant-induced arthritis in male Wistar rats. Complete Freund's adjuvant (CFA)-induced arthritis (AA) is caused by single subcutaneous injection of CFA into the rat's hind paw on day 0. MSC-CM is administered daily (i.p.) and during the 21 days of the study after injection. Hyperalgesia, edema, serum TH-17 and tissue IL-17 and IL-21 levels and spinal IL-15 and -23 activities are assessed on days 0,7,14 and 21 of the study.
(COI: No)

2P-371(Y-26)

Depolarized subicular microcircuits mediate generalized seizure in temporal lobe epilepsy

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Secondary generalized seizure (sGS) is a major source of disability in temporal lobe epilepsy (TLE) with unclear cellular/circuit mechanisms. Here we found that clinical TLE patients with sGS showed the reduced volume specifically in the subiculum compared with those without sGS. Further, using optogenetics and extracellular electrophysiological recording in mice models, we found that photoactivation of subicular GABAergic neurons retarded sGS acquisition by inhibiting the firing of pyramidal neurons. While once sGS had been stably acquired, photoactivation of those neurons aggravated sGS expression *via* depolarized GABAergic signaling. Subicular somatostatin but not parvalbumin subtype GABAergic neurons were not easily depolarized in sGS expression. Finally, photostimulation of subicular pyramidal neurons genetically targeted with proton pump Arch, rather than chloride pump NpHR3.0, alleviated sGS expression. These results demonstrated that depolarized GABAergic signaling in subicular microcircuit mediates sGS in TLE. This may be of therapeutic interest in understanding the pathological neuronal circuitry underlying sGS. (COI: Properly Declared)

2P-372(Y-27)

Mitochondrial fission inhibitor attenuates brain mitochondrial dysfunction in pre-diabetic rats

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Purpose: Excessive brain mitochondrial fission via an increase in Dynamin-related protein 1 (Drp1) activation and mitochondrial dysfunction play a role in neuronal death in obese-insulin resistant condition or pre-diabetes. Therefore, the present study investigated the effects of a mitochondrial fission inhibitor (Mdivi-1) on brain mitochondrial function in obese-insulin resistant rats induced by high-fat diet (HFD) consumption. We hypothesized that Mdivi-1 exerts neuroprotection by improving brain mitochondrial function in pre-diabetic rats.
Methods: Fifteen male Wistar rats were divided into 2 groups to received either normal diet (ND: n=5) or HFD (n=10) for 14weeks. At week 12, HFD-fed rats were assigned to 2 subgroups (n=5/subgroup) to receive either saline or Mdivi-1 (1.2 mg/kg) via intraperitoneal injection for 2 weeks. Then, rat was sacrificed and brain was removed. The metabolic parameters, brain mitochondrial dynamics and function were determined.
Results: HFD-fed rats developed obese-insulin resistance as indicated by increased plasma insulin and HOMA index, as well as impaired brain mitochondrial dynamics and function. Mdivi-1 exerted neuroprotection via improved metabolic parameters, decreased mitochondrial fission, and attenuated mitochondrial dysfunction as indicated by decreased mitochondrial ROS production, depolarization and swelling.
Conclusions: Mdivi-1 exerts neuroprotection via improved mitochondrial dynamics and its function in pre-diabetic rats. (COI: No)

2P-373

Effects of vagotomy and area postrema lesion on induction of emesis by emetine

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We investigated emetine-induced nausea in vagotomized or area postrema lesioned rats. Emetine is the main ingredient of Ipecac that is well-known for its acute emetic effects. Ipecac-induced acute emesis is considered to be induced by gastric mucosal irritation and upregulation of CTZ, however, there is no study elucidate it. The present study focused on the role of vagal nerve activity and neuronal excitability in the area postrema during the emetine-induced nausea. Male Sprague Dawley rats (250~350 g) were used as experimental animals. We performed experiments of conditioned taste aversion (CTA) in 3 groups of animals : intact rats (control), subdiaphragmatic vagotomized rats (VX), and area postrema lesioned rats (APX). Saccharin was used for the conditioned stimulus, nausea induced by emetine injection (1.0 mM, 10ml/body weight, i.p.) was used for unconditioned stimulus. CAT was measured by 1 bottle test after training period of scheduled drinking, i.e. test drinking for 20min, free drinking for 3 h, and 20h 40min water deprivation every day. Although control group acquired CAT, but CTA was absent in the APX group. In the VX group, CTA was reduced as compared with the control group, but not fully be offset. These results suggest that emetine-induced nausea and/or emesis may dominantly depend on the neuronal excitability in the area postrema. (COI: No)

2P-374

Kampo medicine Junchoto promotes intestinal Cl^-/water secretion by cAMP-dependent CFTR activation

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Constipation is a common symptom that often impairs the quality of daily life. Several different medicines, including Japanese herbal (Kampo) medicines, have been used to mitigate constipation. However, the mechanisms of their actions are often not well understood. Here, we aimed to investigate the molecular mechanism underlying the effect of Kampo medicine, Junchoto (JCT), which is empirically prescribed for chronic constipation.
Cl^- channel activity was measured by the patch clamp method. The cAMP was measured by chemiluminescence. Cell volume change was measured by a coulter counter and camera image measurements.
In both CFTR-expressing HEK293T and Caco-2 cells, JCT dose-dependently induced whole-cell currents showing typical biophysical and pharmacological features of CFTR. Endogenous expression of CFTR in Caco-2 cells was confirmed by RT-PCR and western blotting. Luciferase-based measurements revealed that JCT increases the intracellular cAMP level. Treatment with SQ22536 (SQ), CFTR inhibitor-172, or siRNA targeting CFTR suppressed JCT-induced whole cell currents, suggesting that JCT induces elevation of intracellular cAMP and causes activation of CFTR in Caco-2 cells. Finally, blockade of CFTR activity by CFTR inhibitor-172, siRNA-knockdown of CFTR, or application of SQ markedly reduced the cell volume decrease induced by JCT.
JCT can induce a Cl^- efflux through CFTR channel to promote water secretion, and this effect is likely mediated by increased cAMP production. (COI: No)

2P-375

CFTR function and *CFTR* mutations of cystic fibrosis in Japan

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[Purpose] Cystic fibrosis (CF) is a rare disease in Asian including Japanese. More than 2,000 mutations and polymorphisms have been reported in the *CFTR* gene and the mutations detected in Japanese are different from those found in Europeans. In the present study, we have tried to characterize CFTR function and CF-causing *CFTR* mutations in Japan.
[Methods] Clinical data of CF are accumulated by nationwide survey conducted every 5 years from 1994 and CF registry established in 2012. All exons, their boundaries, and promoter region (up to ~1,000 bp upstream) of *CFTR* were directly sequenced and genomic rearrangements were examined by multiplex ligation-dependent probe amplification. Sweat was stimulated by pilocarpine iontophoresis and Cl^- concentration was estimated from conductivity.
[Results] Clinical data of 118 CF patients (60 males, 58 females) have been collected and the median survival time is about 23 years. Thirty-four consecutive patients with definitive CF were analyzed for *CFTR* mutations since 2004. While European type mutations including F508del were found in 18 alleles, Japanese-type mutations were found in 43 alleles. A large genomic deletion spanning exons 16, 17a and 17b (c.2908+1085_3367+260del7201) were detected in 18 Japanese alleles. Sweat $[Cl^-]$ was measured in 29 among 34 patients. Sweat $[Cl^-]$ was 113 ± 48 mmol/L (mean ± SD).
[Conclusion] Very low incidence, poor prognosis, and severe CFTR dysfunction of CF patients in Japan were demonstrated. (COI: No)

2P-376

Characterization of The Most Frequent CFTR-Mutant Found in Japanese Cystic Fibrosis Patients

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Purpose
Cystic Fibrosis (CF) is reported to be very rare among Asians (approximately three per million in Japan) and previous reports suggested that the profiles of CF-caused CFTR mutations found in Japanese CF patients are different from Caucasians. Recently a novel massively deleted mutation lacking the coding sequences along three exons without frameshift (dele 16-17b mutation) has been found in Japanese CF patients with high frequency. In this study, we attempted to characterize effects of the dele 16-17b mutation on CFTR protein inducing Δ(G970-T1122).
Methods
We expressed mutant-CFTR in CHO cells and characterized their expression manner and function using immunoblot analysis, immunocytochemistry with a super resolution microscopy and whole-cell clamp.
Results
The mutant CFTR proteins were synthetized and core-glycosylated but not complex-glycosylated suggesting that the Δ(G970-T1122) mutation can be categorized into the class II mutation like ΔF508. However, VX-809 a CFTR corrector that can help maturation of ΔF508, had no effect on Δ(G970-T1122).
Conclusions
The dele 16-17b mutation produces a class II mutant Δ(G970-T1122)-CFTR. Lumacaftor (VX-809), aclass II mutant CFTR corrector, is of little help. It is unlikely for them to carry out the function of cAMP-regulated chloride channel or respond to CFTR potentiators. It remains an overbearing challenge to overcome defects caused by some mutations such as Δ(G970-T1122). (COI: Properly Declared)

2P-377

Non-morphogenic function of Sonic Hedgehog as a negative regulator of gastric H^+,K^+-ATPase

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In the stomach, Sonic Hedgehog (Shh), a member of Hedgehog family, is mainly secreted from gastric parietal cells, and acts as a morphogen in development and renewal of the gastric epithelium. Here, two types of vesicles were prepared from hog gastric mucosa; that is, the stimulation-associated vesicles (SAV) derived from apical membrane and the intracellular tubulovesicles (TV) of the gastric parietal cells. We found that the cleaved N-terminal fragment of Shh (Shh-N; 19 kDa) was abundantly expressed in SAV but not in TV. In immunostaining of SAV, Shh was observed to co-localize with gastric proton pump (H^+,K^+-ATPase). Interestingly, human Shh-N recombinant significantly decreased the H^+,K^+-ATPase activity (K^+-ATPase activity sensitive to 50 μM SCH28080, an inhibitor of H^+,K^+-ATPase) in the freeze-dried leaky TV in a dose-dependent manner (0.5-2 μg/ml). In contrast, no significant effect of the recombinant (1.5 μg/ml) on the H^+,K^+-ATPase activity was observed in the intact TV. Furthermore, Shh-N recombinant significantly suppressed the H^+,K^+-ATPase activity in the membrane fractions of hog kidney LLC-PK1 cells stably expressing H^+,K^+-ATPase. In the living cells, SCH28080-sensitive $^{86}Rb^+$ uptake was also decreased by the recombinant. These results suggest that N-terminal polypeptide of Shh is highly expressed in the apical membrane of gastric parietal cells and acts as a negative regulator of H^+,K^+-ATPase by binding to an extracellular side of the pump. (COI: No)

2P-378

Aldosterone action on epithelial Na^+ channel trafficking under the insulin-stimulated condition

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Epithelial Na^+ channel (ENaC) regulates renal epithelial Na^+ reabsorption, controlling blood pressure. Aldosterone and insulin also up-regulates blood pressure by increasing the ENaC-mediated Na^+ reabsorption. However, no information is available on the interaction of aldosterone and insulin on the ENaC-mediated Na^+ reabsorption. In the present study, we tried to clarify the aldosterone action on the insulin-stimulated ENaC-mediated Na^+ reabsorption from a viewpoint of intracellular ENaC trafficking. We measured the insulin-stimulated ENaC-mediated Na^+ transport as short-circuit currents using a four-state mathematical ENaC trafficking model in renal A6 epithelial cells with or without aldosterone treatment. We found that aldosterone treatment significantly elevated the ENaC insertion rate to the apical membrane to a 3.4-fold level, and the ENaC recycling rate to a 2.4-fold level, but diminished the degradation rate to a 0.69-fold level. Aldosterone is well known to diminish the endocytotic rate of ENaCs, leading ENaCs to stay at the apical membrane for longer time. However, the present study indicates that this action of aldosterone on the ENaC endocytotic rate is masked by insulin. This observation suggests that insulin has more effective action on the ENaC endocytotic rate than aldosterone. (COI: No)

2P-379

Loss of ezrin causes impaired proximal tubular solute reabsorption in the kidney

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Ezrin is highly expressed in the renal proximal tubules, and is known to form multi-protein complex with a scaffold protein NHERF (Na^+/H^+exchanger regulatory factor 1), and several transporters including Na^+/Phosphate cotransporter NaPi2a. We previously reported that ezrin knockdown (EKD) mice exhibited the hypophosphatemia due to the abnormal membrane localizations of NaPi2a and NHERF1. Since EKD mice exhibited severe growth retardation, we examined the influence of loss of ezrin expression to the membrane localizations of other transporters in the proximal tubules. We performed comprehensive proteomic analysis of renal brush border membrane (BBM) fractions from WT and EKD mice. Totally 1412 proteins including 45 SLC transporters were identified in renal BBM. Interestingly, expressions of Na^+dependent glucose transporters and several amino acid transporters were down-regulated in BBM from EKD mice. EKD mice exhibited hypoglycemia and increased fractional urinary excretion of glucose ($FE_{glucose}$). Urinary leak of some kinds of amino acids was also observed in EKD mice. Furthermore, uptake of FITC-labeled β_2-microglobulin in the proximal tubules was limited in EKD proximal tubules. These results suggest that ezrin plays important roles in the regulation of membrane targeting of transporters and receptor-mediated endocytosis in the proximal tubules, and dysfunction of ezrin might be associated with the onset of Fanconi syndrome. (COI: Properly Declared)

2P-380

Inhibition of prostaglandin E_2-induced Cl^- secretion by dihydropyrazole derivatives in rat colon

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In the colon, Cl^- secretion is stimulated by prostaglandin E_2 (PGE_2) via cAMP pathway. The Cl^- secretion is mediated by CFTR Cl^- channel. Here, we synthesized dihydropyrazole derivatives (KYY-001~014) and examined effects of them on PGE_2-stimulated Cl^- secretion in the colon. To assess Cl^- secretion, isolated mucosa of rat distal colon was set on the Ussing chamber, and short circuit current (Isc), membrane potential (Pd), and tissue conductance (Gt) across the mucosa were measured. To record directly the Cl^- channel current derived from CFTR, whole-cell patch-clamp recordings were applied in the HEK293T cells overexpressing CFTR. First, we tested effects of these compounds (each at 10 μM) on the PGE_2 (0.5 μM)-induced Cl^- secretion. Among them, only KYY-005 markedly attenuated the Cl^- secretion (estimated by changes in Isc, Gt and Pd), whereas other derivatives had only small effects. We suggest that *p*-trifluoromethylphenyl group at position 3 of the 2,3-dihydropyrazole ring of KYY-005 may be essential to elicit the effect. Next, whole-cell Cl^- currents were measured in the CFTR-overexpressing cells. In the presence of intracellular cAMP (30 μM), KYY-005 (30 μM) had no significant effect on the Cl^- current, whereas a CFTR inhibitor (10 μM CFTRinh-172) was effective. Of note, forskolin (1 μM)-increased current was significantly attenuated by KYY-005. These results suggest that KYY-005 inhibits production of cAMP, and then, interferes activation of CFTR Cl^- channel. (COI: Properly Declared)

2P-381

ZO family proteins regulate epithelial polarity independent of Tight Junction strand assembly

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Epithelial barrier and polarity are fundamentally important for epithelial transport, and tight junctions (TJs) play pivotal roles in epithelial barrier formation. It has been believed that TJ strands play important roles in epithelial polarity by acting as a membrane fence to segregate the apical and basolateral plasma membranes (van Meer and Simons, 1986). However, conflicting views exist on the roles of TJs in epithelial polarity (Baas et al, 2004; Umeda et al., 2006; Phua et al., 2014), and whether TJs are required for epithelial polarity or not remains an unsettled issue. Claudin family genes are the major integral membrane proteins of TJs. Claudins interact with ZO family proteins (ZO-1/2/3) through their cytoplasmic tails, and ZO family proteins are required for TJ strand assembly (Umeda et al., 2006). To investigate the roles of TJs in epithelial polarity, we perturbed TJ assembly in MDCK II cells by generating ZO-1/ZO-2 double KO cells and claudin serial KO cells. Both ZO family proteins and claudins were found to be essential for TJ strand assembly and epithelial barrier formation. Intriguingly, epithelial polarity was disorganized in ZO-1/ZO-2 double KO cells, whereas claudins were dispensable. These results demonstrate that ZO family proteins regulate epithelial polarity independent of TJ strand assembly. (COI: No)

2P-382

Establishment of a tight junction-deficient epithelial cell line by genome editing of claudin genes

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Tight junctions (TJs) contribute to epithelial barrier and transport by regulating free diffusion of solutes through the paracellular pathway. Claudins, a family of TJ-associated membrane proteins, constitute functional structures of TJs within the plasma membrane, namely TJ strands. One of the key findings in TJ research to date is that each claudin subtype has its unique barrier/channel property in TJs. This concept has been established mainly by exogenous expression of a certain claudin subtype in epithelial cell lines, followed by evaluation of the functional property of TJs. However, the results of these experiments are often not consistent between different cell lines probably because of the effect of endogenous claudin subtypes in each cell line on the property of TJs. To overcome this problem, we attempted to generate a claudin-deficient epithelial cell line. By TALEN-mediated serial disruption of five claudin genes in MDCK II cells, we established a cell line, designated claudin qKO, which lacks TJ strands in freeze-fracture replica electron microscopy. Measurements of transepithelial electrical resistance and paracellular flux showed impaired epithelial barrier function in claudin qKO cells. Exogenous expression of any claudin subtype among the disrupted five claudins in these cells reconstituted functional TJ strands. Thus, claudin qKO cells are useful for characterization of individual claudin subtypes and for the understanding of the mechanism of TJ formation. (COI: No)

2P-383

Electrogenic K^+ secretion induced by butyrate in rat rectal colon

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Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, supply energy and affect ion transport. However, their roles in ion transport remain unknown. In order to elucidate the roles of SCFAs, we measured short-circuit currents (I_{SC}) and performed RT-PCR and immunohistochemical analyses of ion transporters in rat rectal colon. The application of 30 mM butyrate shifted I_{SC} in a negative direction, but did not attenuate the activity of epithelial Na^+ channels (ENaC). The application of bumetanide, a Na^+-K^+-$2Cl^-$ cotransporter inhibitor, to the basolateral side reduced the negative I_{SC} shift induced by butyrate. The application of XE991, a KCNQ-type K^+ channel inhibitor, to the apical side decreased the I_{SC} shift induced by butyrate in a dose-dependent manner. The I_{SC} shift was independent of HCO_3^- and insensitive to ibuprofen, a Na^+-coupled transporters for monocarboxylates (SMCT1) inhibitor. The mucosa from rat rectal colon expressed mRNAs of H^+-coupled monocarboxylate transporters, MCT1, MCT4, and MCT5. RT-PCR and immunofluorescence analyses demonstrated that KCNQ2 and KCNQ4 channels localized to the apical membrane of surface cells in rat rectal colon. These results indicate that butyrate, which may be transported by MCTs, activates K^+ secretion through KCNQ-type K^+ channels on the apical membrane in rat rectal colon. KCNQ-type K^+ channels may play a role in intestinal ion transport and defense mechanisms in the gastrointestinal tract. (COI: No)

2P-384

Dragon fruit oligosaccharide ingestion enhances mouse intestinal motility

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Dragon fruit oligosaccharide (DFO) has a prebiotic property which improves gut health by selectively stimulating the beneficial gut microbiota. Altering a microbiota composition may affect gut motility, however, there is no DFO effect study on this function. Thus, aim of this research was to investigate DFO ingestion effects on fecal pellet propulsion and spontaneous motility patterns of isolated mouse colon. Administration of 500 and 1000 mg/kg DFO for 1-2 weeks significantly increased fecal pellet weight when compared to control. In gut transit studies by using Evan-blue and charcoal meals, mice treated with 500 and 1000 mg/kg DFO significantly reduced the total gut transit time but increased the distance of upper gut transit when compared to control. In spatiotemporal map of colonic propulsive motility studies, colonic profile was recorded with a video camera, and image analysis was used to construct the map of the intestinal wall motions. DFO significantly increased the number of total colonic contractions, especially non-propagation pattern, and velocity of fecal pellet movement through the whole colon. As a result, it has been defined that in addition to be a prebiotic, DFO also acts as a bulk laxative which increases fecal output, and a stimulant laxative which increases intestinal motility in mice. (COI: No)

2P-386

The Effect of Fermented Milk and Soy for Controling Blood Glucose and Lipid Level on Rats

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Background : Probiotic have a severals benefit for human and livestock like increasing digestibility, and to decrease blood lipid

Objective : This study aims to determine the effect of fermented cow and soybean milk and combination of both with probiotic for controling on blood glucose and lipid level of rats.

Methodology : The experiment applied Completely Randomized Design The female rats was grouped into 5 treatments: and 6 replications , the total was 30 .. The treatments consisted of normal diet (ND)(T0); ND + fermented cow milk (T1); ND + fermented cow milk 75% + fermented soybean milk 25% (T2), ND + fermented cow milk 50% + fermented soybean milk 50% (T3); and ND + Probiotic fermented cows milk (T4). Blood taken at the end of the study (6 weeks).

Result : Result indicated that addition of fermented cow and soybean milk with probiotic bacteria can decrease base line blood glucose also for decreasing the blood lipid.

Conclusion : Addition fermented cow and soybean milk can be recomended for improving the blood glucose and blood lipid

Keywords : probiotic, fermented milk and soy milk,blood lipid, glucose, rats (COI: No)

2P-387

Effects of dragon fruit oligosaccharide on microbiota in proximal and distal colon of mouse

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Prebiotic oligosaccharides are used as the supplements to improve gut health. Oligosaccharide derived from dragon fruit (DFO) is a mixture of fructooligosaccharide (FOS), which has prebiotic properties to increase beneficial bacteria *in vitro*. In this study, we aimed to investigate bacterial community change in colon of mice fed with DFO. Mice were given distilled water, 100, 500 and 1000 mg/kg of DFO, and 1000 mg/kg FOS. The results showed that DFO did not change body weight of mice, but it altered bacterial community in proximal and distal colon. ITS fingerprinting showed different patterns among the treatments. Moreover, it seemed that DFO increase diversity of bacteria in mouse colon. The high proportions of bacteria were *Blautia, Parabacteroides* and *Bacteroides* in all treatments. The results from qPCR showed that Bifidobacteria increased in distal colon of mice treated with 100 and 1000 mg/kg of DFO for 14 days, and Lactobacilli increased in proximal colon of 500 mg/kg DFO treated mice (7 days). In contrast, Enterococci in proximal colon were decreased, when mice were given with 100, 500 and 1000 mg/kg of DFO for 14 days. These results suggest that DFO is capable to increase beneficial bacteria and decrease some harmful bacteria. (COI: No)

2P-388

Daikenchuto ameliorates intestinal fibrosis by activating myofibroblast TRPA1 channel

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Background

Daikenchuto (DKT) is a traditional herbal medicine widely used to mitigate post-operative ileus and constipation. In this study, we investigated antifibrotic effects of DKT on TRPA1 channel in intestinal myofibroblasts.

Method

An intestinal myofibroblasts was stimulated with TGF-beta1 to produce fibrotic conditions. A murine model of chronic colitis model mice was established by weekly intrarectal administration of TNBS. Pathological staining analysis was performed with biopsy samples from non-stenotic and stenotic regions from Crohn's Disease patient's intestines.

Results

Active ingredients of DKT induced Ca^{2+} influxes in InMyoFib, which were antagonized by co-treatment with a selective TRPA1 channel blocker HC-030031. DKT suppressed TGF- beta1-induced expression of Type I collagen and alpha-smooth muscle actin, being accompanied by phosphorylation of Smad-2 and p38-MAPK. Moreover, DKT or Japanese Pepper increased the expression of TRPA1 channels. Inflammation and fibrotic changes in TRPA1-KO mice is severer in wild type than TRPA1 knockout mice. One-week DKT treatment reduced fibrotic lesions in wild-type but not in TRPA1-KO mice. TRPA1 expression was significantly enhanced in stenotic regions.

Conclusion

The effects of DKT on the expression and activation of the myofibroblast TRPA1 channel could suppress intestinal inflammation and fibrosis progression, which would in part account for the reported beneficial actions of DKT on inflamed intestines. (COI: No)

2P-389

The peripheral regulation of rectal visceral sensation by 5-HT_4-cAMP and NO-cGMP pathways

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PURPOSE: The luminal epithelium of gastrointestinal and urinary tracts releases ATP in response to mechanical and chemical stimuli. The released ATP transmits viscerosensory signals to the central nervous system via $P2X_{2/3}$ at afferent nerve terminals. Here we investigated the contribution of 5-HT_4-cAMP and NO-cGMP pathways to the peripheral regulation of ATP release from the rectum.

METHODS: The opened rectum from adult male SD rats was mounted in an Ussing chamber. Hydrostatic pressure (20 mmHg) was applied to the serosal side. ATP content in the mucosal side of chamber was assayed with the luciferin-luciferase method.

RESULTS: RT-PCR analysis revealed that 5-HT_4 mRNA was specifically expressed in the mouse colorectal mucosa. Whereas 5-HT_4 agonist tegaserod reduced the pressure-induced ATP release from the mucosal side of the rectum, 5-HT_4 antagonist GR113808 increased the ATP release. The repressive effect of cAMP on ATP release was confirmed by the experiments using forskolin, rolipram, or 2',5'-dideoxyadenosine. Furthermore, an NOS inhibitor L-NAME facilitated the ATP release whereas a PDE5 inhibitor sildenafil decreased it.

CONCLUSIONS: These results indicated the inhibitory effects of 5-HT_4-cAMP and NO-cGMP pathways on the pressure-induced ATP release from the mucosal side of rectum. These pathways are potential therapeutic targets for colorectal visceral hypersensitivity in IBS. (COI: No)

2P-390

Calcium Oscillation Complexes in Colonic Musculatures of Mice

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Colonic motility is required to change significantly depending on the functional state. During accommodation tonic contractile force is maintained in balance with the normal force of luminal content, while upon transportation and defecation phasic rapid contractions propel the content. To investigate the mechanisms underlying the radical changes of contractile feature, we used transgenic mice, expressing an intracellular Ca^{2+} ($[Ca^{2+}]_i$) indicator (YC-Nano50) in colonic muscle cells. Förster resonance energy transfer (FRET)-based imaging revealed spontaneous generation of Ca^{2+} oscillation complexes consisting of slow and rapid components. A sustained $[Ca^{2+}]_i$ increase was formed by accumulation of rapid Ca^{2+} oscillations. A small increase in basal $[Ca^{2+}]_i$ of ~1.2-folds, shifted the muscle contractile property from a responsive to unresponsive mode, preferable to the functional states of transportation/defecation and accommodation, respectively. The results indicated the second Ca^{2+}dependent switch for shifting the contraction mode, in addition to phasic contractions induced by rapid $[Ca^{2+}]_i$ transients. The latter mode may correspond to the latch-bridge, for economic contraction. (COI: No)

2P-391

Chronic vomiting observed in captive common marmosets

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Common marmosets, a non-human primate model animal in various researches, sometimes present chronic gastrointestinal problems like diarrhea and vomiting. Treatments on chronic vomiting in marmosets have been mainly symptomatic, using gastric antacids and antiemetic. Vomiting may lead to fatal conditions after showing anorexia, hypothermia, and dehydration. The present study reports the long-term evaluation of the treatments on marmosets with chronic vomiting including medication, changes in feeding programs, and restricted feeding time. The adult marmosets showed chronic and intermittent vomiting for more than one year, but no indication of pica. In the early phases of chronic vomiting, they did not lose the weights. The health conditions gradually became serious with dehydration and emaciation. Antacid, antiemetic, and electrolyte were administrated subcutaneously. We confirmed negative results on protozoa and Giardia antigen using fecal samples, and blood sample tests showed no severe problems. Because there were no clear reasons for vomiting, we modified the feeding regimen and added vegetables, fruits, gums, and boiled eggs, while the amount of complete commercial pellet food was reduced. We also removed the feeding dish during evening, to prevent vomiting in the morning. Although primary cause of their vomiting is still unknown, feeding condition would be one of the important factors to control chronic vomiting in common marmosets. (COI: Properly Declared)

2P-392

Clostridium difficile disrupts epithelial barrier function by altering tight junction proteins

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Clostridium difficile is the major cause of nosocomial antibiotic-associated diarrhea. *C. difficile* infection (CDI) is characterized as acute colonic inflammation associated with destruction of epithelial cells and disruption of gut barrier. Tight junction (TJ) is one of the cell-cell junctions and known to have the barrier and fence functions between adjacent cells. To investigate the gut epithelial integrity after infection, we examined the distribution pattern, constitutive proteins, and permeability of TJ both *in vivo* and *in vitro*. Ultrastructural observations based on the ultrathin section revealed that loss of TJs both in the infected murine colon tissues and infected colon cells. The apical distribution of ZO-1, a tight junction maker, was decreased after infection by immunofluorescence staining and measurement of protein levels. Permeability analysis, demonstrated by levels of serum LPS and FITC-dextran, showed that the gut permeability was significantly increased in the infected animals. The disruption of tight junction functionality was revealed by real time cell analyzer in C. difficile infected HT-29 cells. Taken together, our results demonstrate TJs was decreased which associated with the loss of gut integrity during *C. difficile* infection. (COI: No)

2P-393

Characterization of physiological function of IBD-associated gene LRRK2 in mouse intestine

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[Purpose] Inflammatory Bowel Disease (IBD) is a chronic inflammation of gastrointestinal tract. Recent study suggested that dysbiosis of the gut microbiota and dysregulated immune response are involved in the pathogenesis, however, the etiology remain largely unknown. Leucine-rich repeat kinase-2 (LRRK2) is a multidomain structured protein kinase, and has been reported that LRRK2 gene variants are associated with IBD. In the present study, we investigated the physiological function of LRRK2 in intestine.

[Methods] Distribution of LRRK2 in intestine was analyzed by western blotting and immunostaining. Intestinal permeability was determined by the fluorescence of FITC in plasma of wild type (WT) and LRRK2 knockout (LRRK2-KO) mice after oral administration of FITC-dextran. The severity of colitis of these mice was compared using colitis model by administration of 2% dextran sodium sulfate (DSS) water.

[Results and discussion] LRRK2 protein was mainly expressed in the colon, and LRRK2 positive cells were found in intestinal plexus and mucosal lamina propria. Intestinal permeability of LRRK2-KO mice was lower than WT. Furthermore, it was found that disease activity index of DSS-colitis in LRRK2-KO mice was significantly higher than the WT mice. In addition, mRNA expression of TNF-α and IL-6 was significantly increased in LRRK2-KO mice compared with WT mice. These results suggest that loss of function of LRRK2 in the intestinal tissue may increase the susceptibility to colitis. (COI: No)

2P-394

Analysis of the effect of high-fat diet on intestinal barrier using mouse colitis model

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[Purpose] Inflammatory Bowel Disease (IBD) is a chronic inflammation of the intestines. Current understanding of IBD pathogenesis points out the interplay of genetic events and environmental cues in the dysregulated immune response. Recent epidemiologic studies suggest an increasing incidence and prevalence of IBD in developed countries that have implemented a western diet. Therefore, in this study, we investigated the effect of high-fat diet on the intestinal barrier.
[Methods] The normal diet (ND), high-fat diet (HFD) or normal diet supplemented with cholic acid (CA) fed to C57/BL6J mouse for 3 month. The intestinal permeability was determined by measurement of FITC-dextran in the serum after administration of FITC-dextran. The colitis was induced by drinking of 2% dextran sodium sulfate (DSS) water.
[Results and discussion] Unexpectedly, intestinal permeability was decreased in HFD-fed mice compared with the mice fed ND. In contrast, increased permeability of intestine was observed in the mice fed CA. Interestingly, we observed that fecal color was changed to white in HFD-fed mice. Furthermore, it was found that colitis by DSS administration was exacerbated in CA-fed mice compared with ND-fed group, however, in the HFD-fed mice, DSS-colitis was diminished. These results suggest that the increases of bile acid content in the intestinal tract by intake of high-fat diet may increase the intestinal permeability and the susceptibility to intestinal inflammation. (COI: No)

2P-395(Y-28)

Protective effects of dapagliflozin and atorvastatin on renal function in insulin-resistant rats

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Purpose
High-fat high-fructose diet (HFF) can induce dyslipidemia and lipid accumulation in the liver which are related to obesity and metabolic syndrome. Dyslipidemia and lipid accumulation can lead to lipotoxicity, and cellular damage by activating oxidative stress and inflammatory pathways that leads to kidney dysfunction. This study investigated whether dapagliflozin alone or combined with atorvastatin could improve metabolic parameters, lipid profile, and kidney lipid accumulation in HFF-induced insulin resistant rats.
Method
Male Wistar rats were divided into normal diet group and HFF diet group fed with high-fat diet with 10% fructose in drinking water for 16 weeks. After that, the rats were divided into 4 subgroups to receive drug treatment for 4 weeks; vehicle group (HFF), SGLT2i (dapagliflozin; HFFD) group, atorvastatin (HFFA) group, and SGLT2i combined with atorvastatin (HFFDA) group.
Results
HFF rats demonstrated an increase in $AUC_{glucose}$ in OGTT, dyslipidemia, renal lipid accumulation and renal dysfunction. Insulin resistance, lipid profile, renal lipid accumulation and kidney dysfunction were improved in HFFD. HFFDA group did not show the significant differences in metabolic parameters and kidney function when compared to a single drug treatment.
Conclusion
In summary, dapagliflozin treatment showed better efficacy on insulin resistance, lipid accumulation and kidney function than those of the combined treatment in insulin resistance model. (COI: No)

2P-396

Protective Effects of Agomelatine on Inflammation in Obesity-Induced Kidney Injury

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Purpose
Obesity is a major risk factor for several diseases including chronic kidney disease. Adipocyte hyperplasia in obesity can stimulate pro-inflammatory cytokines release and resulting in kidney inflammation. Agomelatine (AGOM) is an antidepressant drug that acts as a melatonin-receptor agonist. There are several biological effects of AGOM such as anti-oxidant and anti-inflammation. However, effect of AGOM on obesity-induced kidney injury are not clearly understood. The current study was designed to investigate the effect of AGOM on inflammation in obesity-induced kidney injury.
Methods
Male Wistar rats were divided into two groups: normal diet (ND) group fed with standard rat chow and high-fat diet (HFD) group fed with high-fat diet for 16 weeks. After that, the HFD group were divided into two subgroups. (1) HFD group (2) HFD plus AGOM at the dose of 20 mg/kg/day for 4 weeks. After 20 weeks, the rats were sacrificed, kidney tissue samples were collected to investigate the beneficial effect of AGOM on kidney function involving inflammatory modulation.
Results
The results showed that the expression of pro-inflammatory markers including TNF-α, IL-6 and NF-kB were trended to be increased in HFD group when compared with ND group. The improvement of renal function was related to the elevation of inflammation. There was a decreasing trend in these parameters after treated with AGOM.
Conclusion
AGOM might protect kidney injury via ameliorating inflammation induced by obesity. (COI: No)

2P-397(Y-29)

Melatonin activates sirtuin 3 to protect the kidney from long-term consequences of bisphenol A

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Mitochondrial oxidative stress and subsequently functional impairment is the prerequisite for renal injury after exposure to bisphenol A (BPA), a precursor for polycarbonate plastics and epoxy resins. Sirtuin 3 (SIRT3) is a key mitochondrial protein that modulates various proteins to control mitochondrial reactive oxygen species (mROS) generation and function. Melatonin has been reported to protect against BPA-induced nephrotoxicity, however, the mechanism behind this protection remains to be elucidated. This study evaluated whether SIRT3 contributed to the protection of BPA toxicity by melatonin. Rats were assigned to receive vehicle or BPA orally for 12 weeks. At the end of week 12, the vehicle-treated group continued to receive vehicle, while the BPA-treated group was subdivided to receive vehicle or melatonin, in addition to BPA, for further 4 weeks. By the end of week 16, melatonin treatment significantly minimized proteinuria, azotemia, glomerular filtration reduction, podocyte structural damages, renal oxidative stress and apoptosis, loss of mitochondrial membrane potential and excess mROS production caused by BPA. These effects of melatonin were associated with increased expressions of SIRT3, AMPK, and PGC-1a. The results suggest that melatonin activates AMPK-PGC-1a-SIRT3 axis to preserve mitochondrial integrity and accelerate functional recovery, thereby limit renal injury after long-term BPA exposure. (COI: No)

2P-398

Effects of chronic renal failure on cognitive function and neurogenesis in rats

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Currently, the number of dialysis patients has continued to increase. This rate of increase is directly proportional to aging. It is reported that the risk of dementia in dialysis patients is higher than that in healthy individuals. However, the detailed mechanism underlying this relationship remains poorly understood. The current study examined the cognitive function of rat renal failure models using histological and behavioral tests. Rat renal failure models were prepared by removing whole right kidney, left kidney ligated one part of the renal artery. Five months following surgery, brains were harvested and fixed and frozen sections were prepared and subjected to fluorescent immunostaining (Ki67, Iba1). There was a decrease in creatinine clearance and an increase in urinary albumin excretion in renal failure rats compared to that of control rats. Behavioral differences between control rats and renal failure rats were observed using the novel object cognitive test. The proportion of search time for the new object significantly decreased in renal failure rats compared to control rats. The number of Ki67 positive cells in the hippocampus was lower in renal failure rats than in control rats. The number of Iba1 positive cells in the hippocampus tended to increase in renal failure rats compared to control rats. These results indicate that increased inflammation caused by chronic renal failure may suppress neurogenesis in the hippocampus, resulting in reduced cognitive function. (COI: No)

2P-399

The application of predictive equation on estimation sodium intake in Hong Kong young adults

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Measuring 24-hour urine sodium is the golden standard for sodium intake investigation. Due to the heterogeneity of salt consumption among the population, the estimation of sodium intake by predictive equation may exist bias or environmental factors. The two estimating equations, Tanaka equation and Kawasaki equation were used to evaluating the correlation between 24-hour urine sodium and spot urine sodium in Hong Kong young adults aged between 18-26. 24-hour urine collections were obtained from 36 participants and urinary spots collected on the same day. The correlation between estimated and measured sodium and creatinine excretion was evaluated by Pearson´s correlation coefficients. Both equations were being used to estimate the creatinine with the actual creatinine and they showed a strong correlation. Tanaka estimated creatinine correlated the measured creatinine with r=0.79 and measured and Kawasaki creatinine correlation is r=0.81. However, both equation showed a weak correlation with the measured 24-hour urine sodium. Measured and Tanaka estimated result showed the correlation with r=0.34, while measured and Kawasaki estimated result showed the correlation with r=0.38. Kawasaki equation showed a closer distribution with actual results than Tanaka. Kawasaki equation has a better estimation than Tanaka equation in creatinine and sodium, however, several validations on different types of population and age group are needed. (COI: No)

2P-400

Withdrawn

2P-401

Inhibitory effect of a novel less-odorous TRPA1 antagonist

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TRPA1 is targeted acute nociception and inflammatory pain and is considered to be a promising target for the development of analgesic agents. 1,8-Cineole and borneol, which are rare natural monoterpene hTRPA1 inhibitors, have not been widely used as analgesic agents because it should be applied with relatively higher doses, which enhance the unpleasant odors.

In this study, we identified a monoterpene alcohol compound, arbanol, by screening for structural analogues of previous inhibitors. Using Ca^{2+}-imaging and patch-clamp methods, we found that arbanol had lower odor and higher inhibitory activity toward hTRPA1 compared to those previous inhibitors. The hTRPA1 currents induced by AITC were inhibited by arbanol in a dose-dependent manner with IC_{50} values of 0.25 mM. This IC_{50} value was similar to that of borneol and lower than that of 1,8-cineole. We also found that S873, T874 and N855 of hTRPA1 residues were slightly involved in the inhibitory effects by arbanol. Moreover, arbanol activated hTRPM8 in a dose-dependent manner with EC_{50} values of 0.25 mM.

An in vivo sensory irritation test showed arbanol conferred an analgesic effect on the sensory irritation produced by menthol. In addition, in vivo olfactory evaluation showed the odor intensity score of arbanol was comparable to that of the solvent without compounds.

We identified arbanol as a hardly odorous and effective analgesic agent. Arbanol might be a strong candidate for the application in analgesics. (COI: No)

2P-402

Regulation of the leak channel NALCN by H_2O_2

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The spontaneously active GABAergic neurons of the substantia nigra pars reticulata (SNr), a major output of the basal ganglia, provide tonic inhibition of downstream brain areas. Recent study showed the sodium leak channel, NALCN, is critical for this firing pattern. One potential regulator for the spontaneous firing is hydrogen peroxide (H_2O_2). Using whole-cell patch clamp techniques, we examined the effects of H_2O_2 on NALCN and explored the signaling mechanisms underlying those efffects. We found that NALCN activities in HEK293T cells were inhibited by H_2O_2 in a dose-dependent manner. Pretreatment with a p38MAPK inhibitor, SB203580 attenuated the H_2O_2 effects on NALCN. In addition, the expression of a constitutively active form of the upstream kinase for p38MAPK, MKK6(EE) inhibited NALCN activation, and H_2O_2 did not further affect NALCN activities. Taken together, our results suggest that H_2O_2 can regulate NALCN activities through p38MAPK. (COI: No)

2P-403

Regulation of reactive oxygen species and calcium by chloride intracellular channel 1 in A549 cells

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Chloride intracellular channel 1 (CLIC1) plays crucial roles in control of cell cycle, apoptosis, proliferation, invasiveness, and metastasis and thus emerges as a promising therapeutic target in cancer. Reactive oxygen species (ROS) and Ca^{2+} are important signaling molecules that regulate cell stress and cell death. However, the role CLIC1 in Ca^{2+} and ROS signaling in cancer cells is not completely understood. Here we show that CLIC1 regulates ROS through Ca^{2+} signaling in A549 human lung cancer cells. Depletion of CLIC1 with shRNAs in A549 cells increased DNA double-strand breaks both in control condition and under putative anticancer agent chelerythrine treatment that are accompanied by a concomitant increase of p-JNK. CLIC1 knockdown significantly increased basal ROS levels which were prevented by BAPTA-AM, the cell permeant Ca^{2} chelator. Consistently, CLIC1 knockdown increased a basal Ca^{2+} level and augmented a chelerythrine-induced Ca^{2+} response in A549 cells, compared to control cells. Suppression of extracellular Ca^{2+} recovered the basal Ca^{2+} level in CLIC1-knockdown A549 cells, suggesting that CLIC1 regulates $[Ca^{2+}]_i$ through a Ca^{2+} entry from plasma membrane. Taken together, our results suggest that CLIC1 inhibition induces ROS elevation and consequent JNK activation through Ca^{2+} signaling. (COI: No)

2P-404

Ferulic acid enhanced L-type Ca^{2+} channel function in rat insulinoma cell line

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Purpose: Ferulic acid is a natural polyphenol richly found in rice bran, whole grains and citrus fruits. Studies showed that this compound induced *in vitro* insulin secretion and decrease blood glucose in diabetic animal model. Using INS-1 cell, a rat insulinoma cell line, we studied the effects of ferulic acid on cell viability and L-type Ca^{2+} channel, a key molecule involved in the initiation and modulation of insulin release.

Methods: Cell viability was assessed with MTT assay and whole-cell L-type Ca^{2+} currents were measured using patch clamp technique.

Results: Treating INS-1 cells with 0.3 – 100 μM ferulic acid for 24 and 48 hours did not affect the cell viability. However, ferulic acid significantly induced a rapid concentration-dependent increase in L-type Ca^{2+} currents (EC_{50}, 1.10 ± 0.001 μM; maximum increase, 66.69 ± 8.37%; mean ± SEM), shifting the conductance-voltage curve in the hyperpolarized direction (control *vs* ferulic acid: $V_{½}$, -15.16 ± 2.11 *vs* -20.75 ± 2.97 mV; n = 7) with decreased slope factor (8.56 ± 0.74 *vs* 6.21 ± 0.35 mV; n = 7), while the voltage dependence of inactivation was not affected.

Conclusion: This is the first electrophysiological demonstration that acute ferulic acid exposure could increase L-type Ca^{2+} current by enhancing its voltage dependence of activation, which may explain the rapid ferulic acid-induced insulin secretion and decreased *in vivo* blood glucose levels. (COI: No)

2P-405

Withdrawn

2P-406

Mechanism of ginsenoside Re effect on SK_{Ca} current in human coronary artery endothelial cell

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Purpose: Ginsenoside Re (Re), a ginseng active ingredient has been shown to increase SK_{Ca} current in human coronary artery endothelial cells (HCAECs) and NO production in other endothelial cells. We aimed to study whether Re increased SK_{Ca} current via glucocorticoid receptor (GR)-PI3K-Akt/PKB signaling pathway, and whether SK_{Ca} is involved in Re-induced NO increase.

Methods: HCAEC current was measured using whole-cell patch clamping. NO production was assessed with colorimetric NO assay.

Results: 1 mM Re significantly increased whole-cell current by 74.91±18.94% (n=9; +80 mV). 100 nM Apamin significantly decreased Re-induced current to 61.85±8.64% (n=4), while apamin-insensitive current could not be increased by Re, indicating that SK_{Ca} was an Re target. Antagonists of GR, PI3K, Akt/PKB and eNOS significantly prevented Re-induced current. Re significantly decreased current when Akt/PKB inhibitor was present, suggesting a possible inhibitory pathway upstream from Akt/PKB. Finally, Re-induced NO production was significantly increased to 244.69±5.10% (n=3). This could be prevented by blockers of SK_{Ca}, GR, Akt/PKB, or eNOS.

Conclusion: Re increased HCAEC SK_{Ca} current and NO production via GR-PI3K-Akt/PKB and eNOS, and NO may directly increase SK_{Ca} current. Data also hinted at possible SK_{Ca} current inhibition by PI3K and a NO-independent SK_{Ca} current activation by Akt/PKB. These results could lead to further therapeutic application for cardiovascular disease. (COI: No)

2P-407

Gq-mediated activation of non-selective cation channels in insulin releasing b-cells

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Glucose-stimulated insulin secretion in b-cells is initiated by closure of the ATP-sensitive K^+ (K_{ATP}) channel, followed by plasma membrane depolarization. In this process, opening of background inward current through non-selective cation channels (NSCCs) might facilitate depolarization after K_{ATP} channel closure. Activation of heterotrimeric Gq-protein stimulates insulin release, however, its mechanisms of plasma membrane excitation remains to be determined. This study aimed to determine whether activation of Gq-protein stimulates insulin release via NSCCs in b-cells. Activation of Gq-protein by acetylcholine (ACh) and oxytocin (Oxt) significantly increased 8.3 mM glucose-induced insulin release without altering basal insulin release at 2.8 mM glucose. Oxt potentiated glucose (8.3 mM)-induced $[Ca^{2+}]_i$ increases in b-cells as monitored by fura-2 microfluorometry, without altering basal $[Ca^{2+}]_i$ levels at 2.8 mM glucose. The b-cell $[Ca^{2+}]_i$ response to Oxt was attenuated by Gq-protein inhibitor YM-254890 and by NSCC blocker flufenamic acid. Oxt activates NSCC currents and depolarizes the membrane potential in b-cells as measured by patch clamp analysis**.** These results demonstrate that Gq-mediated activation of NSCCs promotes b-cell membrane excitability, providing a potential molecular target to treat type 2 diabetes. (COI: No)

2P-408

Polyamine-mediated inward rectification of TRPC4 channel

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Transient Receptor Potential Canonical 4 (TRPC4) channel is a Ca^{2+}-permeable, non-selective cation channel. TRPC4 is expressed in various tissues such as brain, ventricular myocyte, uterus, gonads, and lower GI tracts. Although exact physiological role of the channel at those tissues are still remaining unknown, it has been reported that TRPC4 is essential for ileal smooth muscle contraction stimulated by parasympathetic nervous system[1,2]. With its inward-rectifying I-V relationship and high Ca^{2+} permeability, TRPC4 channels permit Ca^{2+} influx once the channel is opened by muscarinic receptor stimulation by acetylcholine. Molecular mechanistic study for the nature of inward rectification has been conducted in inward-rectifying potassium channels ($Kir_{2.1}$) with Mg^{2+} and polyamines being putative rectification mediators. Meanwhile, we reported that intracellular spermine blocks TRPC4 channel through electrostatic interaction with two glutamate residues. Here, with electrophysiological analysis and structural modelling, we suggest that there are two-different spermine binding sites (shallow site and deep site) in TRPC4 channels. These sites were different from each other in view of voltage-dependency and time-dependency. While time constant for spermine-mediated blocking showed saturation at highly depolarized voltage (over +40 mV) in $Kir_{2.1}$[3], blocking time constant for TRPC4 showed no saturation. (COI: No)

2P-409

Effect of STIM1 knockdown on calcium response in bovine ciliary myocytes

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In bovine ciliary muscle (BCM), M_3-muscarinic stimulation opens a non-selective cation channel (NSCC) with unitary conductance of 100 fS, which serves as a major Ca^{2+} entry pathway during tonic contraction. We previously showed that caffeine (1- 20 mM) caused this channel to open possibly through signals elicited upon Ca^{2+} depletion in the intracellular stores. We studied here the effects of knockdown of STIM1, a sensor of the store site Ca^{2+} content on the caffeine-induced changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). We also examined the expression of Orai1, a putative component of Ca^{2+} release-activated Ca^{2+} release (CRAC) channels, in BCM cells.

Methods BCM cells were obtained by collagenase treatment of dissected bovine ciliary muscles followed by centrifugation through discontinuous Percoll density-gradient of 1.050 and 1.060 g/mL. Transfection of siRNA was performed by electroporation. In flow cytometry, cellular proteins were visualized using ALEXA fluor-conjugated antibodies.

Results and discussion In the BCM cells, flow cytometry revealed co-expression of STM1, Orai1 and M_3-muscarinic receptor proteins. When the BCM cells were cultured for 3 days after transfection of STIM1-targeted siRNA, the expression of STIM1 protein was suppressed by 66 ± 4% (n=7) and caffeine-induced $[Ca^{2+}]_i$ elevation was reduced by 53± 3% (n=4). These results are consistent with the involvement of a CRAC mechanism in the 100 fS-NSCC opening caused by M_3-muscarinic stimulation. (COI: No)

2P-410

TRPM4 channel is involved in cellular damage caused by simulated ischemia-reperfusion injury

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The transient receptor potential (TRP) melastatin-4 (TRPM4) is a subtype of TRP channel. As a member of TRP family, TRPM4 channel also participates in mediating the flux of Na^+ and Ca^{2+} across the plasma membrane into the cytoplasm in accordance with homology, it can be activated by an intracellular accumulation of Ca^{2+}. However, TRPM4 shows no Ca^{2+} permeability, which induces intracellular Ca^{2+} accumulation and overload to cause cell membrane depolarization. It means that activation of TRPM4 would lead to cell damage or death. We previously reported that TRPM4 channel was participated in myocardial ischemia-reperfusion (I/R) injury using rats. In this study, we investigated possible involvement of TRPM4 channel in I/R injury in human by cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs). Application of 22 h-hypoxia (2%-oxygen) followed by 2 h-reoxygenation, which mimicked I/R injury, caused declined cellular viability and myocardial contractility of hiPSC-CMs detected by MTT assay and contractility analysis. Exposure to 750 μM-hydrogen peroxide, which is another simulated condition of I/R, resulted in similar damage of hiPSC-CMs. However, pretreatment with 9-Phenonthrol, a specific blocker of TRPM4 channel, effectively protected the hiPSC-CMs from the damage caused by the simulated I/R injuries. These results suggested that TRPM4 channel may be involved in the pathogenesis of cardiac I/R injury also in human. (COI: No)

2P-411

Withdrawn

2P-412

TRPM2 channel-Stat3 complex regulates the polarity of tumor-associated macrophage

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The tumor microenvironment is a complex tissue composed of various stromal cells including immune cells. Especially, tumor-associated macrophages (TAM) are one of the major components of tumor tissues, and they play a pivotal role in prompting the various tumor growths by producing growth factors and reactive oxygen species (ROS). Previously, we have reported that TRPM2 channel, a ROS-sensitive Ca2+ channel, is abundantly expressed in macrophages and is tuning gene expression via transcription factor NF-kB. Here, we report the significance of TRPM2 channel in the regulation of pro-inflammatory M1 and pro-angiogenic M2 phenotype of TAM. In TRPM2 knockout mice, TAMs around s.c.-injected B16F10 melanoma tumor showed strong expression of M2 phenotypic markers and proangiogenic factor VEGF according to the enhanced activity of Stat3. Interestingly, blood vessels in TRPM2 knockout mice tumor were so-called non-productive, which is characterized by an increase in vascular density and decrease in tissue perfusion and thus the tumor progression was suppressed. We also found that the activation of TRPM2 channel induced by H2O2 suppress the activity of Stat3 in vitro. Importantly, TRPM2 protein showed physical interaction with Stat3 protein, and their complex was degraded gradually in the presence of H2O2. Therefore, our data suggest that TRPM2-Stat3 complex is critical for handling the phenotypes of macrophages depending on the environmental oxygen/redox conditions. (COI: No)

2P-413

Regulation of neuronal excitability by Trim69 E3 ubiquitin ligase

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Small-conductance calcium-activated potassium channels (SKs), either contribute to the afterhyperpolarization that follows an action potential or facilitate repolarization following excitatory postsynaptic potentials. SKs are critical for a variety of functions in the CNS, from learning and memory to rhythmic activity and sleep. Trim69, an E3 ubiquitin ligase, is structurally and evolutionarily conserved in zebrafish, mouse, rat, and human. Although Trim69 is implicated in zebrafish neurogenesis, its role in *mammalian brain* is unclear. Here we demonstrate that Trim69 deficiency leads to neuronal hyperexcitability via modulation of SK activities. The expression study revealed that Trim69 is highly enriched in cortex, hippocampus and cerebellum. Patch clamp recordings in neurons from mice lacking Trim69 function showed that Trim69 deficiency affected neuronal excitability in dentate gyrus granule cells and cerebellar purkinje cells. Based on the electrophysiological analysis, Trim69 seems to regulate neuronal activity at least in part through SK channels. The mechanistic study suggests that Trim69 is required for the modulation of SK channel activity without altering their expression levels. (COI: No)

2P-414

Activation of TRPM6 current by 2-aminoethyldiphenyl borate is impaired by hydrogen peroxide

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To examine the effect of hydrogen peroxide (H_2O_2) on TRPM6 current, murine TRPM6 was stably expressed in HEK293 cells. Whole-cell recordings from TRPM6-expressing cells revealed that the current was rapidly inactivated after making whole-cell configurations (run-down) by yet unknown mechanisms. For this reason, TRPM6 current was firstly activated by using a known activator, 2-aminoethyldiphenyl borate (2-APB), and then the effect of H_2O_2 on the activated TRPM6 current was tested. 2-APB-activated TRPM6 current was rapidly decreased by an application of H_2O_2. It has been reported that TRPM7, a closest homologue of TRPM6, is inhibited by H_2O_2 irreversibly in a $[Mg^{2+}]_i$-dependent manner. In contrast, 2-APB-activated TRPM6 current was inhibited by H_2O_2 reversibly even in the absence of $[Mg^{2+}]_i$. These results indicated that H_2O_2 inhibited TRPM6 current by a different mechanism. Since H_2O_2 was applied with 2-APB, it was plausible that 2-APB is degenerated by H_2O_2. As diphenyl borate (DPB) can be a candidate for a degradated product of 2-APB, we chemically synthesized and purified DPB and then found that DPB failed to activate TRPM6 current in TRPM6-expressing cells. These results suggest that H_2O_2 enhances degradation of 2-APB to DPB and this chemical change causes loss of effectiveness of the activator on TRPM6. (COI: No)

2P-415

Structure-based virtual screening for G protein-gated inwardly rectifying K^+ (GIRK) channel blockers

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GIRK channels regulate the membrane excitability of excitable cells. Recently, *de novo* mutations in the GIRK family member Kir3.2 and somatic mutations in a closely related member Kir3.4 have been reported to evoke Keppen-Lubinsky syndrome and primary aldosteronism. These mutations cause the substitution of amino acids located around the ion selectivity filter, leading the mutants to the loss of ion selectivity. In model mice, the gene elimination of Kir3.2 slightly enhances the susceptibility to seizure-inducing chemicals, but its non-selective mutant expression provokes severe neurodegenerative effects. Therefore, the medications which suppress the mutant activity would have a therapeutic benefit. Previously identified Kir3.2 blocker, a bacteriostat proflavine, shares the mode of binding and chemical property with those of typical K^+ channel blockers. Since the selectivity of drug-channel interaction used to be low, the novel strategy was needed to generate selective Kir3.2 inhibitors.

In an electron density map from a crystal of Kir3.2 mutant, we found unique electron density independent of the polypeptide chain. The perfusion of a model compound fit to the density at the intracellular side of membrane patches inhibited Kir3.2 current, suggesting that the compound is a GIRK blocker and its surrounding region is a novel blocker binding site. Based on these results, we performed structure-based *in silico* drug screening and identified a single chemical as a Kir3.2 blocker. (COI: No)

2P-416

A novel variant of TRPV3 p.A628T in East Asians showing fast sensitization by chemical agonists

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TRPV3, is activated by various chemical agonists. Repetitive stimulation of TRPV3 characteristically induces sensitization; cumulative increase of current amplitudes with a loss of voltage-dependence (i.e. changes in the current-voltage relation from outward rectification to linear shape). A recent genomic analysis revealed non-rare TRPV3 mutation (p.A628T) in the East Asians including the Korean population (variant allele frequency: 0.249 in East Asians, 0.007 in Europeans). To elucidate the functional significance, whole-cell patch clamp was conducted in HEK293T cells overexpressing wildtype TRPV3 (WT) and the variant (p.A628T). Repetitive warming from 23 (RT) to 37 °C for 30 s with 30 s RT interval cumulatively activated outward-rectifying current in both WT and p.A628T, without I/V curve linearization. The repetitive temperature pulses to 43 °C showed tendency of fast sensitization with I/V curve linearization in p.A628T. Repetitive applications of 10 mM 2-aminoethyl diphenylborate (2-APB) or 100 mM carvacrol (30 s with 30 s washout interval) induced faster activation and earlier linearization of I/V curve in p.A628T. However, farnesyl pyrophosphate (FPP, 1 mM), an intrinsic lipid metabolite agonist of TRPV3, induced only partial activations of outward rectifying currents in both WT and p.A628T. In fura-2 microspectrofluorimetry, the 2-APB pulses induced faster increase of $[Ca^{2+}]_i$ in p.A628T than WT. (COI: No)

2P-417

Structure analysis of the binding between Cav1.2 channel and calmodulin

Masaki Kameyama; Etsuko Minobe; Jianjun Xu; Qinghua Gao (*Kagoshima University, Japan*)

AIM. It is known that calmodulin (CaM) is involved in the Ca^{2+}-dependent facilitation (CDF) and inactivation (CDI) of Cav1.2 channels. Although conformations of the carboxyl terminal (CT) of the channel-Ca^{2+}/CaM complex have been reported, they are still under debate. In this study, we have investigated the conformation of Cav1.2 channel in the activated (or CDF) state. *Methods.* Computer simulation was done with software's, I-TASSER (http://zhanglab.ccmb.med.umich.edu/), ClusPro (https://cluspro.bu.edu/), MOE (Walters et al., Biophys J 2004) and MDWeb (http://mmb.irbbarcelona.org/MDWeb/). GST pull-down assay was performed between mutated proximal C-terminal peptides of the channel (pCT) and CaM. *Results.* Computer simulation showed several relevant conformations of the binding of pCT and Ca^{2+}-free (apo) CaM. The preIQ region of pCT consisted of two a-helices, A and C region, with a bent between the two regions. Based on the predicted contact residues between pCT and CaM, we have carried out pull-down assay with mutated pCT. *Conclusion.* A conformation model for the binding of apoCaM and pCT in the activated state of channel has been proposed, in which apoCaM binds to IQ region in a parallel fashion. (COI: No)

2P-418

Voltage-clamp fluorometry analyses of voltage-dependent gating of ATP receptor channel P2X2

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P2X2 is a ligand-gated ion channel activated by extracellular ATP. This receptor shows a complex gating depending on both [ATP] and voltage, in spite of the absence of a canonical voltage sensor domain. It remains unknown how the structural rearrangements occur during voltage dependent gating. Thus, in the present study we aim to analyze the structural rearrangements of P2X2 receptor upon voltage- and ATP-dependent gating, by voltage-clamp fluorometry (VCF). We used a fluorescent unnatural amino acid (fUAA) named Anap as a fluorophore which can be directly incorporated into the channel protein in *Xenopus* oocytes. In addition to that, to improve the VCF recording optical signal by decreasing the intrinsic background fluorescent of oocytes, a small molecule kinase inhibitor named HG-9-91-01 (SIK inhibitor) was applied. We then observed Anap fluorescence intensity changes associated with voltage changes at Ala337 and Ile341 in the 2nd transmembrane domain (TM2). The changes from Ala337Anap and Ile341Anap showed a much faster kinetic than the current activation. Moreover, both changes showed a linear voltage-dependent behavior. This fast kinetics in sub-millisecond and linear voltage-dependence of the fluorescence intensity changes are thought to be due to an electrochromic phenomenon. Taken together, these results could reveal the first insight of the voltage-dependent gating properties of P2X2 receptor from the optical recording point of view. (COI: No)

2P-419

Functional Coupling of Metabolic Sensors, TRPM2 and Sirtuin

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TRPM2 is a thermosensitive non-selective cation channel expressed in various tissues including brain, spleen and β-cells where TRPM2 is continuously affected by core body temperature. We have previously reported the regulatory mechanism of TRPM2 at body temperature whereby H_2O_2, a kind of ROS, decreases the temperature threshold for TRPM2 activation leading its activation at body temperature. In addition, recently, TRPM2 roles in body temperature regulation has been reported. Therefore, TRPM2 activity at body temperature is thought to be regulated depending on systemic/cellular metabolic state.

Additional metabolic sensors, sirtuins, are a group of NAD^+-dependent deacetylase to regulate energy homeostasis, circadian rhythm and longevity, etc. We are interested in functional cooperation between TRPM2 and sirtuins because sirtuins generate o-acetyl ADPR (OAADPR), a TRPM2 activator, along with its protein deacetylation, especially in SIRT1 which is present in cytoplasm to interact with TRPM2 in plasma membrane.

Intracellular Ca^{2+}-imaging of TRPM2/SIRT1-expressing HEK293T cells have revealed that a SIRT1 activator leads TRPM2 activation. Notably, physical interaction between TRPM2 and SIRT1 has been observed in an immunoprecipitation study. These results suggest an effective functional coupling of TRPM2 and SIRT1.

We'd like to discuss possible regulatory mechanisms and physiological functions of metabolic sensors, TRPM2 and SIRT1. (COI: No)

2P-420

Examination of the contribution of SLCO2A1 to maxi-anion channel currents in murine cells

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Anion-selective channels with large unitary conductance of over 300 pS are expressed in variety types of animal cells and are called maxi-anion channels (MACs). Maxi-Cl exhibits strong discrimination for anions over cations and ATP release activity and is a representative channel among MACs. We reported that the molecular identify of a core factor of Maxi-Cl is *Slco2a1*, which encodes a prostaglandin transporter (PGT), and that several PGT antagonists including bromosulfophthalein (BSP) inhibited Maxi-Cl currents (EMBO J, 2017). However, contributions of endogenous expression of *Slco2a1* to Maxi-Cl currents were testified only in a murine cell line C127 derived from mammary adenocarcinoma. In this study, we examined the relationship between expression of *Slco2a1* and generation of MAC currents in mouse L929 cells derived from subcutaneous adipose tissues and mouse embryonic fibroblast (MEF) cells. By inside-out patch-clamp, MAC currents in both L929 and MEF cells were found to exhibit BSP sensitivity and anion selectivity, although the generation rate of patch excision-induced MAC events in L929 cells was higher than that in MEF cells. RT-PCR studies showed that the expression level of *Slco2a1* in L929 cells was much prominent than that in MEF cells. Effects of siRNA-mediated knockdown of *Slco2a1* on MAC activities in L929 and MEF were also found distinct from each other. Currently, we are examining ATP release activities in relation to *Slco2a1*expression in both cells. (COI: No)

2P-421(Y-30)

Protein arginine methyltransferase 1-dependent regulation of slow delayed rectifier K^+ current

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Arginine methylation is one of the most important post-translational modifications. It is mediated by protein arginine methyltransferases (PRMTs). In mammals, nine PRMTs have been characterized. PRMT1, originally identified as a histone H4 methyltransferases, methylates many non-histone proteins and it is implicated in diverse cellular processes. However, there is little information on the effects of PRMT1 on ion channel function and membrane excitability.We recently found that PRMT1 regulates the slow delayed rectifier K current (IKs) channel via PIP_2 affinity control. The *IKs* channel is mainly formed by *KCNQ1,* a pore-forming α-subunit and *KCNE1, an* auxiliary subunit. PRMT1 inhibition with furamidine, a PRMT1 specific inhibitor decreases KCNQ1/KCNE1 channel activity in HEK 293T cells. The decrease of KCNQ1/KCNE1 channel activities are recovered by exogenous PIP_2 addition. Finally, the mechanistic study suggests that KCNE1 might be a target for PRMT1 modulation. (COI: No)

2P-422

Effects of chemical chaperone on surface expression of PHHI mutant K_{ATP} channel (SUR1/A28VKir6.2)

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Gain or loss function of K_{ATP} channel results in neonatal diabetes or congenital hyperinsulinism respectively. A28V is a novel mutation in KCNJ11 encodes Kir6.2 leads to persistent hyperinsulinemic hypoglycemia of infancy (PHHI). Diazoxide is the first line of treatment for patients with PHHI. Unfortunately, 90% of the neonatal forms of PHHI are resistant to diazoxide, and severe PHHI usually requires near-total pancreatectomy. Carbamazepine, a K_{ATP} blocker, corrects the surface expression defects caused by a subset of SUR1 mutations in congenital hyperinsulinism. Importantly, the combination of diazoxide and carbamazepine led to enhanced mutant channel function. Our preliminary data found that metformin, the antidiabetic drugs for type II diabetics, increases surface expression of K_{ATP} channels in rat insulinoma cells. We investigate whether if carbamazepine, glibenclamide or metformin rescues the surface expression of A28VKir6.2/ SUR1. HEK293 cells were transfected with A28VKir6.2/ SUR1 followed by surface biotinylation and immunoblotting. Our preliminary results confirmed a reduction of surface SUR1 protein in A28VKir6.2 transfected cells as compared to control cells. Dose-or time-dependent of chemical chaperone will be conducted to see if there is an increase in surface SUR1 expression. (COI: Properly Declared)

2P-423

Effects of antihistamine drugs on G-protein-gated inwardly rectifying K^+ channels

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G-protein-gated inwardly rectifying K^+ channel (GIRK) regulates several physiological functions, such as the excitability of cardiomyocytes and neurons. GIRK channel is known to be activated by the $G_{\beta\gamma}$ subunit released from the stimulated G_i-coupled GPCRs. Recently, we found that some of antihistamine drugs inhibit the activity of GIRK channels. To clarify the inhibitory mechanisms of GIRK channel by these drugs, we performed electrophysiological experiments and observed the results as follows. (1) By examining the effects of several antihistamine drugs, including terfenadine (TER), astemizole (AST), desloratine (DES), ebastine (EBA) and loratadine (LOR), we observed that only GIRK1 is sensitive to TER, AST and DES, and both GIRK1 and GIRK4 are sensitive to EBA and LOR. (2) By mutagenesis analyses, we found that Phe137, an unique amino acid residue located in the pore helix of GIRK1, is critical for TER-mediated inhibition. (3) We introduced the mutations into the corresponding position Ser148 on GIRK2, and observed that homomeric GIRK2 Ser148Phe, as well as GIRK2 Ser148Tyr and GIRK2 Ser148Trp, show high Na^+ permeability. (4) GIRK2 Ser148Phe&Trp106Ala double mutant shows more permeability to Na^+ than the case of GIRK2 Ser148Phe. Taken together, our present data show the effects of the novel inhibitors on GIRK channels and the roles of the pore helix residue in the regulations of the channel activity as well as the ion selectivity. (COI: No)

2P-424

Measurements of water flux across a lipid bilayer membrane with evaluation of unstirred water layer

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Water flux across the membrane is an important physiological process ubiquitous for all the cell types. Water flux has been measured, but quantitative evaluation is difficult by the emergence of the unstirred water layer (UWL) accompanying water flux. Here we established a method to evaluate water flux across a lipid bilayer under the osmotic gradient. In this method, the inner surface of a glass capillary was made hydrophobic, and a lipid bilayer was formed by introducing a lipid-containing oil gap between electrolyte columns. Accordingly, free-standing and movable bilayer is formed across the capillary. Under the osmotic gradient between two electrolyte columns, bulk flow of water across the membrane is visualized as movements of the membrane when one of the electrolyte columns is closed. First, water flux across a bare diphytanoylphosphatidylcholine membrane was measured, in which the bilayer area was evaluated by capacitance measurements under voltage clamp. For measurement of UWL, gramicidin was incorporated into the bilayer, and a current-voltage curve was obtained. The streaming potential was obtained as a by-product. The ion concentration at the vicinity of the membrane in both sides was evaluated from the asymmetry of the current-voltage curve. The osmotic permeability coefficient of water was evaluated after correction of the UWL. (COI: No)

2P-425

in bulla channel synthesis and functional expression system under applied membrane potentials

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Cell-free protein synthesis provides an experimental platform to examine processes of functional protein formation. For membrane proteins, membrane fractions such as liposomes and nanodiscs are involved in the *in vitro* synthesis such that synthesized proteins are incorporated into the membrane. In the case of ion channels, these channel reconstituted membranes are delivered to a pre-formed lipid bilayer for functional studies under voltage-clamped single-channel current recordings. To circumvent this two-step procedure of synthesis and recordings, here we developed a concurrent channel expression and functional detection system. Channel proteins are synthesized in a water-in-oil droplet lined with a monolayer and bilayer (*in bulla* synthesis), and synthesized channel proteins are spontaneously incorporated into lipid bilayer under application of the membrane potential. This is mimicking bacterial synthesis system via unassisted pathway (without using translocon for membrane insertion) in the presence of a resting membrane potential. Newborn functional channels are readily detected as a single-channel current. Channel synthesis was initiated by raising the temperature to 37 °C, and a first functional single channel appeared 20 min after initiating the synthesis. We found surprisingly that this first appearance time shortened substantially as the membrane potential was increased in both polarities. (COI: No)

2P-426

Regulation of TRPV1 and TRPA1 function by free fatty acid receptor

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G-protein-coupled receptor 40 and 120 (GPR40 and GPR120) are suggested to function as a transmembrane receptor for polyunsaturated fatty acids. Recent study showed GW9508, a dual agonist for GPR40 and GPR120, reduced mechanical allodynia in pain animal models. However, the role of polyunsaturated fatty acids and GPR40/120 in pain regulation is still unclear. The transient receptor potential vanilloid 1 and ankyrin 1 (TRPV1 and TRPA1) channel are mainly found in primary nociceptive afferents whose activity has been linked to pathophysiological conditions including pain, itch and inflammation. Using patch clamp and molecular techniques, we determined the effects of GPR40 and GPR120 on TRPV1 and TRPA1 activities. In mouse dorsal root ganglion (DRG) neurons, both GPR40 and GPR120 are co-localized with TRPV1 and TRPA1. Capsaicin and N-methyl maleimide (NMM) increased intracellular Ca2+ level in these cells via TRPV1 and TRPA1, respectively. Capsaicin-induced Ca2+ responses were potently attenuated by pretreatment of these neurons with a GPR40/120 dual agonist (GW9508), but not with GPR40- and GPR120-specific agonists. In contrast, NMM-induced Ca2+ responses were inhibited by both GW9508 and compund A, a GPR120-specific agonsit. These data imply that the anti-TRPV1 effects of GW9508 require activation of both GPR40 and GPR120 and that TRPA1 activities were regulated mainly by GPR120, suggesting that GPR40/120 agonists might serve as a mew class of analgesics for treating pain. (COI: No)

2P-427

Cav1.2 channel inactivation induced by two molecules of calmodulin

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AIM Activity of Cav1.2 channels requires calmodulin (CaM). Conformations of the carboxyl terminal (CT) of the channel-CaM complex have been reported, but they are still in debate. In this study, we have investigated the conformation of Cav1.2 channel in the inactivated state. Previously we have reported that channel activity increases and decreases in a CaM concentration dependent manner at fixed [Ca^{2+}]. Inactivation induced with CaM dose-up (CaM dependent inactivation) would give a hint to understand Ca^{2+} dependent inactivation (CDI). *Methods* We prepared two channel mutants of Cav1.2 a1C: channels deleted in the distal CT tail and linked to CaM with glycine chains (CaM-linked channel), and CaM-linked channel deleted in the amino terminal (NT) tail (delN-CaM-linked channel). These mutant channels were expressed in HEK 293 cells, and activity of the channels was measured in inside-out mode of patch-clamp. GST pull-down assay was performed between NT and/or CaM-linked CT peptide and CaM in different Ca^{2+} and CaM concentrations. *Results* The CaM-linked channel showed both Ca^{2+} and CaM dependent inactivation, while the delN-CaM-linked channel showed only CaM-dependent inactivation but not CDI. *Conclusion* The inactivation induced by one CaM may require NT of the channel, while two CaM induced inactivation may take place without NT, but within CT of the channel. Thus, there might be two types of conformation of CDI of Cav1.2. (COI: No)

2P-428

Dipole Potential Evaluated by Hydrophobic Ions using the Contact Bubble Bilayer Method

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Generally, the transmembrane potential is imposed for all the cells as a result of the electrochemical potential gradient and permeability of relevant ion species. In addition, another physical potential inherent in the membrane is imposed. This is called the dipole potential generated from electrical properties of phospholipids with their charged head groups and backbone dipoles. Physiologically, channel gating is affected by the dipole potential. Here we evaluated the dipole potential using the contact bubble bilayer (CBB) method. In a defined lipid composition of diphytanoylphosphatidyl-choline, hydrophobic ions, tetraphenylphosphonium (TPP+) and tetraphenylborate (TPB-), were used as carriers of transient currents upon voltage steps. TPP+ or TPB- was added in the aqueous solution, which is spontaneously partitioned into the membrane interface. Upon a step voltage change, these molecules transfer across the hydrophobic core of the membrane towards the other side of the interface, yielding transient currents. The geometry of the CBB allows rapid voltage clamp via a small series resistance, and the fast transient current was readily recorded. From the voltage and concentration dependence of the transient current, the dipole potential profile inside the membrane was evaluated through an application of a simple kinetic model. (COI: No)

2P-429(Y-31)

TTYH family encodes the pore-forming subunits of the volume-regulated anion channel in the brain

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In the brain, a reduction in extracellular osmolality causes water-influx and swelling, which subsequently triggers Cl^- and osmolytes-efflux via volume-regulated anion channel (VRAC). Although LRRC8 family has been recently proposed as the pore-forming VRAC which is activated by low cytoplasmic ionic strength but not by swelling, the molecular identity of the pore-forming swelling-dependent VRAC ($VRAC_{swell}$) remains unclear. Here we identify and characterize Tweety-homologs (TTYH1, TTYH2, TTYH3) as the major $VRAC_{swell}$ in astrocytes. Gene-silencing of all *Ttyh1/2/3* eliminated hypo-osmotic-solution-induced Cl^- conductance ($I_{Cl,swell}$) in cultured and hippocampal astrocytes. When heterologously expressed in HEK293T or CHO-K1 cells, each TTYH isoform showed a significant $I_{Cl,swell}$ with similar aquaporin-4 dependency, pharmacological properties and glutamate permeability as $I_{Cl,swell}$ observed in native astrocytes. Mutagenesis-based structure-activity analysis revealed that positively charged arginine residue at 165 in TTYH1 and 164 in TTYH2 is critical for the formation of the channel-pore. Our results demonstrate that TTYH family confers the *bona fide* $VRAC_{swell}$ in the brain. (COI: No)

2P-430(Y-32)

The Arginine in the side portal determines the physiological $[pH]_o$ sensing of TALK1

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TALK1, a two-pore domain potassium channel, involves in glucose stimulating insulin secretion. Extracellular pH ($[pH]_o$) regulates the TALK1 channel activity; however the detailed mechanism of $[pH]_o$ sensing is mostly unknown. In our study, we aimed to probe the structural basis of $[pH]_o$ sensing of TALK1 channel by sitedirected mutagenesis and electrophysiological assays. *De novo* protein structure prediction indicated that TALK1 channel contains side portals and positively charged residue arginine at position 233 within the side portals might be a $[pH]_o$ sensor. Neutralizing this arginine residue eliminated the activation at alkaline $[pH]_o$, which further corroborated the critical role of R233 in sensing $[pH]_o$. Interestingly, converting arginine to lysine restored the value of $pK_{1/2}$ to close pKa of lysine (10.5). Our TALK1 structure indicated the arginine residue at position 233 forming an elaborative hydrogen bond network with S97 and F100. Mutating either of these 2 residues disrupted the $[pH]_o$ sensing, suggesting that the microenvironment formed by S97, F100 and R233 act as the $[pH]_o$ sensing pocket on the TALK1 channel. Tandem TALK1-dimer carrying opposite charges at the 233 position exhibited the U-shape of $[pH]_o$ activation, indicating two side portal gates operates independently. In conclusion, our study shows that the R233 within side portals of TALK1 is the critical residue for sensing $[pH]_o$ and shifting the pH sensing curve towards physiological ranges. (COI: No)

2P-431

Down-regulation of $K_{Ca}3.1$ K^+ channels by the treatment with VDR agonists in mouse pre-osteoblasts

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[Purpose]

Vitamin D (VD) plays important roles in calcium homeostasis, mineral metabolism, and bone development indirectly via control calcium absorption in the intestine and reabsorption in the kidney. However, several in vitro studies showed that VD can directly suppress the cell proliferation of mouse osteoblasts. The intermediate-conductance Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ regulates intracellular Ca^{2+} signaling pathways and is associated with cell proliferation in various types of cells including pre-osteoblasts. This study was performed to clarify the effects of treatments with VDR agonists on the expression and activity of $K_{Ca}3.1$ in mouse pre-osteoblast MC3T3-E1 cells.

[Methods]

Intracellular Ca^{2+} concentration was measured by Ca^{2+} indicator, fura-2/AM. Cell viability was measured by WST-1 assay.

[Results]

Treatments with VDR agonists markedly decreased the expression levels of $K_{Ca}3.1$ transcripts and proteins in MC3T3-E1 cells, resulting in the significant inhibition of Ca^{2+} rises induced by DCEBIO, a $K_{Ca}3.1$ activator. Treatments with VDR agonists also significantly decreased the expression levels of several transcriptional regulators of $K_{Ca}3.1$ such as histone deacetylase 2 (HDAC2) and Fra-1.

[Conclusion]

Our results suggest that $K_{Ca}3.1$ is a new downstream target of VDR signaling and the down-regulation of $K_{Ca}3.1$ through the transcriptional repression of $K_{Ca}3.1$ contribute, at least partly, to the antiproliferative effects of VDR agonists in mouse pre-osteoblasts. (COI: No)

2P-432

Cell imaging with magnetic particle with on a diamond sensor

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Paramagnetic centers in semiconductors may serve as microscopic high-sensitivity magnetometers. In particular the negatively charged nitrogen-vacancy (NV^-) center in diamond has many advantages in application to biological imaging. By utilizing the fluorescence of the NV^- center, its spin state can be polarized and detected by optical means such as a single-molecule fluorescence microscopy and can be manipulated by microwave pulses. This technique, ODMR (optically detected magnetic resonance), can be used to measure the effect of magnetic field in biological specimens. Our recent interest is to observe dynamic reactions of living cells. Magnetic particles with a diameter less than 1 μm are placed over live cells on a diamond substrate containing high density NV^- centers in the surface layer. The depth of the NV layer is required to be less than the magnetic particles' diameter for a maximum effect on the ODMR spectrum. The spectrum is measured by projecting the fluorescence from the NV^- centers to an image sensor thorough an optical microscope. Analysis of the ODMR spectrum at every pixel allows to determine the positions/angles of the magnetic particles, to extract the live cell reaction. A theory predicts a temporal resolution of <1 s combined with a spatial resolution of <1 μm for the recently obtained density and quality of the NV^- centers. (COI: No)

2P-433

A novel mechanism responsible for the intracellular zinc-sensing

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As an important trace element, zinc is required for the normal cellular structure and function and impairment of zinc homeostasis is associated with a variety of health problems. In addition to zinc transporters and zinc binding molecules, zinc sensing also plays an important role in the regulation of zinc homeostasis. Metal-response element-binding transcription factor-1 (MTF-1) is the only well-established intracellular zinc sensor during zinc repletion in mammals. Curiously, however, little is known about the zinc sensing mechanism during zinc deficiency in mammals. Here we report that under the condition of normoxia or hypoxia/reoxygenation, zinc deficiency increased STAT3 phosphorylation at Tyr^{705} and the activated STAT3 promoted transcription of the genes coding for the Zip family zinc transporters (zinc importers) after its translocation to the nucleus in cardiac H9c2 cells or in mouse hearts, indicating that STAT3 is crucial for the zinc-sensing in the setting of intracellular zinc deficiency. Further studies demonstrated that protein inhibitor of activated STAT3 (PIAS3) and ER stress contribute to STAT3's zinc-sensing. These data suggest that STAT3 senses decreases in intracellular free zinc via ER stress and PIAS3, and thereby promoting the expression of genes that are required for zinc import leading to the protection against intracellular zinc deficiency. (COI: No)

2P-434

TRPA1 receptors mediate the hypoxia-induced surfacing response of goldfish

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The present study investigates whether transient receptor potential cation channel subfamily A member 1 (TRPA1) receptors are involved in hypoxic responses in goldfish.

To record the surfacing responses to hypoxia, the fish were individually housed recorded for individual fish in dissolved oxygen in an experimental tank with a DO of 0.5, 1.5, 2.4, 3.4 and 4.3 mg/L. The surfacing response was defined as the first instance of the fish's nose reaching the surface, and was quantified as numbers of air gasps for 1 min.

Compared with control conditions (DO = 4.3 mg/L), exposure to heavy hypoxia (DO = 0.5, 1.5 mg/L) shortened the surfacing response latency and increased the number of air gasps on the surface, whereas mild hypoxia (DO = 2.4, 3.4 mg/L) did not produce any such responses.

Pre-exposure to the TRPA1 receptor antagonist, HC-030031 inhibited hypoxic responses in a dose-dependent manner, when DO=0.5. A lower concentration (0.007 μM) of the TRPA1 receptors agonist allyl isothiocyanate (AITC) produced a hypoxic response and a decreased respiratory rate, both of which were inhibited by the TRPA1 receptor antagonist in a dose-dependent manner. Higher concentrations of AITC (0.07-0.1 μM) increased the respiratory rate, whereas concentration greater than 0.3 μM decreased the respiratory rate and produced no hypoxic response.

These results demonstrate that the TRPA1 receptor is involved O2-sensing mechanisms of hypoxia in goldfish. (COI: No)

2P-435

MicroRNAs in mouse salivary glands as a putative Bio-Marker of stress-dependent diseases

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MicroRNAs are small non-coding RNAs that play key roles in the regulation of gene expression. The microRNAs are secreted in saliva. We investigated a new diagnostic method using the microRNA of salivary gland and saliva as a stress biomarker. MicroRNA expression patterns in salivary glands of ICR mice were investigated and 39 miRNAs were identified. Expression patterns of these microRNAs in mice treated with various hormones were analyzed by quantitative real-time PCR. It is known that anti-stress hormone (glucocorticoid) is secreted when a hypothalamus recognizes stress. Dexamethasone-administration to adrenalectomized mice combined with castration increased microRNAs of miR-21a and miR-141 of the salivary glands. Castration caused remarkable decrease of these microRNAs. These results suggest that glucocorticoid affected to the microRNA expression *via* androgen receptors under stress state. On the other hand, these microRNAs did not change for the long acceleration of the sympathetic nerve by the sequential epinephrine administration by the use of micro-osmotic pump (Alzet, Model 1002). In conclusion, the change in expression patterns of miR-21a and miR-141 was expected to make diagnosis of stress-dependent diseases *via* hypothalamus. In addition, the state of stress-dependent diseases *via* hypothalamus was distinguished from the state of the sympathetic nerve acceleration by using these microRNAs as biomarker. This works was supported by JSPS KAKENHI Grant Number 18K19757. (COI: No)

2P-436(Y-33)

Circadian gene Clock post-transcriptionally regulates mitochondrial morphology and functions

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Purpose: Many daily activities are under the control of circadian clock, including nutrition metabolism and energy generation. Mitochondria undergo changes in quantity and morphology to adapt to the demand for energy. We aimed to explore the effect of circadian gene Clock on mitochondria. **Methods:** Mitochondrial were labeled with ad-Cox8a-GFP and specific markers to observe their morphology. To estimate mitochondrial functions, we used DCF and JC-1 dyes to evaluate mitochondrial ROS content and membrane potential. Moreover, mitochondria respiration and ATP generation were measured by seahorse assay. **Results:** We found that Clock$^{\Delta 19}$ leads to fragmented mitochondria accompanied with the loss of membrane potential, excessive ROS accumulation and decreased mitochondrial respiration and ATP generation. Clock$^{\Delta 19}$ mice exhibit disordered lipid metabolism and evident non-alcoholic fatty liver disease (NAFLD), which are rescued by treatment with the mitochondrial fission inhibitor Mdivi-1. We demonstrated that circadian gene Clock affects the number, architecture and function of mitochondria via posttranscriptional regulation of Drp1 by binding on its mRNA 3'UTR region. Moreover, its PAS domain plays a critical role in CLOCK's mRNA binding function. **Conclusion:** These results suggest a strong relationship between Clock's post transcriptional regulation role, mitochondrial dynamics and metabolic diseases and provide a new perspective on disordered circadian clock and related diseases. (COI: Properly Declared)

2P-437

Improvement of genetically encoded probe to measure Ca^{2+} dynamics in subcellular compartments

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The endoplasmic reticulum (ER) is the organelle that plays critical roles in regulating intracellular Ca^{2+} dynamics. The Ca^{2+} concentration in the ER lumen ($[Ca^{2+}]_{ER}$) not only affects the rate of Ca^{2+} release to the cytoplasm, but also controls Ca^{2+} influx from outside in many types of cells. For the measurement of $[Ca^{2+}]_{ER}$, genetically encoded fluorescent probes with the ER-targeting signal sequence can be used. Fluorescence signal of the probe, however, will not report $[Ca^{2+}]_{ER}$ correctly, if a fraction of the expressed probe proteins remain not to be transported from the cytosol into the ER. In the present study, we improved the subcellular localization of a probe for $[Ca^{2+}]_{ER}$, D1ER, by adding the destabilizing domain (DD) of FK506-binding protein, which leads the protein remained in the cytosol to be degraded by proteasomes. When mouse eggs expressing original D1ER were treated by ionomycin to release Ca^{2+} from the ER, fluorescence signal changed so as to apparently suggest the transient increase in $[Ca^{2+}]_{ER}$, probably due to the fluorescence from the residual probes in the cytosol. In contrast, fluorescence signals in the eggs expressing DD-D1ER reported rapid decrease in $[Ca^{2+}]_{ER}$ upon ionomycin treatment. Together with the results of measurement of $[Ca^{2+}]_{ER}$ during Ca^{2+} oscillations in mouse eggs, it was shown that the addition of DD to the genetically encoded probe significantly improves the specificity of Ca^{2+} measurement in subcellular compartments. (COI: No)

2P-438

Method to Record Single-Molecule Fluctuations and Conformational Changes in Proteins

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We have been studying single-molecular dynamics of KcsA potassium channel by diffracted X-ray tracking. In this method, a gold nanocrystal is utilized as a probe to report the motion of a protein. One side of the protein is fixed on a plate surface and the other side harbors a gold nanocrystal. The sample is irradiated with synchrotron white X-rays and the diffraction spots from the nanocrystal are tracked on the plane of a 2D X-ray detector. The motion of the spots is translated as the motion of protein; the rotational and radial motions of the spots represent twisting and bending motions of the protein, respectively. During the last decade, we have introduced (1) X-ray focusing mirror and high-speed camera to achieve sub-millisecond time resolutions; (2) ultraviolet laser for flash-photolysis of caged compounds to change the solution conditions; and (3) infrared laser and thermal camera to record dynamic response to the thermal stimuli. Recently, we succeeded in measuring the focused X-ray intensity profiles and attenuated profiles by using metal plates, fabricating low X-ray scattering chamber composed of silicon nitride membrane, and employing software for a large volume of images with low signal-to-noise ratio. These developments can enable us to minimize the X-ray dose for observations, thus, preventing radiation damage. Herein, we detail the method and its applicability for other proteins to study their dynamic properties at high spatial and temporal resolutions. (COI: No)

2P-439

Development of a photo-activatable CaMKII and its application to the study of synaptic plasticity

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Calcium/calmodulin-dependent protein kinase II (CaMKII) is believed to play central roles in synaptic plasticity which underlies some forms of learning and memory. To study the function of CaMKII for synaptic plasticity, we developed a photo-activatable CaMKII (paCaMKII) by utilizing light oxygen voltage (LOV) domain from phototropin1. Blue light or 2-photon excitation can induce the conformational change of paCaMKII from the closed form to the open form, leading to the activation of paCaMKII. To indentify the function of CaMKII in spines, we expressed paCaMKII in CA1 pyramidal neurons of hippocampal slices and activated it in single dendritic spines by 2-photon excitation. We found that the direct photoactivation of paCaMKIIα succesfuly induced the Cdc42 activation, the recruitment of AMPA receptors, and spine enlargement in the stimulated spine. Moreover, the spine enlargement induced by CaMKII activation lasts over 4 hours in a protein synthesis-dependent manner, suggesting that CaMKII activation is sufficient to trigger the synaptic plasticity. (COI: No)

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Truncated dystrophin ameliorates the dystrophic phenotype by sarcolipin-mediated SERCA inhibition

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Duchenne muscular dystrophy (DMD) and the less severe Becker muscular dystrophy (BMD) are due to mutations in the *DMD* gene. Previous reports show that in-frame deletion of exons 45-55 produces an internally shorted, but functional, dystrophin protein resulting in a very mild BMD phenotype. In order to elucidate the molecular mechanism leading to this phenotype, we generated exon 45-55 deleted dystrophin transgenic/*mdx* (Tg/*mdx*) mice. Muscular function of Tg/*mdx* mice was restored close to that of wild type (WT) mice but the localization of the neuronal type of nitric oxide synthase was changed from the sarcolemma to the cytosol. This led to hyper-nitrosylation of the ryanodine receptor 1 causing increased Ca^{2+} release from the sarcoplasmic reticulum. On the other hand, Ca^{2+} reuptake by the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) was restored to the level of WT mice, suggesting that the Ca^{2+} dysregulation had been compensated by SERCA activation. In line with this, expression of sarcolipin (SLN), a SERCA-inhibitory peptide, was upregulated in *mdx* mice, but strongly reduced in Tg/*mdx* mice. Furthermore, knockdown of SLN ameliorated the cytosolic Ca^{2+} homeostasis and the dystrophic phenotype in *mdx* mice. These findings suggest that SLN may be a novel target for DMD therapy. (COI: No)

2P-441

Flonicamid affects insect proprioception and feeding through 5-HT_7 receptors

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Pymetrozine and flonicamid control plant-sucking pests by disturbing their coordination and ability to feed. Defining the molecular targets of these two insecticides will contribute to the safety evaluation and risk assessment of insecticides with similar mode of action. It will also promote the development of novel insecticides. In this study, four types of serotonin receptors from the green rice leafhopper, *Nephotettix cincticeps*, were functionally expressed in HEK-293 cells. We found that pymetrozine had no effect on any of them. However, flonicamid selectively activated Nc5-HT_7 at micromolar concentrations and does-dependently activated Nl5-HT_7 from the brown planthopper, *Nilaparvata lugens*. Flonicamid treatment impaired negative geotaxis and feeding behaviors of wild type Drosophila. The deletion of *5-HT_7* gene not only rescued the gravity-sensing partially, but also completely rescued the antifeedant effect in poisoned flies. In addition, 5-HT_7 receptors are expressed in several types of proprioceptive neurons in the chordotonal organ, campaniform sensilla and hair plates. Therefore, our results indicated that the 5-HT_7 receptor is one of the molecular targets of flonicamid and mediates some toxic effects. (COI: No)

2P-442

Analysis of electrically-modulated molecules that enhance bone marrow stromal cell proliferation

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Bioelectricity such as membrane potential may play an important role for controlling proliferation and differentiation of bone marrow stromal cells (BMSCs). We previously reported that depolarization induced by TRPC6 channel activation effectively facilitated the cell cycle progression of BMSCs (*Br. J. Pharmacol.*, 171(23): 5280-5294, 2014). In a subsequent study employing platinum electrodes, we found that electrically-induced depolarization of resting membrane potential enhanced BMSC proliferation without affecting the expression level of TRPC6 (*J.Physiol.Sci.* 66(Suppl.1), 2016, *J.Physiol.Sci.* 67(Suppl.1), 2017). In this study, to explore the mechanism underlying this electrically enhanced BMSC proliferation, we performed a comprehensive analysis of electrically-modulated molecules in BMSC by using the DNA microarray and real-time PCR analyses. At an early stage of electrical stimulation (before BMSC proliferation was accelerated), molecules including a few miRNAs were up- or down-regulated. We then chased the time-dependent changes in these miRNAs expressions during a long course of electrical stimulation by real-time PCR. The altered expression pattern of miRNAs observed at the early stage of electrical stimulation disappeared when the stimulation was sustained . These results suggest that the initial increase/decrease of miRNA expression induced by electrical stimulation may be responsible for accelerated BMSC proliferation that may involve multiple complex processes. (COI: No)

2P-443

Involvement of VNUT-exocytosis in TRPV4 ion channel-dependent ATP release from colonic epithelium

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Adenosine triphosphate (ATP) modulates mechanosensitive vagal afferent nerves in the gastrointestinal tract. ATP is stored in secretory vesicles via the ATP transporter VNUT. Recently, the bisphosphate clodronate was reported to inhibit VNUT and was suggested to be a safe potent therapeutic option for chronic pain. Transient receptor potential vanilloid 4 (TRPV4) is activated by mechanical stimuli and some epoxyeicosatrienoic acids and becomes sensitized under inflammatory conditions. We have previously reported that TRPV4 and VNUT are expressed in mouse esophageal keratinocytes and that TRPV4 activation induces ATP release in gastric epithelial cells. Here we show the expression of TRPV4 and VNUT in normal human colonic cell derived cell lines, CCD 841 and in tissues from Wild-type mice. TRPV4 agonists (GSK101 or 8,9-EET) induced an increase in cytosolic Ca2+ and/or current responses in mouse primary colonic epithelial cells and CCD 841 cells, but not in cells isolated from TRPV4-KO mice. Clodronate did not inhibit GSK101-induced cytosolic Ca2+ responses. TRPV4 agonists (GSK101 or 5.6-EET) induced ATP release in and CCD 841 cells, which could be blocked by the VNUT inhibitor, clodronate. Thus, VNUT inhibition with clodronate could represent a novel therapeutic option for visceral pain. (COI: No)

2P-444

Essential role of Ca^{2+} and pH for in vitro cornification in isolated mouse stratum granulosum cells

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The skin is the largest organ of our body. The outermost layer of skin called the stratum corneum (SC) is a crucial air-liquid interface barrier of epidermis. SC is generated from SG1 cells which are the uppermost living layer of stratum granulosum (SG), via unique cell death called cornification. However, cell biological analysis of cornification has been difficult due to the lack of appropriate *in vitro* culture system.To perform single cell analysis, we generated EGFP-knockin mice into the SASPase gene locus which contains SG1-specific promoter. SASP(EGFP) mice showed SG1-specific expression of GFP in the epidermis. Confocal microscopic analysis revealed that these isolated EGFP-positive SG1 cells have polygonal domed saucer-like morphology, suggesting the maintenance of their unique *in vivo* cell shape. We next examined various culture conditions with different $[Ca^{2+}]$ and pH. High $[Ca^{2+}]$ under weakly acidic pH induced gradual degradation of keratohyalin granules (KHGs) during cell death, whereas low $[Ca^{2+}]$ under neutral pH did not. Under high $[Ca^{2+}]$, the timing and duration of KHGs-degradation were dependent on the pH. These results suggest that the initiation of cornification process is regulated by the extracellular environment of SG1 cells such as $[Ca^{2+}]$ and pH. Furthermore, this novel culture system will help us understand the detailed molecular mechanisms of cornification. (COI: No)

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CTLA4-Ig suppressed intracellular calcium oscillation and inhibited murine osteoclast formation

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[Background] Abatacept (CTLA4-Ig) is a biologics drug for Rheumatoid Arthritis. CTLA4-Ig contains of the extra-articular domain of CTLA4 and Fc domain of IgG1. It binds to CD80/86 complex in surface of antigen presenting cells, and blocks T cell activation. Axmann reported CTLA4-Ig directly inhibited the Osteoclast differentiation, and IDO/Tryptophan pathway were proposed as a part of the mechanism. Nevertheless, the whole suppressive mechanism in osteoclast differentiation remains to be solved.

[Purpose] To examine the negative effect of CTLA4-Ig for Osteoclast differentiation, and clarify the mechanism.

[Method] *In vitro* study, bone marrow macrophages (BMMs) derived from murine bone marrow were cultured with M-CSF and RANKL four days with or without recombinant Mouse CTLA4-Ig. Osteoclasts were counted as TRAP positive cells, and the expression of *NFATc1* were examined with real time PCR. Next, real-time intracellular calcium oscillation of BMMs with RANKL or CTLA4-Ig were detected with Fura-2 and made an analysis of frequency spectrum.

[Results] CTLA4-Ig inhibited Osteoclast differentiation *in vitro*. RT-PCR data resulted in the less expression of *NFATc1* with the higher concentration of CTLA4-Ig.

The Fura-2 fluorescence ratiometry data showed that CTLA4-Ig suppressed the calcium oscillation especially in high frequency range.

[Conclusion] CTLA4-Ig inhibited intracellular calcium oscillation and down-regulated *NFATc1* expression. (COI: No)

2P-446

Metabotropic glutamate receptor mGlu2 regulates signaling via Gq-coupled serotonergic receptor

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Serotonin is thought to play important roles in the higher order brain function. It has been reported that Gq-coupled serotonin receptor 5-HT2aR forms a complex with Gi/o-coupled metabotropic glutamate receptor mGlu2 in mammalian brain and that the complex formation alters their signaling profiles. However, effects of the crosstalk on Gq and Gi/o signaling have not been elucidated yet. Here we analyzed the functional coupling of mGlu2 with 5-HT2aR in HEK293T cells, by monitoring the Gq and Gi/o signaling. Gq signaling was monitored as the activity of phospholipase C which causes the translocation of the pleckstrin homology (PH) domain from membrane to cytosol. Activation of 5-HT2aR by application of serotonin (0.1-10 μM) induced the translocation of the PH domain, while the serotonin-induced translocation was inhibited by co-expression of mGlu2 in the absence of glutamate. Interestingly, additional application of glutamate (0.2 mM) unexpectedly induced the translocation of the PH domain. We also analyzed Gi/o signaling by recording the G protein gated inwardly rectifying K^+ channel current upon the activation of mGlu2. When co-expressed with 5-HT2aR, the amplitude of the glutamate-induced current was decreased in the absence of serotonin. In summary, we showed that Gq signaling via 5-HT2aR and Gi/o signaling via mGlu2 were inhibited each other when they were co-expressed, and that the inhibitory effect of mGlu2 was attenuated by the application of glutamate. (COI: No)

2P-447

Altered expression of taste signaling elements in jejunal tissue of obese patients

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Purpose: More than 300 million adults are clinically obese worldwide, making obesity a major health problem. It is generally accepted that obesity is associated with low-grade systemic inflammation. Previous studies have shown that taste signaling proteins such as G protein gustducin (GNAT3) and phospholipase C, beta 2 (PLCb2) are expressed in the human gut, especially, in intestinal tuft cells, which play a critical role in innate epithelial immunity against parasitic infection. Additionally, it has been shown that taste signaling controls metabolism, including the regulation of body weight and adiposity via intestinal tuft cells and taste cells in mice. This study aimed to determine if taste signaling is altered in patients with extreme obesity.

Methods: The mRNA expression level of PLCb2 and GNAT3 in the proximal discarded jejunum obtained from patients with obesity who underwent bariatric surgery (BMI under 40, 40-49, and 50-59) and was examined using PCR. After reverse transcript PCR, the intensity of the amplified bands was measured by using imageJ. Preliminary results of the initial 15 patients were analyzed.

Results: The intensity of the amplified PLCb2 product from BMI 50-59 patients was lower than those with BMI<50. The intensity of GNAT3 bands was similar across the three BMI groups.

Conclusions: Our preliminary data suggest that taste signaling elements may be altered in patients with extreme obesity, and dysregulation of taste signaling may contribute to obesity. (COI: No)

2P-448

The intracellular C-terminal domain is responsible for cell surface expression of mGluR6

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The intracellular C-terminal domain (ICD) is crucial for various cellular functions, including intracellular signaling and trafficking. Very little is known about the mechanism of membrane expression of metabotropic glutamate receptor type 6 (mGluR6), which are exclusively localized in dendritic tips of retinal bipolar cells. In order to determine whether the surface expression of mGluR6 depends on its ICD, we analyzed the subcellular localization of mGluR6 carrying C-terminal deletions in transfected HEK293 cells and primary cultured hippocampal neurons by using immohistochemistry and cell surface biotinylation assay. We showed that deletion of the last 15 amino acids of the mGluR6 ICD dramatically reduces cell surface expression. We also showed that cell surface expression of mGluR6 is restored by deletion of additional 6-amino acids while it is again disrupted by further removal to the boundary between the transmembrane domain 7 and ICD. These results suggest that the ICD of mGluR6 is crucially involved in its cell surface expression. (COI: No)

2P-449

Effects of PCSK9 inhibitor and atorvastatin on mitochondria of red muscle fibers in obesity

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Purpose Obesity and dyslipidemia lead to mitochondrial dysfunction in skeletal muscle. PCSK9 inhibitors and statins are widely used as anti-dyslipidemic drugs, yet their effects on skeletal muscle remain unclear. Low dose atorvastatin impaired mitochondrial function of white muscle fibers but not red muscle fibers. However, the effects of PCSK9 inhibitors and high dose atorvastatin on mitochondria of red muscle fibers have not been identified.
Methods We compared soleus muscle mitochondria in 4 groups of female Wistar rats: normal diet with vehicle (NDV), high-fat diet with vehicle (HFV), high-fat diet with 3 wks of 4 mg/kg/day of PCSK9 inhibitor (HFP), and high-fat diet with 3 wks of 40 mg/kg/day of atorvastatin (HFA).
Results High-fat diet fed rats weighed heavier than the NDV group. The HFV and HFA groups showed higher ROS production and membrane depolarization in soleus muscle mitochondria compared to the NDV group. However, there were no such differences between the HFV and HFA groups. Interestingly, ROS production and membrane potential in soleus muscle mitochondria of the HFP and NDV groups were not different.
Conclusions Obesity increases oxidative stress and impairs mitochondrial function of red muscle fibers. High dose atorvastatin neither improves nor worsens obesity-induced mitochondrial dysfunction in red muscle fibers. On the other hand, PCSK9 inhibitor attenuates obesity-induced mitochondrial oxidative stress and mitochondrial dysfunction in red muscle fibers. (COI: No)

2P-450

Intracellular calcium responses to mechanical stimulation in mouse and human synoviocytes

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Purpose: Fibroblast-like synoviocytes (FLS) are present in the synovium and are constantly exposed to mechanical stimulation (MS) with joint movement. MS to FLS contributes to development of joint inflammation, however, the receptive mechanisms of FLS to MS remain to be elucidated. The purpose of this study is to compare the intracellular calcium response to MS between mouse and human FLS.
Methods: Normal mouse and human FLS were used in experiments. Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) of FLS in mouse and human were measured with loaded Fluo-3 AM. MS were performed by touching with a glass micropipette or by ejecting nitrogen gas from a glass micropipette.
Results: Both touching and shear stress stimulations elicited immediate $[Ca^{2+}]_i$ increasing responses in both FLS in the presence of extracellular Ca^{2+}. These responses of FLS in mouse and human remained in Ca^{2+}-free conditions.
Conclusions: These results indicated that different MS elicited similar intracellular calcium responses in mouse and human FLS and suggested the possibility that Ca^{2+} release from the intracellular store contributes to the responses of FLS. (COI: No)

2P-451

Global analysis of specific gene expression in thymus gland of AQP11 null mice

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Aquaporin11 (AQP11) is one of aquaglyceroporins which are permeable to water and glycerol. The representative expression patterns are kidney, brain, testis and thymus. Especially, AQP11 null mice have characteristics that the phenotype of thymic involution is began from juvenile and they are dead from polycystic kidney disease within one month. The purpose of this study is to elucidate the role of AQP11 in thymus. Therefore, microarray analysis was employed to determine altering gene expression. Thymus gland were isolated from AQP11 null mice and wild type mice. Gene expression was available to calculate quantitatively as the fold change (FC). Database searches were performed using the David Bioinformatics Resources 6.8(beta). The annotation analysis was performed 1.5 or more and 0.5 or less for each FC. Representative gene was analyzed RT-qPCR quantitatively.
By this analysis, we identified 66 up-regulated genes which were mainly participate in PI3K/Akt signaling pathway. Moreover, 55 gene were identified as down-regulated gene which were regulated PPAR signaling pathway. RT-qPCR analyses revealed the enhanced expression of Egfr, Itgb4 and Il2ra and the diminished expression of AQP7, Pck1 and Ucp1. These observations suggest that up-regulated genes support immunity system for early thymic involution and down-regulated genes inhibit cytokine in AQP11 null thymus. Furthermore, the thymus in AQP11 null mice might not be able to regulate glycerol transportation of AQP7. (COI: No)

2P-452

Different expression of Olig2 and O4 in cultured mouse brain cells

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Objective: Olig2 and O4 are widely used for identification of oligodendrocytes. It is thought that immature oligodendrocytes express Olig2 and relatively mature oligodendrocytes express O4. The present study investigated if Olig2 and O4 are appropriate to identify oligodendrocytes and to estimate the oligodendrocyte developmental stage. **Methods:** Cells isolated from the mouse brain were cultured. They included neurons and glial cells (oligodendrocytes, astrocytes and microglial cells). Cell dynamics such as cell division was observed using time-lapse microscopy. Then cells were immunostained with antibodies against Olig2, O4, MAP2 (a neuronal marker), and GFAP (an astrocyte marker). **Results:** Approximately 12% of total cells were positive for Olig2, whereas less than 0.5% were positive for O4. Olig2-positive cells were neurons and astrocytes other than oligodendrocytes, whereas O4-positive cells were exclusively oligodendrocytes. A part of oligodendrocytes, neurons and astrocytes after cell division were Olig2-positive. Oligodendrocytes immediately after cell division were all O4-positive. **Conclusion:** Different from the previous view, Olig2 is likely expressed in immature neurons and astrocytes as well as oligodendrocytes. O4 is expressed in exclusively oligodendrocytes, not only mature oligodendrocytes but also immature dividing oligodendrocytes. These results may help the identification of oligodendrocytes in various developmental stage. (COI: Properly Declared)

2P-453

Withdrawn

2P-454

MitoQ protects endothelial barrier injury and inflammation by inhibiting ROS and autophagy in HUVECs

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Chronic obstructive pulmonary disease (COPD) is a common disease characterized by persistent airflow limitation. Pulmonary vascular endothelial barrier injury and inflammation is increasingly considered to be important pathophysiological processes in cigarette smoke extract (CSE)-induced COPD, but the mechanism is still unclear. To identify the cellular mechanism of endothelial barrier injury and inflammation in CSE-treated human umbilical vein endothelial cells (HUVECs), we investigated the mitochondria-targeted antioxidant mitoquinone (MitoQ) on endothelial barrier injury and inflammation. We demonstrated that MitoQ, which restored endothelial barrier integrity by preventing VE-cadherin disassembly and actin cytoskeleton remodeling, as well as decrease in inflammation by NF-κB and NLRP3 inflammasome pathway in endothelial cells. In addition, MitoQ also maintained mitochondrial function by reducing production of ROS and excess autophagy. Inhibition of autophagy by 3-MA exacerbates cytotoxicity induced by CSE in HUVECs. Taken together, our study indicated that mitochondrial damage is a key promoter in the induction of endothelial barrier dysfunction and inflammation by CSE, the protective effect of MitoQ is related to the inhibition of ROS and excess autophagy in CSE-induced HUVECs injury. (COI: No)

2P-455

Withdrawn

2P-456

Nardilysin in hepatocyte regulates adaptive thermogenesis in brown adipose tissue

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Thermogenesis is enhanced not only by cold exposure but also by feeding, which is considered as a partial defense mechanism against obesity. However, the molecular mechanism of diet-induced thermogenesis has remained elusive. Whole-body deficient mice of metallopeptidase nardilysin (NRDC) show hypothermia (1.5 ℃ lower at room temperature) and cold intolerance. NRDC is involved in body temperature homeostasis through the control of central set point, heat dissipation and thermogenesis in brown adipose tissue (BAT). To elucidate the tissue-specific role of NRDC in body temperature homeostasis, we established several lines of conditional knockout mouse. Among them, hepatocyte-specific NRDC deficient (LKO) mice showed unexpected intriguing phenotypes including 1) elevation of thermogenic genes in BAT, 2) decrease in lipid accumulation in BAT, and 3) increase in whole-body energy expenditure. These results suggested that the loss of NRDC in hepatocyte enhances adaptive thermogenesis in BAT via inter-organ metabolic network. Notably, the phenotypic difference between control and LKO mice was completely eliminated by hepatic vagotomy. On the other hand, NRDC expression in liver is increased by fasting and decreased by re-feeding in wild-type mice. Taken together, these results indicated that diet controls NRDC expression in liver, which in turn regulates BAT thermogenesis via vagal nerve. In conclusion, NRDC, a possible sensor of nutrition, mediates diet-induced thermogenesis. (COI: No)

2P-457

Structure Development of Oxolinic Acid, a Novel Inhibitor of Type 1 Ryanodine Receptor

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Type 1 ryanodine receptor (RyR1) is a Ca^{2+} release channel on the sarcoplasmic reticulum in the skeletal muscle. Mutations in RyR1 cause various muscle diseases including malignant hyperthermia (MH) and central core disease (CCD). Although dantrolene is the only therapeutic drug for MH, it cannot be used for CCD due to its lower solubility and side effects. It is therefore urgent to discover novel RyR1 inhibitors. We have recently developed an efficient high-throughput screening platform for RyR1 inhibitors using Ca^{2+} measurements in the endoplasmic reticulum (ER). By screening a library of well-characterized drugs, we successfully identified oxolinic acid as a novel RyR1 inhibitor (Murayama et al., Mol Pharmacol, 94: 722-730, 2018). However, affinity of oxolinic acid was much lower than that of dantrolene. In this study, we designed and synthesized a series of quinolone derivatives using oxolinic acid as a lead compound. Dose-dependent inhibitory effects were evaluated by ER Ca^{2+} measurement using HEK293 cells expressing R-CEPIA1er, a genetically-encoded EC Ca^{2+} indicator, and RyR1 carrying an MH mutation (R2163C). The most potent derivative so far exhibited >50 and 4-fold greater affinity than oxolinic acid and dantrolene, respectively. These compounds may be good candidates for treatment of RyR1-related diseases. (COI: No)

2P-458

Ribosome binding protein GCN1L1 controls cell cycle and is essential for embryonic development

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Protein kinase GCN2 regulates translation in response to amino acid-starvation by phosphorylating eukaryotic initiation factor 2 alpha (eIF2α), leading to elevated translation of ATF4 that regulates amino acid synthetic enzymes and transporters. Although yeast GCN1 is essential for GCN2 activation by amino acid-starvation, function of mammalian GCN1L1 remains to be elucidated. We generated *Gcn1l1* knockout mice and found that mouse GCN1L1 is also necessary for amino acid-starvation-induced ATF4 activation in *Gcn1l1*-/- mice embryonic fibroblast cells (MEFs). *Gcn1l1*-/- embryos showed growth retardation and perinatal lethality due to respiratory failure. Administration of progesterone to pregnant *Gcn1l1*+/- female crossed with *Gcn1l1*+/- male prolonged the gestational period for 24 hours and rescued the expression of lung differentiation markers and the lethality of *Gcn1l1*-/- embryos. These results suggest that growth retardation including the delay of lung development results in the lethality in *Gcn1l1*-/- embryos. We further examined the cause of growth retardation using MEFs and found that *Gcn1l1*-/- MEFs exhibited reduced proliferation and G2/M arrest accompanying decrease of Cdk1. Additionally, IGF-1 signaling, regulator of cell proliferation and survival, was impaired in *Gcn1l1*-/- MEF, that might relate to the cause of growth retardation. Altogether we showed that GCN1L1 is necessary for embryonic developement as well as for the response to amino acid-starvation. (COI: No)

2P-459

Malignancy of cancer cell lines correlates with NKCC1 expression and intracellular Cl^- concentration

Hiroaki Miyazaki (*Department of Life Science, Setsunan University, Japan*)

Our previous study demonstrated that the reduction of intracellular Cl^- concentration ($[Cl^-]_i$) brings the inhibitory effect on the cell proliferation of MKN28 gastric cancer cell. In general, $[Cl^-]_i$ is determined by the balance of activities of Cl^- transporters and channels in cells. Therefore, the $[Cl^-]_i$ regulated by Cl^- transporters and channels would be one of critical messengers which regulate cell proliferation.

In this study, we confirmed the expression levels of Na^+-K^+-2Cl^- cotransporter, K^+-Cl^- cotransporter and CLC Cl^- channels (CLC-2 and CLC-3) in human gastric cancer cell lines (MKN28 and MKN45) with different malignancies to clarify the correlation between the cell proliferative activity and the expression levels of Cl^- transporters and channels. The protein expression level of NKCC1, which is a major pathway for Cl^- uptake into cells, in MKN 45 (poorly differentiated type) was much higher than that in MKN 28 (moderately differentiated type). In order to clarify the involvement of NKCC1 on cell proliferation, we confirmed the influence of NKCC inhibitors (furosemide or bumetanide) on cell proliferation. The proliferation of MKN45 cells was highly suppressed compared to that of MKN28 cells by the application of NKCC inhibitors. In addition, $[Cl^-]_i$ in MKN45 cells was maintained higher than that in MKN28 cells. These findings suggest the regulation of NKCC1 function and $[Cl^-]_i$ is a novel, unique therapeutic strategy for gastric cancer treatment. (COI: No)

2P-460

Structure of bound water in myofibril suspension: A role of ATP

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The dynamic structure change of water molecules surrounding contractile proteins might play an important role in cross-bridge cycling during contraction. The spin-spin relaxation process of ^{1}H-NMR signals from suspension of myofibrils prepared from rabbit could be well represented by the summation of several exponentials indicating that water molecules in the suspension could be conveniently grouped into several components based on the relaxation time constant (T_2). In the M or MT state, myofibril affects water molecules within 500 nm from its surface differently from water molecules in the bulk solution, and releases many water molecules in the MDPi or MD state.

This may suggest that the potential of the water molecules that surround myofibril proteins are dynamically changed during cross-bridge cycling. (COI: No)

2P-461

mTORC2 signaling is critical for lysosomal activation by isorhamnetin treatment in J774.1

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[Objective] Lysosome is the principal cellular organelle for the ultimate degradation of intracellular macromolecules delivered by several pathways. Remarkably, the lysosomal dysfunction in macrophages has been reported to be responsible for the development of atherosclerosis. In this study, we searched for polyphenols contained in dietary vegetables which could enhance the lysosomal activity in macrophages, and investigated its molecular mechanism.

[Methods & results] We established the assay system using DQ-BSA reagent, a fluorogenic substrate for proteases, to evaluate the lysosomal activity in J774.1 mouse macrophage-like cell line. Cells were treated with each polyphenol at 10 mM for 29 hours, and incubated with DQ-BSA for another hour. Fluorescence laser scanner enabled high-throughput cell-based assay, which identified isorhamnetin as the most active compound among a total of 54 candidates.We assumed that mTORC1 or mTORC2 signaling should be related to the regulation of the lysosomal activity, since Torin1, an mTOR inhibitor, also showed the potent activity. Raptor or rictor-KO cells generated by a CRISPR/Cas9 system led us to the conclusion that the lysosomal degradation of DQ-BSA was mainly controlled by mTORC2 signaling. Interestingly, isorhamnetin inhibited the phosphorylation of NDRG1 and Akt, mTORC2 substrates. Based on these results, it is suggested that isorhamnetin might activate lysosomal activity by suppressing mTORC2 activity. (COI: No)

2P-462

Novel RyR1 Inhibitors Identified by High-Throughput Screening Using ER Ca^{2+} Measurement

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Genetic mutations in type 1 ryanodine receptor (RyR1), a Ca^{2+}-release channel on the sarcoplasmic reticulum of skeletal muscle, cause various muscle diseases including malignant hyperthermia (MH). Because the main underlying mechanism of the pathogenesis is overactive Ca^{2+} release by gain-of-function of the RyR1 channel, inhibition of RyR1 is expected to be a promising treatment for these diseases. We have recently developed an efficient high-throughput screening (HTS) platform for RyR1 inhibitors using Ca^{2+} measurements in the endoplasmic reticulum (ER) and successfully identified three novel compounds from a library of well-characterized drugs (Murayama et al., Mol Pharmacol, 94: 722-730, 2018). However, only oxolinic acid was found to be specific to RyR1 and other two compounds inhibited both RyR1 and RyR2, a cardiac isoform. To explore another RyR1-specific inhibitor, we performed HTS from a library of larger numbers of compounds. ER Ca^{2+} of HEK293 cells expressing RyR1 carrying an MH mutation (R2163C) was monitored with R-CEPIA1er, a genetically encoded ER Ca^{2+} indicator, using FlexStation3 fluorometer. Compounds which inhibit RyR1 would increase ER Ca^{2+} by preventing Ca^{2+} leak via the mutant RyR1. We successfully identified several RyR1-specific compounds that are structurally different from oxolinic acid or dantrolene, a known RyR1 inhibitor. These compounds may be good candidates for treatment of RyR1-related diseases. (COI: No)

2P-463

Regulation of cell cycle by N^6-methyladenosine modification in cancer cells

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[Background] N^6-methyladenosine (m^6A) modification is the most abundant modification in messenger RNA (mRNA). m^6A modification regulates the stability, localization, and translational efficiency of mRNA, which contributes to a diverse physiological and pathological processes. Recently, aberrant m6A modification has been implicated in cancer development, but the molecular mechanism has been largely unknown. In this study, we investiged the role of m^6A in cancer cell growth.

[Methods] Oral cancer cells were treated with siRNAs targeting obesity-associated gene (FTO), a m^6A demethylase. Total RNA was subjected to RNA-seq to analyze the landscape of transcriptome. To analyze m^6A sites in mRNA, mRNA was purified from total RNA, followed by immunoprecipitation and RNA-seq. In addition,total RNA was purified from clinical samples obtained from oral cancer patients. Expression pression level of FTO and cell growth-related genes were examined by qPCR.

[Results] FTO-deficiency suppressed expression of genes related to cell cycle. The m^6A modification was detected in mRNAs related to cell cycle, which induced degradation of mRNA. m^6A modification of cell cycle gene was differentially regulated during cell cycle. The mRNA level of cell cycle-related gene was significant correlated with FTO mRNA level in oral cancer samples.

[Conclusions] Our study suggests that m^6A modification of mRNA regulates the stability of mRNA and contributes to the promotion of cell cycle. (COI: No)

2P-465

Inhibition of the frequency of airway ciliary beating by PDE1 activation in Down syndrome mouse

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Down syndrome is caused by trisomy of human chromosome 21. Down syndrome patients have respiratory problems. We examined the effects of 1 nM procaterol (an β2-agonist, proc) on ciliary bend angle (CBA) and ciliary beat frequency (CBF) in airway ciliary cells of Down syndrome mouse (Ts1Rhr). The CBA and CBF were measured in airway ciliary cells isolated from lungs using a video microscopy equipped with a high-speed camera (500Hz). The time course of CBF increase, not CBA increase, stimulated by proc was much slower in the Ts1Rhr than in the wild type mouse (WT). The previous study demonstrated that the slower CBF increase stimulated by proc is caused by activation of Ca^{2+}/calmodulin dependent PDE1A in the cilia. The delay in the CBF increase was abolished by 8-methoxymethyl-IBMX (40 μM, a specific inhibitor of PDE1) and calmidazolium (25 μM, a specific inhibitor of calmodulin). Pcp4 (Purkinje cell protein 4), which locates within Ts1Rhr region, is the gene regulating calmodulin. The CBF increase stimulated by proc in Ts1Rhr; $Pcp4^{+/+}$(Pcp4 knockout Ts1Rhr mice) was similar in the time course to that in WT. These observations suggest the CBF suppression in Ts1Rhr caused by an excessive PDE1 activation. PDE1 activity appears to be Pcp4 dose-dependently maintained at a high level. (COI: No)

2P-466

Microscale liquid layer on the olfacrory receptors affects on the vapor chemical detection

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The extraordinary olfactory capability of the animal has long been used for biologically functional odorant identification and discrimination. Although signal transduction pathway and molecular machinery for odorant detection has been identified from various species across phyla, the function of olfactory mucus in odor perception is poorly understood. It has been reported that olfactory mucus secretion-impaired mice showed elevated threshold sensitivity to odorants, and that ligand repertoire in in vivo recording with vapor olfactory stimulation differed from that of heterologous olfactory receptor (OR)-expressing cells with vapor olfactory stimulation. To examine the function of olfactory mucus in vapor chemical detection, I address the technique to measure the mass transfer processes of gaseous odorants to the microscale liquid thin layer on the surface of mammalian cells. In the system, insect ionotropic OR complex was utilized as the odorant-sensor. ORs were functionally expressed in HEK293T cells. Vapor odorant stimulation was performed by using an olfactometer, and monitored by using a photoionization detector. Thickness of liquid layer on the surface of cells was measured by the scanning of impedance of microelectrode on the surface of liquid layer. Odorant-induced calcium influx was measured by using calcium imaging. The results demonstrated that 20 μm increase in thickness of surface liquid layer on the cell resulted in the decreased olfactory response by half. (COI: No)

2P-467

Differential effects of Fe^{2+} and Fe^{3+} on the proliferation and differentiation of osteoblasts

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Iron overload is known to be associated with osteoporosis with higher incidence in several diseases that require chronic blood transfusion or aberrant intestinal iron uptake, e.g., hemochromatosis, β-thalassemia, and sickle cell anemia. Since iron potentially generates free radicals, iron toxicity from an increase in non-transferrin bound iron could explain bone loss in iron-overloaded patients. However, it was unclear whether ferric (Fe3+) or ferrous (Fe2+) were responsible for an impaired osteoblast function. In this study, we used ferric ammonium citrate (FAC) and ferrous ammonium sulfate as donors of ferric and ferrous, respectively, and exposed them to osteoblast-like UMR-106 cells. It was found that both iron species decreased cell survival and proliferation, while increasing apoptosis. Stronger effects were observed in ferric-treated groups. Further study by using atomic absorption spectrophotometry and polymerase chain reaction showed that the intracellular iron content was elevated along with a downregulated expression of genes for iron influx and upregulated expression of genes for iron efflux. Alkaline phosphatase activity, an indicator of osteoblast differentiation, became lower after exposure to FAC. In conclusion, the two iron species had negative effects on osteoblast proliferation and differentiation, and they were more sensitive to ferric than ferrous. A chelator that targets ferric is probably beneficial to iron-overloaded patients with high ferric levels. (COI: No)

2P-468

Synergistic effect of histone deacetylase inhibitors in intravesical instillation of bladder cancer

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Bladder cancer is common malignancy with high tumor recurrence rate even after tumor resection. Intravesical instillation (IVI) of chemotherapy drugs or Bacillus Calmette-Guerin is recommend for preventing tumor recurrence. Study of bladder tumor revealed that the histone deacetylases (HDACs) might play the key role in regulation of cell cycle, cell apoptosis, and malignancy of bladder cancer which suggests the potential therapeutic role of HDAC inhibitors in bladder cancer. In this study, we analyzed the synergistic effect and molecular mechanism of HDAC inhibitors in vitro with MTT, apoptotic, and cell cycle assays. The synergistic effect was further confirmed via IVI in autochthonous rat bladder cancer model (Approval by Chung-Shan Medical University Experimental Animal Center, No. 1920). Our study evidenced the synergistic effect of histone deacetylase inhibitors combined with chemotherapy drugs with significant increased cell death rate, sub-G1 percentage, caspase activity, and related protein expression. These results were further evidenced with autochthonous rat bladder cancer model which revealed less residue tumor of combination therapy compared with chemotherapy along or control group. The mechanism observed in cell model was further confirmed with tumor section of animal model via immunohistochemistry staining. Our study evidenced the role of HDAC inhibitors via IVI treatment for bladder cancer which might provide new insights to promote further clinical trials. (COI: Properly Declared)

2P-469

Neferine selectively alters LPS-induced inflammatory responses in RAW 264.7 macrophages

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Neferine is a major alkaloid derived from lotus seed embryo. It is used as wide range in traditional medicine. In this study, we investigated the effect of neferine on free radical scavenging and inflammatory response in lipopolysaccharide (LPS) induced RAW264.7 macrophage cells. Neferine showed a potential antioxidant activity in dose-dependent manner for 2,2-diphenyl-1-picryl-hydrazyl-hydrate(DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activities. Subsequent studies demonstrated that neferine potentially reduced the reactive oxygen species (ROS) production but not nitric oxide (NO). The finding was further correlated with the suppression of mRNA levels of pro-inflammatory cytokines such as interleukin 1β (IL-1β), interleukin 6 (IL-6) and cyclooxygenase 2 (COX-2). On the other hand,neferine had no effect on tumor necrosis factor α (TNF-α) and inducible nitric oxide synthase (iNOS) expression. These finding provide scientific evidences that neferine attenuated LPS-induced ROS damage and inflammatory cytokines in RAW 264.7 cell, representing a relevant bioactive compounds from lotus seed embryo with promising benefits for treatment and prevention of various inflammatory diseases.

There is no actual or potential conflict of interest in relation to this presentation.

Keywords: Neferine, anti-inflammation, ROS, IL-6, IL-1β, COX-2 (COI: Properly Declared)

2P-470

The influence of KATP channel abnormality on calcium handling of endoplasmic reticulum

Hiroki Takanari (*Tokushima University Hospital, Japan*)

Adenosine triphosphate (ATP) sensitive potassium channel (KATP channel) is expressed on plasma membrane and intracellular organelle like endoplasmic reticulum (ER). The role of KATP channel on ER is not well understood. We examined the influence of KATP channel on ER Ca^{2+} handling. Wild type (WT) human skin fibroblast (FB) and FB with gain-of-function (GOF) mutation of KATP channel were obtained. The cells were loaded with Fluo4-AM to observe intracellular Ca^{2+} ($[Ca^{2+}]_i$) transition by live imaging. Steep upstroke of $[Ca^{2+}]_i$ and rapid decline thereafter were elicited by 100 μM histamine in both WT and mutant FBs. However, the behavior of $[Ca^{2+}]_i$ after that was different in the two types of FBs; $[Ca^{2+}]_i$ quickly decreased to the baseline level within 5 minutes in WT, whereas $[Ca^{2+}]_i$ prolonged at 40% of histamine-induced maximal $[Ca^{2+}]_i$, suggesting that Ca^{2+} re-uptake of ER was impaired by GOF mutation of KATP channel. Next, we depleted extracellular calcium from the cells and added 1 μM thapsigargin (TG), which inhibits ER Ca^{2+} re-uptake, to examine ER Ca^{2+} content. Apparent increase in $[Ca^{2+}]_i$ by TG was confirmed in WT, while only a slight increase in $[Ca^{2+}]_i$ was observed in GOF mutant. Subsequently, we increased the extracellular Ca^{2+} to 5 mM. We confirmed that $[Ca^{2+}]_i$ increased sharply up to a few mM in GOF mutant, whereas $[Ca^{2+}]_i$ increase in WT was very few. Our findings showed that GOF abnormality of KATP channel influence $[Ca^{2+}]_i$ handling via decreasing ER Ca^{2+} content. (COI: No)

2P-471

Dinaciclib inhibits Aurora A expression and proliferation of prostate cancer cells

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Aurora A plays crucial roles in cell cycle control, especially in mitosis. Since Aurora A activation is required by tumor growth, it becomes a therapeutic target in recent years. Cyclin-dependent kinases (CDKs) also play important roles in controlling all stages of cell cycle. Dinaciclib is newly synthesized inhibitor to CDKs with low effective doses and currently under clinical evaluation. However, the application of Dinaciclib in treating prostate cancer remains unclear. **Purpose:** To investigate the effects and mechanism of Dinaciclib to prostate cancer cell proliferation. **Methods:** Base on drug treatment to LNCaP cell culture and identify following protein expression, distribution, and cell proliferation. **Results:** The authors found that Dinaciclib effectively inhibited proliferation of prostate cancer cell line, LNCaP, as well as affected the levels of some cell cycle-related proteins. The activities of CDK1, 4, 5, and 6 in LNCaP cells were inhibited after Dinaciclib treatment. Interestingly, mRNA and protein levels of Aurora A were both declined after Dinaciclib treatment, while its protein decrease was primarily contributed to nuclear fraction. In addition, the abnormal phenotype of centrosome was observed after Dinaciclib treatment. **Conclusion:** These evidence showed that Dinaciclib effectively inhibited LNCaP cell proliferation and might be correlated the defect of Aurora A's biological function in mitosis. (COI: No)

2P-472

Dose-response relationship of free radical scavenging activity of dexmedetomidine

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Purpose Dexmedetomidine (DXM) is clinically used for sedation in perioperative patients. Recently, it has been reported that DXM might act as anti-oxidant. Thus we evaluated dose-response relationship of direct scavenging activity of DXM against multiple free radical species.

Methods Eight kinds of free radicals were generated in test tubes, and direct scavenging activity of DXM were evaluated by electron spin resonance spectroscopy with spin-trapping method. IC_{50} was calculated from dose-response curve of each radical. Inhibitory effect of DXM on intracellularly generated hydroxyl radical was also evaluated by hydroxyphenyl fluorescein in HEK293 cells.

Results DXM scavenged the following free radicals in dose-dependent manners (IC_{50}): hydroxyl radical (2.2 ± 0.4 mM), superoxide anion (17 ± 8 mM), *t*-butoxyl radical (0.96 ± 0.10 mM), singlet oxygen (0.15 ± 0.01 mM), ascorbyl free radicals (36 ± 1 μM). No scavenging activity was observed against *t*-butyl peroxyl radical, nitric oxide and DPPH. DXM significantly inhibited intracellular generation of hydroxyl radical by adding Fe^{2+} or antimycin to cultured cells.

Conclusions It is speculated that DXM has direct scavenging effect on multiple kinds of oxygen-centered radicals but not on nitrogen-centered radicals. Inhibitory effect of DXM on intracellular generation of hydroxyl radical could be attributable to direct scavenging activity against hydroxyl radical and superoxide anion. (COI: No)

2P-473

Airway ciliary beating activated by enhanced Ca^{2+} signal in Hochu-ekki-to (TJ-41) treated mice

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Hochu-ekki-to (TJ-41, Bu Zhang Yi Qi Tang), a Chinese traditional medicine, is known to stimulate airway ciliary beatings. The ciliary beat frequency (CBF) and ciliary bend angle (CBA) were measured in airway ciliary cells isolated from lungs using a video microscopy equipped with a high-speed camera. In the test mice, TJ-41-containing water was administered for 4-6 weeks (1.8g/kg/day), while in the control mice, only water was administered. The basal CBF and CBA are high in the TJ-41 treated mice compared with the control mice. In TJ-41 treated mice, the basal CBF was maintained by Ca^{2+} signal, not cAMP signal, whereas, in the control mice, the basal CBF was maintained by both cAMP and Ca^{2+} signals. CBF is regulated by the Ca^{2+}-dependent PDE1 in airway ciliary cells. The Ca^{2+} signal may inhibit a cAMP accumulation by activating PDE1 in airway ciliary cells. The experiments using procaterol (1 nM, an β_2-agonist) and an inhibitor of PDE1 revealed that the TJ-41 treatment stimulates PDE1 in airway ciliary cells, suggesting the enhancement of Ca^{2+} signal. Moreover, the concentration response studies of acetylcholine and ionomycin exhibited that the TJ-41 treatment shifted the CBF concentration-response curves to lower concentrations. These results suggest that the TJ-41 treatment enhances CBF and CBA by stimulating Ca^{2+} signal. (COI: No)

2P-474

Influence of TRPC knockout on mouse pupillary sphincter

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Since vision is an important sensory organ for mammals, mechanisms to properly capture visual information are developed very well. The iris has a role of adjusting the size of the pupil to adjust the amount of light incident on the retina, the pupillary sphincter contained therein is only involved in the miosis and is dominated by parasympathetic nerves. To maintain the light intensity properly, the iris needs to rapidly change the diameter of the pupil (rapid phase) and maintain the pupil for a long time (sustained phase). Extracellular calcium influx is required for intraocular smooth muscle, but there are some molecular entities and mechanism of action, but details are unknown. So far, we have shown that calcium influx in sustained phase is mediated by two nonselective cation channels (NSCC) with different unit conductance by experiments in bovine ciliary muscle. Expression of TRPC1, TRPC3, TRPC4, TRPC6, Orai1, etc. has been confirmed as a molecule candidate, but it is difficult to apply gene knockdown to bovine material, details of the relationship with NSCC have been clarified absent. Therefore, experiments were carried out using TRPC 3 and TRPC 6 knockout mice and double knockout mice based on these, as experimental materials, using mice which are relatively easy to genetically modify. We report the results of the miosis analysis by light reflex which can measure the contraction of pupillary sphincter non-invasively. (COI: No)

2P-475

The inhibitory effects of microRNA-107 on p35/CDK5-regulated prostate cancer cell growth

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Prostate cancer is one of the most common cancer in men. The greater age of the male has the higher possibility of prostate cancer occurrence. Previous study found that microRNA-107 can influence the cellular migration by inhibiting the gene expression of Cyclin-Dependent Kinase 5 (CDK5) activator, p35. In addition, our previous studies show that CDK5 can regulate cancer cells, such as prostate cancer by regulating the activity of androgen receptor. Purpose: Based on our *in silico* research between microRNA-107 and CDK5. Our aim of the research is to investigate whether microRNA-107 could inhibit the activity of AR through CDK regulation in prostate cancer. Methods: We exam p35 mRNA and protein level by Western blotting and Q-PCR as well as growth by WST-1 assay. Result: Our study shows that microRNA-107 may sabotage p35 mRNA and make its protein level decrease; therefore, microRNA-107 could diminish the activity of CDK5 as well as Ser81-AR phosphorylation level, which caused the protein level of AR decline. Eventually, the growth of prostate cancer was inhibited. Conclusion: Thus, microRNA-107 may inhibit the growth of prostate cancer through down-regulating p35 mRNA and protein level. Thus, we may be able to use microRNA-107 as a biomarker on cancer diagnosis in the future. (COI: No)

2P-476

The inhibitory effects of valproic acid on androgen receptor and prostate cancer cell growth

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Prostate cancer is one of the most common cancer in men. Binding of androgen to androgen receptor (AR) induces dissociation from HSP (heat shock protein) in order to promote growth. It has been reported that VPA, a drug for epilepsy treatment, not only inhibits AR gene expression but also its transcription activity. Purpose: Clinical analysis shows that the patients who were treated with VPA may increase serum androgen concentration. However, it is unclear of the VPA effects on AR protein stability. Method: We investigate whether AR is regulated under VPA treatment by Western blotting; in addition, we also observed AR stability by cycloheximide or MG132 treatment. Result: VPA significantly reduced AR protein level and phosphorylation of S81-AR as well as its protein stability in LNCaP cells. Moreover, VPA treatment led to the dissociation of HSP90 and AR and increased AR ubiquitination and proteasomal degradation. On the other hand, VPA decreased both nuclear and cytosolic AR protein level and inhibited AR translocation to nuclear, resulting in down-regulation of AR and PSA mRNA levels. The proliferation of prostate cancer cells was therefore inhibited. VPA suppressed prostate cancer cell proliferation by targeting AR protein stability. Conclusion: VPA suppressed prostate cancer cell proliferation by targeting AR protein stability. We hope this finding can be used as the reference for future medication. (COI: No)

2P-477

CDK5 promotes androgen receptor transactivation under Akt inhibition stress

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Aberrant activation of PI3K/Akt signaling has been found in different human cancer cells and supports their proliferation. Androgen receptor (AR) is critical in the early stage of prostate cancer growth. The authors' previous findings indicate CDK5 promotes prostate cancer cell growth through androgen receptor, STAT3 and p21 regulation in various cancer cells. Purpose: Our previous finding indicates CDK5 is able to increase AR stability and its transactivation as well as prostate cancer cell growth. However, the relationship between these two major growth regulators in androgen-dependent prostate cancer, AR and Akt, remains unclear. Methods: Akt inhibitor, LY294002, and inactive Akt mutant were used to treat cancer cells. The levels of mRNA and protein were respectively measured by Q-PCR and Western Blotting. Result: We found that Akt inhibition increased AR activity through CDK5-related signaling regulation in prostate cancer LNCaP cells, which suggests that AR could be activated by Akt-dependent Cdk5 activation, while p35 upstream regulator, Egr1, was also paralleled regulated. Conclusion: Although Akt inhibition is detrimental to cell growth, Cdk5-dependent AR activation becomes an alternative pathway to maintain the growth of prostate cancer cell. This finding implies that Cdk5 might serve a critical linkage between the pathways of Akt and AR and play an important role in regulating growth of prostate cancer cells under stress. (COI: No)

2P-478

CDK5 down-regulates p21 expression through inhibiting STAT3

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p21 is cyclin-dependent kinase inhibitor (CKI), as a negative cell cycle regulator. Previous study shows that human cancer activated by CDK5 through STAT3 and CDK5 could inhibit p21. Purpose: Based on the study on CDK5 and p21 in cancer. We investigate the detail mechanism between CDK5 and p21. Method: We observed whether CDK5 could interact with STAT3 by using Immunoprecipitation. Also CDK5-regulated p21 gene expression was observed by Western blot and Q-PCR. Result: we found that physical interaction of CDK5 and STAT3 in MCF-7 cells, and decrease of nuclear STAT3 in wild-type CDK5-overexpressing cells but not in cells with mutant CDK5. This suggest that activation of CDK5 decrease STAT3. In contrast, inhibition of CDK5 could increase STAT3. Next, we used transient transfection to observe CDK5-induced p21 expression decline through STAT3. Conclusion: CDK5 overexpression can decrease nuclear STAT3 expression, eventually affect p21 expression. Finally, these results suggest that p21 transcription might be regulated by Cdk5 through inhibiting STAT3 activity. (COI: No)

2P-479

Circadian rhythms in nicotinamide adenine dinucleotide concentration in mouse liver

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Nicotinamide adenine dinucleotide (NAD^+) is an electron mediator and is a coenzyme in various physiological pathways. NAD^+ plays a critical role in regulating numerous biological and physiological pathways including circadian clock, energy metabolism, inflammation, cancer, and aging. Previous studies report that NAD^+ levels decline with aging and that administration of NAD^+ precursors exhibit beneficial effects against aging-related metabolic disorders in animals. In addition, NAD^+ levels are reported to show a mild circadian oscillation due in part to circadian expression of nicotinamide phosphoribosyltransferase, which is the rate-limiting enzyme in the salvage pathway of NAD^+ synthesis. Here we report the results of measuring the NAD^+ levels in the liver of 2-month-old mice. The mice were maintained under 12h light:12h dark conditions. To capture reproducible rhythmicity, liver NAD^+ levels were profiled over 2 days at 6-h intervals. We observed that the levels of the liver NAD^+ show a circadian increase during the activity/feeding phase with an amplitude of about 1.5-fold. In the present study, we used a simple HPLC-UV (high-performance liquid chromatography with ultraviolet detection) method to accurately measure the levels of NAD^+. We will also show a detailed validation of this HPLC-UV-driven method with tandem mass spectrometry (LC-MS/MS). (COI: No)

2P-480

Integrins are involved in mechano-electrical transduction in arterial baroreceptors

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Purpose Arterial baroreceptors act as a mechano-electrical transducer to sense the changes in blood pressure and transduce the mechanical force into discharge of nerve endings. The underlying mechanism is unclear. The connection between integrins and RGD or other ligands may be involved.

Methods Carotid sinus-sinus nerve (CS-SN) specimens isolated from rabbits were used to record nerve discharges while perfusing the vessel and controlling the intra-vascular pressure. All the animals were used following the protocol approved by the Animal Ethics Committee of Capital Medical University (Beijing China) in accordance with the recommendations of the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Results Pressure-dependent discharges of sinus nerve were successfully recorded and RGDF peptide could reversely and does-dependently block the pressure-dependent discharges. IKVAV/YIGSR, integrin ligands on laminin, and PHSRN, integrin ligand on fibronectin showed similar effects. Focal adhesion plaque (FAP) is an important structure for integrins to transport extracellular signals into cells, and focal adhesion kinase (FAK) is an important component involeved in. FAK inhibitor GSK2256098 also blocked pressure-dependent discharges of sinus nerve.

Conclusions All these results suggested the connection between the integrins and the adjacent RGD or other ligands is necessary for the machano-electrical signal transduction in arterial baroreceptors. (COI: No)

2P-481

Vapor detection and discrimination with a panel of odorant receptors

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Mammalian olfactory systems have evolved the extraordinary capability to detect and discriminate volatile odorous molecules (odorants) in the environment. Fundamentally, this process relies on the interaction of odorants and their cognate olfactory receptors (ORs) encoded in the genome. Here, we conducted a large-scale cell-based screen using over 800 mouse ORs against seven odorants using a luciferase reporter gene assay. Cells were directly stimulated by various odorants dissolved in media. This initial screening resulted in the identification of a set of high-affinity and/or broadly-tuned ORs for the tested odorants. We then went on to ask whether heterologously expressed ORs respond to odors presented in vapor phase. To test this, we selected a diverse set of 31 ORs and expressed them individually to measure cAMP responses against vapor phase odor stimulation. This resulted in identifying ORs responsive to odors at concentrations as low as one to one million. Comparison of response profiles between various odors and ORs demonstrates this platform is capable of discriminating between structural analogs. Lastly, co-expression of carboxyl esterase Ces1d expressed in olfactory mucosa resulted in marked changes of specific OR activation in an odorant specific manner. Altogether, these results establish a cell-based volatile odor detection and discrimination platform and form the basis for an OR-based volatile odor sensor. (COI: No)

2P-482

Metabolic alterations in cells transformed by oncogenic Lck kinase

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We showed previously that both oncogenic Lck kinase and the downstream STAT5 transcription factors were highly activated and translocated into mitochondria in a mouse leukemic cell line LSTRA that mimics human large granular lymphocytic leukemia. In the present study, we further defined their effects on mitochondrial activity and cell metabolism. We compared LSTRA with EL4 cells that exhibit normal Lck and STAT5 activity without mitochondrial localization. Consistent with the "Warburg effect" known in many cancer cells, LSTRA cells have reduced mitochondrial oxidative phosphorylation as shown by less oxygen consumption and less ATP production. Metabolomic study also revealed reduced levels of many metabolites in the citric acid cycle in LSTRA cells. It suggests that a global change of mitochondrial metabolism is linked to altered mitochondrial respiration in LSTRA cells. Because STAT5 binds to the transcriptional control region of circular mitochondrial DNA, we also examined the expression levels of mitochondrial-encoded transcripts of electron transport chain components. Real-time RT-PCR confirmed reduced expression of mitochondrial genes from all three promoters in mitochondrial DNA. All together, our results established a link from altered gene expression, to respiration, and finally to metabolism in LSTRA mitochondria. Non-canonical activity of Lck and STAT5 in mitochondria may contribute to metabolic shift in LSTRA leukemia and, potentially, other human cancers. (COI: No)

2P-483

Effects of chloride ion channel blocker on the adipogenic differentiation of rabbit ASCs

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Adipose tissue derived stem cells (ASCs) is known to have the potential into many kinds of functional cells. However, there are few studies about Cl^- channels of rabbit ASCs.

In this study, We isolated adipose tissue from rabbits and confirmed gene expressions of ASCs markers (CD44, SMA, Vimentin) with RT-PCR. Further, we visualized "lipid droplets (LDs)" in differentiated cells with fluorescent dyes, to evaluate this adipogenic differentiation. Large lipid droplets were found with adipogenic medium. Cl^- channel blockers (NPPB and DIDS) suppressed LDs accumulation and changed intracellular distribution of LDs. Rab8a, which regulates LDs fusion, was downregulated with NPPB. Thus, LDs fusion contributed by Rab8a might be inhibited.

Intracellular ClCs are known as Proton-Chloride exchanger rather than Cl^- channel and expressed in endosomes. Acidification of intracellular compartments primarily occurs through actions of the V-ATPase acting in parallel with a Cl^- conductance. We showed that ClC-5,6 expressions decreased after NPPB treatment.

These results imply Cl^- channel play a important roles in lipid droplets fusion through Rab8a of rabbit ASCs. (COI: No)

2P-484

ITAM receptors regulate two frequency components in calcium oscillations during osteoclastogenesis

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It has been known that RANKL induces Ca^{2+} oscillations mediated by ITAM costimulatory receptors, DAP12 and FcRgamma, resulting in osteoclastogenesis. Although Ca^{2+} oscillations are considered as essential factors for the RANKL-induced osteoclastogenesis in osteoclast precursors (OCPs), little is known how ITAMs mediate Ca^{2+} oscillations. In the present study, we developed a novel method to analyze the spectrum of oscillations, and demonstrated that each ITAM regulates two frequency components in Ca^{2+} oscillations during osteoclastogenesis. OCPs obtained from bone marrow cells in four phenotypes of mice were subjected to experiments; wild type (WT), FcRgamma-, DAP12-, and ITAMs-KOs. Ca^{2+} oscillations in OCPs were measured by Ca^{2+} imaging with Fura-2 and analyzed in the frequency spectrum using software R.

Spontaneous Ca^{2+} oscillations in the absence of RANKL in OCPs observed from all phenotypes mice. We decomposed Ca^{2+} oscillations in frequency domain, and separated frequency spectrum into high (HFR) and low (LFR) frequency range components. In OCPs from WT and FcRgamma-KO, RANKL induced HFR oscillations after 48 h stimulation. LFR peaks were detected in DAP12 or ITAMs-KO OCPs, but not in WT or FcRgamma-KO OCPs. RANKL stimulated osteoclastogenesis in OCPs from WT and FcRgamma-KO mice, but not from DAP12- and ITAMs-KO mice.

These results indicate that the fine tuning of RANKL-induced Ca^{2+} oscillations is necessary, especially HFR for osteoclastogenesis. (COI: No)

2P-485

RNF20/BRE1a regulates proliferation and differentiation of GBM cancer stem-like cells

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A quiescent slow-growing state of Glioblastoma multiforme (GBM) stem cells (GSCs) subpopulation in GBM is thought to underlie the tumor propagation, drug resistance, and relapse. Recent evidence indicates that GSCs share similar phenotypes with neural stem cells. However, the underlying mechanism of how epigenetic modification controls stemness features in GSCs remain poorly understood. In the previous report, a posttranscriptional modification of histone H2B monoubiquitylation (H2Bub1) mediated by the ring finger protein 20 (RNF20/BRE1a), a histone H2B E3 ubiquitin ligase plays a role in the maintenance of neural precursor cells and determination timing to promote neural differentiation. Therefore, we postulate that a strong relationship between genetic changes with epigenetic modification via RNF20/H2Bub1 may contribute to the maintenance of GSCs. The H2Bub1 expression positively correlated with the level of RNF20. The RNF20 over-expression exhibit higher mRNA expression of stemness (SOX2, OCT4) and GSCs (CD133) markers while RNF20 knockdown down-regulated SOX2. The proliferation rate of RNF20 over-expressing cells is much faster than the control. In contrast to the RNF20 over-expression, the RNF20 knockdown suppressed GSCs proliferation. Besides, the RNF20 over-expression is prone to promote astrocytic-differentiation in GSCs. Our findings suggest that RNF20 might be important as an epigenetic regulator in regulating proliferation and differentiation of GSCs. (COI: Properly Declared)

2P-486

Analysis of the mechanism regulating intercellular transport of silencing RNA in *C. elegans*

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Recent studies suggest that RNA is transported between cells and has a role in cell to cell communication. Molecular mechanisms that underlie the intercellular transport of RNA remain largely unknown. In the nematode, *Caenorhabditis elegans*, double-stranded RNA (dsRNA) introduced into cells or provided in diet can be spread throughout the body triggering RNA interference (RNAi) in cells distant from the cells where dsRNA is initially introduced. This is referred to as systemic RNAi and its process conceptually consists of secretion from donner cells and uptake into recipient cells of silencing RNA. To understand the molecular basis of systemic transport of silencing RNA, we conducted a genetic screen for mutants that have defects in RNAi triggered by ingested dsRNA. We have identified two genes from our screen. One is a gene encoding a protein that lacks known functional domains and has no homologs outside of nematodes. The other is a gene encoding a TBC domain containing protein, which is known to be a membrane trafficking regulator, indicating possible involvement of a certain membrane trafficking pathway in the transport of dsRNA. Future studies to elucidate the role of factors identified from our screen in the systemic RNAi may provide novel insights into the underlying mechanism of intercellular transport of silencing RNA. (COI: No)

2P-487

Swallowing reflex-inducible stimulations in rats

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Mechanical and chemical stimulations of the pharyngolaryngeal mucosa elicit reflex swallowing in humans and animals. The swallowing mechanism has not been clarified. Hence, we explored effective stimulations to elicit swallowing reflex in the pharyngolaryngeal region in rats. Adult male Wistar rats were used under anesthesia with urethane (1.3 g/kg, ip). Stimulating solution was applied to the pharyngolaryngeal region using an infusion pump. Swallowing movement was identified by EMG activity of the mylohyoid muscle and visual observation of the laryngeal movement. The number of swallows and latency from the onset of infusion to the first swallow were analyzed. Water stimulation elicited swallowing. NaCl solution at 0.15 M decreased the number of swallowing compared with water, but Na-gluconate solution at 0.15 M elicited swallowing as well as water. Cold or hot water did not affect the swallowing compared with water at room temperature. Carboxymethyl cellulose-containing solutions (0.1% and 1%) elicited swallowing as well as water. Mineral oil did not elicit swallowing, but mineral oil with 10 μM capsaicin or 30 mM AITC elicited swallowing. Olive oil did not also elicit swallowing, but extra virgin olive oil, known to contain TRPA1 agonist, elicited swallowing. These results suggest that water-induced swallowing reflex is suppressed by Cl^- in rats, and thermal, viscosity and oil-induced mechanical stimulations are ineffective to swallowing reflex. (COI: No)

2P-488

Intracellular Ca^{2+} source for SK channels in cartwheel cells of the mouse dorsal cochlear nucleus

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The intracellular Ca^{2+} is a crucial second messenger which modulates directly or indirectly ion channels in excitable cells, including neurons. Some of the modulations are mediated by the activation of Ca^{2+}-activated K^+ channels, resulting in the change of firing properties. We previously reported that the activation of large-conductance, voltage- and Ca^{2+}-activated K^+ channels by Ca^{2+}-induced Ca^{2+}-release (CICR) through ryanodine receptors can control the generation of burst firings in cartwheel inhibitory interneuron of the mouse dorsal cochlear nucleus (Irie and Trussell, 2017). In this paper, we also found unexpectedly that the CICR does not activate small-conductance, Ca^{2+}-activated K^+ (SK) channels of cartwheel cells. This observation contrasts with the Ca^{2+} signaling seen in the SK channel activation of other types of cells, raising the question how intracellular Ca^{2+} is supplied in order to activate SK channels in cartwheel neurons. To answer the question, I recorded SK currents from mice cartwheel cells using *in vitro* patch-clamp recording, and explored the Ca^{2+} source pharmacologically. I will present data on what channels/receptors are involved in the gating of the SK currents, and on how the activation contributes to the firing mode in cartwheel cells. (COI: No)

2P-489

Investigation into functions and molecular mechanisms of hesperetin on human cancer cells

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Hesperetin (3', 5, 7-trihydroxy-4'-methoxyflavanone), a compound found in citrus fruits, has been shown to have anticancer effects in previous in vivo and in vitro studies; however, the underlying molecular mechanisms remain unclear. The aim of this study is to elucidate the anticancer effects of hesperetin. Induced NO synthase (iNOS) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) have been linked with cell death, and glucose transporter 1 (GLUT1) is reportedly overexpressed in cancer cells. We analyzed the expressions of these molecules on hesperetin-treated cell lines, including human neuroblastoma GOTO cells, lymphoma U937 cells, and osteosarcoma U2OS cells. We used cell viability and DNA ladder assays and cell cycle analysis to analyze cellular functions and western blotting and immunocytochemistry to analyze protein expression. We found that hesperetin was antiproliferative and modified cell cycle activity in cancer cells. DNA ladder assay indicated that hesperetin did not induce apoptosis in U2OS cells. Western blotting suggested hesperetin-mediated changes in GAPDH and GLUT1 expression; in the GOTO cells, immunocytochemistry indicated localization of GAPDH in the nuclei of a subpopulation of iNOS-immunopositive cells. Therefore, hesperetin may exert antiproliferative effects via a GAPDH and GLUT1-mediated pathway that modifies cell cycle activity. (COI: No)

2P-490

STARD10 promotes lipid droplet formation cooperatively with LPCAT1

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[Purpose] Lipid droplet (LD) is surrounded by phospholipid monolayer mainly composed of phosphatidylcholine (PC). Lipid accumulation in hepatocyte associated with excessive LD formation can induce inflammation and fibrosis in the liver. STARD10 (steroidogenic acute regulatory protein-related lipid transfer domain containing 10) has been shown to transfer PC between membranes *in vitro*. We have previously shown that STARD10 is highly expressed in the liver and *Stard10*$^{-/-}$ mice accumulated less cholesterol and triglycerides when fed a high fat diet. The purpose of this study was to elucidate the role of STARD10 in LD formation.

[Methods and Results] We hypothesized that the size of LD should depend on the surface to volume ratio. Lysophosphatidylcholine acyltransferase1 (LPCAT1) catalyzes the conversion of lysoPC to PC. STARD10 was partly co-localized with LPCAT1 at the surface of LD in the mouse liver. We showed that STARD10 interacts with LPCAT1 in Hepa1-6 cells. Interestingly, the number of small LDs was increased by LPCAT1 overexpression, while the number of large LDs was increased by the overexpression of both STARD10 and LPCAT1. Next, we analyzed livers of WT mice, *Stard10*$^{-/-}$ mice and *Lpcat1*$^{-/-}$ mice fed high-fat diet. We found that single LD size and total LD area were smaller in *Stard10*$^{-/-}$ and *Lpcat1*$^{-/-}$.

[Conclusions] STARD10 and LPCAT1 are involved in LD formation by regulating surface to volume ratio through its interaction and its activity of PC transfer and synthesis. (COI: No)

2P-491

ATP dependent H+transport in endoplasmic reticulum membrane

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It is thought that the pH regulation of endoplasmic reticulum (ER) is important for its function, e.g. Ca^{2+}transport, protein synthesis. However little has been known about pH regulation in ER. To investigate the change of pH in ER responding to various stimulations, we modified genetically encoded fluorescent ratiometric pH indicator, mCherrySEphluorin (*Koivusalo M et al. J Cell Biol. 188(4):547-63,2010*) to localize in ER lumen and performed pH imaging in isolated nuclear envelopes (containing peri-nuclear ER membrane) from the probe-expressing HEK293 cells.
It is known that SERCA concomitantly exports 2 or 3 protons from ER with importing one $Ca^{2+}$to ER lumen. So, we explored whether the pH changes in the ER with SERCA activations. Surprisingly, it was indicated that pH was dropped with ATP stimulations. Moreover the ATP dependent lowering of pH had thapsigargin resistant property. And this phenomenon was observed in bafilomycinA existing conditions. These results indicated that the change of pH is SERCA and vacuolar type ATPase independent.
These experiments were carried out in KCl solution. When the potassium was exchanged with $NMDG^+$, the lowering of pH with ATP application was diminished. The changing of pH could be cytosolic K^+ion dependent at least in part. The mechanism is not yet clear, our finding is possibly novel ATP dependent H^+transport in ER membrane. (COI: Properly Declared)

2P-492

Highly localized pH sensing on the outer membrane of cells using surface enhanced Raman spectroscopy

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Regulation of intracellular pH is critically important for many cellular functions. The quantification of proton extrusion in different physiological conditions is pivotal to fully elucidate the mechanism of pH homeostasis in living cells. Here we show the use of gold nanoparticles (AuNP) to create a high spatial resolution sensor for measuring extracellular pH in proximity of the membrane proteins of different types of cells. The conjugation of AuNP was specifically designed to efficiently target the membrane surface proteins of the cells and to measure pH using surface enhanced Raman spectroscopy (SERS). Experimental data based on laser scanning microscopy (LSM), SERS and transmission electron microscopy (TEM) clearly proved that we succeeded in attaching the nano-pH-sensor to the cell outer membrane surface. Measurements of surface pH were obtained from HepG2 human liver cancer cells, MKN28 gastric cancer cells, A549 lung cancer cells, human bronchial ciliary cells and human epidermal keratinocyto. The nanometer size of the AuNP sensor attached to the proteins and the use of SERS enabled us to visualize highly localized variation of pH induced by H^+ and/or HCO_3^- transport and to unfold the dynamics of pH homeostasis in several types of cells exposed to different treatments and/or different physiological conditions. (COI: No)

2P-493

High-level of homocysteine alters cell viability of endothelial cell and Müller cell

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Purpose: To investigate the effects of homocysteine on vascular endothelial cells and Müller cells, we established in vitro endothelial cell and Müller cell models to examine the possible mechanisms involved in homocysteine-evoked cellular changes. Methods: Human vascular EA hy926 endothelial cell and QNR/K2 Müller cell were cultured with different concentrations of homocysteine. The effects of homocysteine were studied by examining cell viability, cell apoptosis, and DNA fragmentation. The expression of signal transduction molecules were also evaluated by Western Blot and immunofluorescence staining. Results: In certain range of concentrations, homocysteine evoked vascular capillary sprouting by inducing endothelial cell proliferation. In some cases, however, cell viability was reduced in vascular endothelial cells and Müller cells when cultured with different concentrations of homocysteine.DNA fragmentations was also significantly increased after cultured with homocysteine in these cells too. The mechanisms were slightly different between endothelial and Müller cells, but then confirmed later that homocysteine caused cell apoptosis via activating caspase 3 and caspase 8 pathways in these cells. Conclusion: High level of homocysteine reduced cell viability, promoted DNA fragmentation, and induced cell apoptosis via caspase pathways in vascular endothelial cells and Müller cells. (COI: No)

2P-494

Expression of Mechanosensitive Ion Channel in Osteoblasts

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[Introduction]
Mechanical stress is an important regulatory factor in bone homeostasis. Although mechanical stimulation to osteoblasts elicits an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), their detailed mechanism of the mechanosensitive processes remains unclear. The present study investigated the biophysical and pharmacological properties of direct mechanical stimulation-induced $[Ca^{2+}]_i$ response in osteoblasts.
[Method]
Mouse osteoblast-like cells MC3T3-E1 were cultured for 12 to 24 hours in 5 % CO_2 at 37 ℃, and loaded with Ca^{2+} fluorescent indicator for 1 hour. The standard extracellular solution was Krebs solution, and we measured $[Ca^{2+}]_i$ responses during the following three types stimulations. 1) direct mechanical stimulation with a glass micropipette, 2) direct mechanical stimulation in the presence of Gd^{3+} , or 3) in the presence of GsMTx4.
[Result]
When direct mechanical stimulation was applied, $[Ca^{2+}]_i$ was increased and showed significant desensitizing effects. Extracellular Gd^{3+} and GsMTx4 reversibly inhibited mechanical stimulation-induced $[Ca^{2+}]_i$ increases. When the concentration of Gd^{3+} or GsMTx4 were changed, the increases of $[Ca^{2+}]_i$ changed depending to their.
[Discussion]
Gd^{3+} and GsMTx4 are inhibitors of mechanosensitive ion channels, and significantly suppressed $[Ca^{2+}]_i$ increases induced by direct mechanical stimulation of osteoblasts. Mechanosensitive ion channels might be involved in the mechanosensitive processes of osteoblasts. (COI: No)

2P-495

Exploratory search for therapeutic target genes to cure MELAS using CRISPR activation

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[Purpose] Mitochondrial disease is caused by dysfunctional mitochondria, and show various symptoms including skeletal muscle and central nervous system disorders. The disease is recognized as intractable diseases, and development of the effective treatments is required. Among mitochondrial diseases, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) has the largest patient number. Thus, the aim of this study is to conduct a screening to search for therapeutic target genes to cure MELAS.
[Methods] We utilized a genome-wide transcription activation screening using the CRISPR-Cas9 system, targeting 23,430 human genes. MELAS patient-derived 2SD cells were used to screen therapeutic target genes of MELAS. The cells have an A to G mutation at the position 3243 of the mitochondrial DNA, which is present in 80% of MELAS patients. Unlike wild-type cells, 2SD cells died when grown under low glucose conditions due to mitochondrial dysfunction. Therefore, we used a screening system for CRISPR-activated genes that enable 2SD cells to survive under low glucose condition to identify the therapeutic target genes.
[Results] To prepare for the screening, we set up CRISPR activation experimental system and confirmed that we can upregulate desired genes. We also optimized glucose concentration to be used for 2SD cells.
[Conclusions] We are now ready to perform our screen for therapeutic target genes to cure MELAS. (COI: No)

2P-496

The effect of benzodiazepine on proliferation and survivals of CNS cells

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Benzodiazepines (BZDs) are commonly prescribed as anxiety, epileptic discharge, insomnia, muscle-relaxing, sedatives, and anti-convulsants. BZDs increase the conductance of $GABA_A$ receptor ($GABA_AR$) and promote a state of CNS depression. Recent studies raised concern about the safety because some clinical reports have shown that chronic BZD treatment increases the risk of dementing disorder in aged patients. Additionally, it was reported that the proliferation and survival of CNS cells was impaired by BZD treatment, and cognitive function was attenuated. However, detail mechanism of BZD effect on proliferation and survivals of CNS cells were still unknown. In this study, the effect of BZD treatment on proliferation and survival of BV2 cells, microglia-like cell line, was investigated. The proliferation of BV2 was impaired but survivals was not affected by DZP (a benzodiazepine) treatment. The BZD-induced impairment of BV2 proliferation was not affected by bicuculine-methiodide, $GABA_AR$ antagonist. These results suggested that DZP impaired BV2 proliferation without involvement of $GABA_AR$ activities. We discuss about the effect of other several BZDs (lorazepam or midazolam) on proliferation and survival in other neuronal or astroglia-like cell line. (COI: No)

2P-497(Y-34)

The impact of DNA methyltransferase 3A in erythrocytic differentiation

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The continuous production of erythrocytic cells is important for maintenance of homeostasis. Previous studies identify the importance of DNA methylation in the plasticity and lineage specification of hematopoietic cells as well as in globin gene expression. However, which one of DNA methyltransferases (DNMTs) playing a critical role in erythropoiesis is still unclear. Previous efforts on identifying genomic alterations in acute myeloid leukemia (AML) demonstrate *DNMT3A* mutations with biological, clinical and potential therapeutic relevance in AML and other hematopoietic malignancies. Our COSMIC database mining also find that DNMT3A mutations are predominant, however, mutations in other DNMTs are rare in leukemia patients. Thus, we were interested in whether DNMT3A exhibit a particular role in erythrocytic differentiation. Our cell models demonstrated the increasing of DNMT3A expression in erythrocytic differentiation. Knockdown of DNMT3A further promoted differentiation. Due to the importance of MAPK in erythrocytic differentiation, we found that DNMT3A can be co-immunoprecipitated with ERK1/2. Our results not only showed the essential role of DNMT3A in erythrocytic differentiation, but also linked the MAPK pathway and DNMT3A. Further studies are ongoing to study the *in vivo* role of DNMT3A, and the impact of ERK1/2 in DNMT3A function in erythropoiesis. (COI: No)

2P-498

Calcium response in human synovial cells induced by shear stress in normal and rheumatoid arthritis

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Purpose: Previous studies have reported that calcium-release activated calcium channel (CRAC) is involved in the inflammatory response of synovial tissue in rheumatoid arthritis (RA). However, its mechanism has not been clarified. In the present study, we focused on the contribution of CRAC to the response of human synovial cells to shear stress (SS).
Methods: Experiments were conducted using human synovial cells in normal and RA. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of synovial cells was measured with loaded Fluo-3 AM by adding twice SS with nitrogen gas ejected from a glass micropipette.
Results: Each SS elicited immediately $[Ca^{2+}]_i$ increasing response in the presence of extracellular Ca^{2+}. When the CRAC inhibitor (YM-58483) was administered, the ratio of the second one to the first response in synovial cells in RA were significantly smaller than that in normal.
Conclusions: The result indicated that CRAC was involved in $[Ca^{2+}]_i$ increasing response with SS in human synovial cells more dominantly in RA. In the future we will examine the involvement of $TRPV_4$ channel. (COI: No)

2P-499

Relationship between dehydration and amount of drinking water before shifts : a preliminary study

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Dehydration has risks of reducing cognitive function, attention, and concentration. Because shift worker needs to pay attention to their works, it is necessary to determine how they are dehydrated before their shifts to reduce the risks and improve work environment. However, there has been no study to determine the dehydration state of shift workers before starting their morning shifts. This study aimed to determine the hydration state before starting the morning shifts. Urinary specific gravity was measured in 33 nurses at 8 o'clock before starting the morning shifts. The nurses were considered to be in a dehydration state when the urine specific gravity exceeded 1.020. Additionally, body weight was surveyed and followings were asked by a questionnaire: about the amount of water consumed, if breakfast was taken before the shift, and if alcohol was consumed the previous night. This study was conducted according to the protocol approved by the Ethical Committee of the Graduate School of Nursing, Chiba University. In total, 19 nurses (54%) were dehydrated before their shifts and 14 nurses (46%) were well-hydrated. The dehydration group had taken significantly lesser water before their shifts than the non-dehydration group ($p = 0.03$). For other variables, significant differences were not observed. We conclude that over half of nurses starts their shift in a dehydration state, and we recommend drinking water before shifts to prevent this dehydration state. (COI: No)

2P-500(Y-35)

Hearing status of Rickshaw's drivers in Karachi, Pakistan assessed by Pure tone audiometry

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Workplace health is a key concern now a days and excessive exposure to occupational noise results in loss of hearing and is now a recognized professional hazard. The aim of our study is to assess the hearing status of the rickshaw drivers of Karachi using pure tone audiometry to assess the impact of noise pollution. In this cross-sectional survey, a total of 256 subjects including 128 control and 128 rickshaw drivers with minimum experience of 1 year were selected and audiometry was performed on them. Data gathered through structured questionnaire after consent followed by history and local examination to rule out presence wax or any abnormality like perforation in tympanic membrane. The average age of the rickshaw drivers was 46.25±15.20 years. Among them, hearing impairment was observed in 87.5% (112/128) drivers. Hearing impairment was significantly associated with age groups, experience and use of protective device. From our results it is concluded that most of the public transport drivers are suffering from noise induced hearing loss. This hearing impairment among the rickshaw drivers may jeopardize their safety as they may not be able to hear warning signals or horns from other vehicles on the roads thus leading to road traffic accident. It is suggested that government and regulatory bodies should take necessary actions in reducing noise pollution as it may decrease the probability of road traffic accidents secondary to impaired hearing among drivers. (COI: No)

2P-501

A corticohypothalamic neural pathway that drives sympathetic responses to psychological stress

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Psychological stress causes a variety of sympathetic responses in mammals, which help the body react quickly and effectively to the stressor. We have reported that stress induces thermogenesis in brown adipose tissue (BAT), hyperthermia and tachycardia by activating a monosynaptic neural pathway from the dorsomedial hypothalamus (DMH) to the rostral medullary raphe (rMR). Recently, we found that the ventral medial prefrontal cortex (vmPFC) is a major source of the stress signals to the DMH, by performing *in vivo* physiological, optogenetic and neuroanatomical experiments. Furthermore,selective ablation of vmPFC-DMH projecting neurons eliminated BAT thermogenesis and hyperthermia induced by social defeat stress, a sociopsychological stress model. These results indicate that the vmPFC-DMH monosynaptic excitatory pathway provides the DMH with stress signals to activate the DMH-rMR sympathoexcitatory pathway driving sympathetic stress responses. In addition, we sought for the upper neurons that provide psychological stress signals to the vmPFC to elicit sympathetic responses. Rats exposed to social defeat stress exhibited increased expression of Fos, a marker for neuronal activation, in vmPFC-projecting neurons in brain regions including piriform cortex layer 2 and paraventricular thalamic nucleus. These upper brain regions likely provide psychological stress signal inputs to the vmPFC for the drive of sympathetic and possibly behavioral responses. (COI: No)

2P-502

Expanded plasma volume after a bout of exercise increases erythropoietin secretion to hypoxia

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Purpose: Erythropoietin (EPO) secretion is a critical determinant for hematological adaptation to hypoxia, which is associated with the level of hypoxemia. Plasma volume (PV) expansion and the resultant hemodilution are observed after a bout of exercise especially in warm conditions. Therefore we tested the hypothesis that EPO secretion to hypoxia was enhanced with an expanded PV after a bout of exercise in a warm condition. Methods: Eight healthy young men underwent two identical trials which differs only ambient temperature (Ta) during exercise. Baseline blood sample was taken in a thermoneutral condition, then subjects performed a 72-min intense-intermittent exercise (8 sets of 4 min at 80% VO2max - 5 min at 20% VO2max) under cool (Ta of 20˚C) and warm (Ta of 30˚C) conditions, 23 hours after exercise, they exposed to hypoxia (14.4%) for 3 hours and blood sample was taken at the end. We determined percent change in PV (%ΔPV) from baseline and serum EPO. Esophageal (Tes) and mean skin temperature (Tsk) were measured during exercise. Results: During exercise, Tsk were higher ($p < 0.05$) in warm than cool trial while Tes was comparable between trials. %ΔPV at 23 hours after exercise was higher in warm than cool trial ($p < 0.05$). Serum EPO increased in both trials and we found significant effects of interaction (trial x time) in serum EPO. Conclusions: EPO release to hypoxia was enhanced when PV was expanded after a bout of exercise especially in a warm condition. (COI: Properly Declared)

2P-503

The effect of aging on event-related potentials during mild-hyperthermia

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Purpose: The cognitive function is generally decreased with healthy aging. It has been reported that the cognitive processing evaluated by using electroencephalographic event-related potentials (ERPs) is decreased with heat stress in young people while remained unknown in elderly people. We tested the hypothesis that the cognitive processing would be decreased prominently in the elderly compared with the youth during mild hyperthermia. Methods: Five elderly and 11 young healthy men performed auditory oddball paradigms under normothermia (NT) and mild hyperthermia (HT) with esophageal temperature increased by ~1.0˚C attained by passive heating with lower legs immersion of 42˚C and with water perfusion suits of 40˚C. During both thermal conditions, after baseline measurement, the reaction time and the latency and amplitude of P300 to the auditory oddball paradigms were measured. Results: The reaction time was decreased during HT compared to NT ($P < 0.05$) while the latency remained unchanged with heat stress in both age groups. We found a significant effect of interaction (age x thermal condition, $P < 0.05$) in the amplitude which increased in the young group while decreased in the elderly group with heat stress, and the amplitude during HT was lower ($P < 0.05$) in the elderly than the young group. Conclusions: The cognitive processing evaluated by ERPs decreased in the elderly than in the young subjects during mild hyperthermia. (COI: No)

2P-504

Thermosensory changes in heat resistant tadpoles of Ryukyu kajika frogs inhabiting hot springs

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Temperature is a critical environmental factor for organisms, and extreme heat or cold causes detrimental effects. However, some of the species acquired resistance to harsh thermal environments and thereby occupying the niches where most other species are unable to utilize. Tadpoles of Ryukyu kajika frog (*Buergeria japonica*) possess extreme heat resistance and even inhabit geothermal hot springs, thereby suitable for examining the molecular basis of thermal adaptation processes to such extreme environments. In the present study, we focused on the role of thermosensory system in the acquisition of heat resistance in *B. japonica* by examining their behavioral responses to heat. Upon exposure to a heat ramp, *B. japonica* tadpoles tolerated high temperature up to about 42 degrees C and, above that temperature, they showed an abnormal swimming behavior. We then characterized thermal property of TRPV1 and TRPA1 channels which serve as heat sensors by electrophysiological approaches. Both channels showed reduced heat responses to heat stimulation although their channel functions were retained. All these observations suggested that *B. japonica* tadpoles reduced thermal sensitivity to noxious heat, which enables them to reside in extreme thermal environments such as hot springs. Therefore, the evolutionary change in thermosensory system partly contributed to the acquisition of heat tolerance in *B. japonica*. (COI: No)

2P-505

Influence of combined stimulus of cold, hypoxia and dehydration status on thermoregulation in rats

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Ambient temperature (T_a) and arterial O_2 content decreases with an increasing of altitude. Cold exposure induces peripheral vasoconstriction, while acute exposure to hypoxia has been demonstrated to result in vasodilation to prevent hypoxaemia. Therefore, cold-induced vasoconstriction and hypoxia-induced vasodilation may cause counteractive responses at high-altitude. Additionally, heat dissipation caused by fluid redistribution is important in thermoregulation, suggesting that dehydration status may also play an important role to maintain core body temperature (T_{core}). We sought to investigate combined effects of cold, hypoxia, and dehydration status in rats. Wistar rats were exposed under eight conditions for 24h each; T_a (24°C or 10°C) × oxygen (21% or 12% O_2) × hydration (euhydration or dehydration). Normoxia (21% O_2) with euhydration did unchanged T_{core} irrespective of T_a. However, hypoxia (12% O_2) with euhydration decreased T_{core} both 24°C and 10°C; moreover, this decreasing rate of the T_{core} was accentuated at 10°C compared to 24°C. In normoxia at 24°C, dehydration status did not affect T_{core}. Conversely, dehydration at 10°C with normoxia caused a lower trend in the T_{core} compared with euhydration. Furthermore, dehydration in hypoxia enhanced reductions in the T_{core} at both 24°C and 10°C compared with euhydration. These results suggest that dehydration status may accentuate the decreases in the T_{core} under low T_a and hypoxia. (COI: No)

2P-506

Possible central mechanism of acquired heat tolerance in exercise-trained rats

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In humans and rodents, chronic heat exposure and/or exercise training have been well known to induce heat acclimation that improves heat tolerance. We have previously reported that heat exposure promotes progenitor cell proliferation and neural differentiation in the hypothalamus and the inhibition of hypothalamic neurogenesis impaired the ability of heat tolerance in rats. The aim of the present study was to investigate whether exercise training induces hypothalamic neurogenesis in rats. Male, 6-week-old Wistar rats were initially maintained at an ambient temperature of 24 °C for 10 days. They were then kept for 30-40 days in cages either with a running wheel (exercise rats, EX) or with a locked wheel (control rats, CN). Bromodeoxyuridine (BrdU) was intraperitoneally injected daily for 5 consecutive days after commencing exercise training. After the training period, rats' brain was used for immunohistochemical analysis. The numbers of BrdU-immuno-positive (BrdU+) cells in the hypothalamus of EX were significantly greater than that of CN. Moreover, the number of hypothalamic BrdU+ cells double-stained with neuronal differentiation marker, doublecortin, of EX were significantly higher than that of CN. These results suggest that exercise training facilitates proliferation of neuronal progenitor cells and promotes differentiation into neurons in the hypothalamus, which might have a certain role in acquired heat tolerance of exercise-trained rats. (COI: No)

2P-507

Estimation of basal body temperature from breast skin temperature during sleep

Shuri Marui; Kei Nagashima (*Faculty of Human Sciences, Waseda University, Japan*)

Background The menstrual cycle changes arising from the basal body temperature are a key marker of health in women of reproductive age, yet it remains difficult to measure accurately and easily during sleep. This study measured three points of breast skin temperatures during sleep to estimate the basal body temperature.
Methods Data were collected from eleven healthy young women. Three points of breast skin temperatures (i.e., lower left breast, side of left breast, and upper left breast) were measured every 15 minutes during sleep using three temperature sensors attached to clothing and sublingual temperature (i.e., basal body temperature, BBT) was measured in the morning using a 10-second digital thermometer. Both the breast skin and sublingual temperatures were measured every day throughout the menstrual cycle.
Results The temperature of the lower left breast was higher than the temperatures of the side and upper left breast among all subjects. The average temperature of the lower left breast until two hours before waking up was significantly associated with the BBT. The average temperature of the lower left breast also demonstrated the biphasic pattern of the BBT. However, the average temperatures of the side and upper left breast were not associated with the BBT.
Conclusion It was demonstrated that the BBT could be estimated from the temperature of the lower left breast. (COI: No)

2P-508

Wearable patch-type sensors for core temperature monitoring by a modified dual-heat-flux method

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Wearable technology is now being adopted, but a system that accurately measures core temperature using wearable devices has yet to be reported. We propose a model based on a dual-heat-flux method that predicts core temperature using data from patch-type sensors on the chest. We performed experiments that compared our predicted temperatures (T_{pre}) with actual temperatures, both esophageal (T_{eso}) and rectal (T_{rec}), during exercise in hot conditions. Eighteen males walked for 60 min at 5 km/h (at 35°C, 50% RH); nine subjects wore standard work uniform and nine wore protective clothing.
In the work uniform group, T_{eso}, T_{rec}, and T_{pre} increased from 37.1 ± 0.2°C, 37.0 ± 0.2°C, and 37.1 ± 0.3°C to 37.9 ± 0.1°C, 37.9 ± 0.1°C, and 37.9 ± 0.1°C (mean ± SD), respectively, during exercise. The difference between T_{pre} and T_{eso} was 0.01 ± 0.18°C and that between T_{pre} and T_{rec} was 0.26 ± 0.26°C, using data sampled at 5-minute intervals during exercise. In the protective-clothing group, T_{eso}, T_{rec}, and T_{pre} increased to 38.2 ± 0.2°C, 37.9 ± 0.3°C, and 38.0 ± 0.2°C after exercise. In this case, the difference between T_{pre} and T_{eso} was –0.20 ± 0.23°C and that between T_{pre} and T_{rec} was 0.30 ± 0.31°C.
Error ranges for our model are similar to those in previous studies involving noninvasive core temperature measurements. T_{pre} values during exercise tended to be lower than T_{eso} values and higher than T_{rec} values. (COI: No)

2P-509

Operant behaviors affected by warm ambient temperature are task-dependent and hippocampus involved

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Thermal stress from the change of ambient temperature (Ta) has an impact on behavioral function, however, little is known about how the increased Ta may affect operant conditioned behavior and its underlying neural mechanisms. We investigated the effects of high Ta on operant behaviors maintained on a fixed-ratio 1 (FR1) and a differential reinforcement for low-rate responding 10 second (DRL 10-sec) schedule of reinforcement. The rats were randomly assigned to three groups receiving the acute exposure to Ta of 23 °C, 28 °C, and 35 °C, respectively, for behavioral tests. The results showed that total responses of FR1 behavior were decreased only under 35 °C as compared to the control of 23 °C; but those of DRL 10-sec behavior was significantly reduced in the groups of 28 °C and 35 °C. Distinct patterns of inter-response time (IRT) distribution of DRL behavior appeared among three groups which between-group differences of behavioral changes produced by high Ta were confirmed by quantitative analyses of IRT data. Via western blot assays of dopamine D1 and D2 receptor, dopamine transporter (DAT) and brain-derived neurotrophic factor (BDNF), significant Ta-related effectiveness was only revealed in the hippocampus: D1 receptor increased in 28 °C group and DAT increased in 35 °C group. Together, these data indicate that the performance of operant behavior affected by high Ta is task-dependent which may be regulated by D1 receptor and DAT in the hippocampus. (COI: No)

2P-510

The effect of environmental temperature on spontaneous exercise in mice

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Introduction

When humans are asked to run with a similar subjective intensity (i.e. self-paced exercise), the objective intensity of the exercise decreases in heat. However, the mechanism remains unclear yet. In the present study, we aimed to establish mice model for the self-paced exercise in heat to clarify the mechanism.

Methods

Male mice (n=13, age of 7 w, C57BL/6J3) were used. IC-chip sensors for temperature and acceleration was placed in the abdominal cavity. Mice were individually housed at an ambient temperature (T_a) of 25°C During experimental period, a mouse was placed in a semi-closed chamber with a running wheel, where abdominal temperature (T_{abd}), counts of movement, rotation number of wheel, and metabolism by indirect calorimetry was continuously determined. And, a mouse was exposed to heat at 33°C (Hot trial) or remained at 25°C (Control trial) at the time of day of 19:00-1:00.

Results

The number of wheel rotation decreased in the Hot trial than in the Control trial between 19: 30-1:30 (Hot: 14 ± 30 vs Control: 273 ± 21), counts of movement was significantly lower in the hot trial. T_{abd} was significantly lower in the Hot trial between 20:30 and 1:30 (Hot: 36.9 ± 0.4 vs Control: 37.9 ± 0.8°C).

Conclusions

The number of wheel rotation and counts of movement of mice decreased in heat; although T_{abd} decreased. Therefore, the suppression of exercise observed in the present study may be induced by thermal signals from the body surface. (COI: No)

2P-511

Function of polyunsaturated fatty acid in thermoregulation

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Thermoregulation is crucial for maintenance of homeostasis in animals, but the molecular mechanism of thermoregulation was not completely understood. To reveal the regulatory mechanism of thermoregulation, we focused on the polyunsaturated fatty acid (PUFA) that is supposed to play the important role in neuronal functions by modulating the fluidity of cellular membrane as well as by producing PUFA-derived signaling molecules.

First, we found that supplementation of culture medium with linoleic acid (18:2) caused a decrease in preferred temperature in *Drosophila melanogaster.* Next, to manipulate the linoleic acid content in a tissue specific manner, we generated a transgenic line of *Drosophila* expressing *Caenorhabditis elegans* Δ12 fatty acid desaturase (FAT-2) which catalyzes the production of linoleic acid from oleic acid (18:1). By neuron-specific expression of FAT-2 using GAL4/UAS system, the content of linoleic acid was increased in the central nervous system and the preferred temperature was decreased. By genetic screening, we identified the thermosensitive neuron whose responsiveness to temperature change was altered by the ectopic expression of FAT-2, resulting in the decrease in preferred temperature of the FAT-2 expressing *Drosophila*.

These results suggest that PUFA especially linoleic acid is one of the determinants of preferred temperature via modulating the activity of thermosensitive neurons in *Drosophila.* (COI: No)

2P-512

Cold induced sleep-related sympathovagal imbalance and sleep fragmentation in rats

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Purpose: Many cardiovascular disease events occur before morning awaking and are also more frequent in winter. Based on this, we hypothesized that a cold temperature impairs sleep quality causing an autonomic imbalance towards the sympathetic side and a loss of the wake-to-sleep-related depressor response. These events might produce an increase in cardiovascular events.

Methods: Polysomnographic recordings were performed on freely moving Wistar-Kyoto rats that were housed in thermo-regulated chambers at one of three different ambient temperatures. Three ambient temperature ranges used in this study were: 15 °C, 18 °C, 23 °C; one of these temperature was randomly selected.

Results: Lower temperatures of 18°C and 15°C led to a higher blood pressure (BP) and to higher vascular sympathetic activity, lower RR, lower parasympathetic activity and lower baroreceptor reflex sensitivity (BRS) during sleep together with more interruptions and a lower delta power of sleep. Sleep-related lower BP and vascular sympathetic activity in association with higher RR, parasympathetic activity, and BRS were significantly impaired at lower temperatures. Core temperature declined during sleep, but there were no significant differences among three different ambient temperatures.

***Conclusions*:** Cold temperature impairs sleep quality and BRS and results in an autonomic imbalance towards the sympathetic side; this may play an important role in the cold-related increased frequency of cardiovascular events. (COI: Properly Declared)

2P-513

A mouse model that can evaluate fever and hyperalgesia due to peripheral inflammation

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[Purpose] Inflammation is associated with fever and hyperalgesia. These reactions involve the centrally produced prostaglandin E_2 (PGE_2) as a common mediator. PGE_2 is produced using arachidonic acid (AA) as an initial material. In 2011, it was demonstrated that monoacylglycerol lipase (MGL) rather than phospholipase A_2 is involved in the AA supply in neuroinflammation. In this experiment, we aimed to establish a mouse model that can evaluate both PGE_2-dependent fever and hyperalgesia as a basis for investigating the involvement of MGL in these responses.

[Methods] Zymosan was subcutaneously injected into the plantar of the hind paw of wild mice. Subsequently, we recorded their abdominal temperature under free moving state. In a separate group of mice, brains were sampled to measure brain cyclooxygenase-2 (COX-2) and PGE_2 levels.

[Results] Administration of zymosan caused swelling of the hind paw and elevated body temperature in the light period. COX-2 and PGE_2 levels in the brain were elevated. Expression of COX-2 in cerebrovascular endothelial cells was confirmed by immunostaining.

[Conclusion] Subcutaneous injection of zymosan into a mouse hind paw seems to be a model suitable for studying central PGE_2-dependent fever and hyperalgesia due to peripheral inflammation. In the future, we will study the involvement of MGL in fever and hyperalgesia using this model. (COI: No)

2P-514

Induction of long-term torpor by enhancing the adenosine receptor signal via PPARs activation

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The phenomena of a depression in metabolic rate like daily torpor and hibernation are broadly seen among mammals. Therefore, it is suspected that many homeothermic species retain the ability of decreasing metabolism and body temperature (Tb). To investigate the mechanism for such metabolic regulation, some pharmacological methods to induce artificial reversible torpor (called synthetic torpor) is developing in laboratory animals. Among them, N^6-Cyclohexyladenosine (CHA), the adenosine A1 receptor agonist, is a strong candidate to induce the torpor state. However, since the effect of CHA with single dose is transient, developing new methods for long-term synthetic torpor is required. In this study, we report a new method by the treatment of CHA combined with peroxisome proliferator-activated receptor (PPAR) agonists. In our protocol, mice were fed with diets supplemented with either bezafibrate or pioglitazone, PPARα and γ agonists respectively, for 10 days, and then CHA was intraperitoneally injected. Tb was measured using the telemetric system in which devices were implanted in the peritoneal cavity of mice. Both bezafibrate and pioglitazone treatments prolonged CHA-induced hypothermia at an ambient temperature of 23 °C. In conclusion, PPARs activation by the agonists prolongs the CHA-induced hypothermia through the enhancement of the adenosine A1 receptor signaling pathway. (COI: No)

2P-515

Involvement of the vagus nerve in autonomic thermoregulation responses induced by TRPM8 agonist

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Purpose: To elucidate whether TRPM8 expressed in nerves besides the cutaneous sensory nerves, such as the vagal afferent nerve, is also involved in thermoregulation, we aimed to investigate whether TRPM8 expressed in the vagal afferent nerve is involved in the changes in body temperature induced by ingestion of TRPM8 agonist.

Methods: Male mice (C57BL/6, 7wks) were anesthetized with urethane (1.4 g/kg) and thermistors were set on the tail skin, colon, and intrascapular brown adipose tissue (IBAT). A cannula was then inserted into the stomach or jugular vein to administer the solution. The temperature was measured for 10 min before administrating the 1,8-cineole or vehicle and for 180 min after administration. M8-B was intraperitoneally administered as TRPM8 antagonist at 30 min before the agonist administration. Vagotomy was conducted at just below the diaphragm.

Results: Intragastric administration of 1,8-cineole increased IBAT and colonic temperatures, and M8-B-treatment inhibited these responses. Intravenous administration of 1,8-cineole also showed similar effects to those by intragastric administration. In vagotomized mice, the responses induced by intragastric administration of 1,8-cineole were attenuated.

Conclusions: Vagus nerve is involved in the changes in body temperature induced by ingestion of TRPM8 agonists via TRPM8. (COI: No)

2P-516

Aurelia Aurita venom evoke hyperpolarization and SOCS1 expression in toad urothelium membrane

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Aurelia Aurita venom as the membrane pore-forming peptides may involve in the urothelium permeability and cellular membrane functions. In modified Ussing chamber, membrane was initially activated by Ca^{2+} in serous layer, further treated by *Aurelia Aurita* crude venom or the *CfTX-1* analogues peptide respectively. Urothelium layer potential variations were recorded by unipolar lead and computerized by fast Fourier transform technique. The SOCS1 redistributions were observed by membrane centrifuge artificial gravity strain method. Ca^{2+} caused hyperpolarization on urothelium apical side. However this can be blocked by Nifedipine. Crude venom and *CfTX-1* analogues peptide evoked this peak. 2) *CfTX-1* analogues peptide enhanced this hyperpolarization under Cl^- symporter blocker intervene, which suggested hyperpolarization were the Cl^- inflow from serous side flow into urothelial apical side. 3) Muscarinic receptor antagonist prompted *CfTX-1* analogues peptide induced hyperpolarization, in which G-protein coupled cellular signaling were involved in this hyperpolarization. 4) Centrifuge artificial gravity strain (10g, 10min) not influenced *CfTX-1* analogues peptide induced hyperpolarization. 5) Crude venom but not *CfTX-1* analogues peptide generated a considerable up regulation SOCS1 expression in membrane. As the conclusions, *CfTX-1* analogues peptide were membrane potential effective type compound, while crude venom was cellular signaling regulation type compound.

(COI: No)

2P-517

Withdrawn

2P-518

Seasonal differences in cardiac autonomic nervous activity during exercise in obese men

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Light is the strongest synchronizer controlling circadian rhythms. The intensity and duration of light changes throughout the year, thereby influencing body weight, food preferences, and melatonin secretion. Obesity is a significant health problem in the world and it has been rapidly increasing over the past two decades. Obese people have an increased risk factors for developing cardiovascular, renal, and hormonal diseases and sleep disorders. These incidences were increased in winter season in cold environmental temperature and during short days. In this study, we investigated that seasonal differences of cardiac autonomic nervous activity during exercise in obese and non-obese subjects. Six of non-obese men and 5 of obese men participated in summer and winter. Electrocardiogram (ECG) was recorded continuously using a standard device. The R-R intervals were analyzed by spectral analysis using the maximal entropy method for cardiac sympathetic nervous activity (LF/HF) and cardiac parasympathetic nervous activity (HF). Heart rates were increased during pre-exercise and exercise in winter than in summer in obese. LF/HF components in pre-exercise were increased in summer than in winter in non-obese. Heart rates were increased in obese subjects in winter season, suggesting that exercise in winter season may increase heart rates in obese subjects. (COI: No)

2P-519

PAI-1 is crucial in osteoblastic differentiation of mesenchymal stem cells

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[Purpose] Plasminogen activator inhibitor-1 (PAI-1) is known as an inhibitor of fibrinolytic system. We reported that PAI-1 is involved in the pathogenesis of osteoporosis in mice. It was reported that mesenchymal stem cells (MSCs) from fibrodysplasia ossificans progressive, a genetic disorder with progressive skeletal muscle ossification, highly express PAI-1 compared with control MSCs. However, the roles of PAI-1 in early stage osteogenic differentiation have remained unknown. In the present study, we investigated the roles of PAI-1 in osteoblastic differentiation of MSCs derived from wild type and PAI-1-deficient mice.

[Results] MSCs were isolated from bone marrow or adipose tissues of 8-10-week-old male mice. PAI-1 mRNA levels were increased with time during osteoblastic differentiation of MSCs or mouse mesenchymal ST2 cells. PAI-1 deficiency significantly blunted the expression of osteogenic gene, such as Osterix and Alkaline phosphatase enhanced by BMP-2 in MSCs. Moreover, a reduction in endogenous PAI-1 levels by siRNA significantly blunted the expression of osteogenic genes in ST-2 cells. Meanwhile, deficiency or reduction of PAI-1 did not affect the phosphorylation of Smads induced by BMP-2 and TGF-β in MSCs and ST2 cells.

[Conclusion] We first showed that PAI-1 is crucial for the differentiation of MSCs into osteoblasts in mice. (COI: No)

2P-520

Regenerative capacity of stem cells in the skeletal muscle: Comparison between human, mouse and pig

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Purpose: We have reported the isolation method of the mouse and human skeletal muscle-derived stem cells (Sk-SCs) and clarified their differentiation/regenerative capacity, for the purpose of the establishment of autograft stem cell therapy. A large animal study is essential before moving to clinical trials, and the pig is considered to be an ideal preclinical model. Here we tried to determine which the pig Sk-SCs is closer to human or mouse, to be clarified the validity of the pig study.

Methods: Sk-SCs were isolated from GFP-Tg micro-mini pig (provided by Shizuoka Prefectural Research Institute of Animal Industry) using conditioned collagenase solution, and were then sorted as $CD34^-/CD45^-/CD29^+$ (Sk-DN/29^+) and $CD34^+/CD45^-$ (Sk-34) cells, in a similar manner as for the previous mouse and human Sk-SCs. Both cell fractions were appropriately expanded for about 2 weeks. Differentiation potentials in vitro and in vivo were examined during cell culture using RT-PCR, and transplantation into the severely damaged muscles and the sciatic nerve crash injury model.

Results: At 6 weeks after transplantation, the pig Sk-SCs showed comparable differentiation potential to the reported mouse and human Sk-SCs, showing differentiation into skeletal muscle cells, Schwann cells, perineurial/endoneurial cells, and vascular cells.

Conclusion: The pig Sk-SCs is closer to the human's, and thus, useful to the pre-clinical large animal trial as the Sk-SCs therapy. (COI: No)

2P-521(Y-37)

Alpha-5 integrin mediates simvastatin-induced osteogenesis of bone marrow mesenchymal stem cells

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Simvastatin (SVS), an HMG-CoA reductase inhibitor, is known to promote osteogenesis of mesenchymal stem cells (MSCs). However, the mechanism underlying SVS-induced osteogenesis is not well understood. In this study, we hypothesize that α5 integrin mediates SVS-induced osteogenesis. Mouse bone marrow MSCs (BMSCs) derived from BALB/C mouse, D1 cells, is used. Alizarin Red S staining and alkaline phosphatase (ALP) activity staining were used to evaluate the SVS-induced osteogenesis of D1 cells. The mRNA expression of α5 integrin and osteogenic marker genes (Runx2, bone morphogenetic protein 2/BMP2, type I collagen, ALP and osteocalcin/OC) were detected. Surface expressed α5 integrin were detected using flow cytometry analysis. Protein level of α5 integrin and phosphorylation focal adhesion kinase (p-FAK), the downstream of α5 integrin was detected. Results showed that SVS enhanced the gene expression of osteogenic markers as well as the subsequent ALP activity and mineralization in D1 cells. The up-regulated p-FAK is accompanied with the increased protein level of α5 integrin at first 12hr after SVS treatment. The surface expressed α5 integrin was up-regulated 3 days after SVS treatment. Depletion of α5 integrin expression by siRNA significantly suppressed the SVS-induced osteogenic gene expression, mineralization and ALP activity in D1 cells. These results identify a critical role for α5 integrin in SVS-induced osteogenic differentiation of MSCs. (COI: No)

2P-522

Molecular network search for *bcl-7* related factors

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The human BCL7 gene family functions as a tumor suppressor, and is involved in cancer development and progression. However, there are still many unclear points about the mechanisms. We previously reported that the *C. elegans bcl-7* gene, which is the only homolog of the BCL7 gene family, is involved in the regulation of self-renewal ability as stem cells, and acts in the Wnt signaling pathway. However, because several phenotypes of the *bcl-7* mutants are different from those of mutants for Wnt signaling, there is a possibility that other pathways than Wnt also work with BCL-7.

In this study, using RNA interference (RNAi) experiment, we sought the relationship between *bcl-7* and the *lin-28/let-7* pathway that plays critical roles in epigenetic regulation and cancer stem cell biology. We found that *bcl-7* RNAi alleviated the sterile phenotype of the *lin-28* mutants, while *lin-28* RNAi did not drastically affect that of *bcl-7* mutants. This suggested that *bcl-7* may be a suppressor gene for *lin-28* sterility. To address the relationship between epigenetic regulation and BCL-7, we are currently examining expression patterns of genes involved in histone modification (*e.g.* histone methylation) as well as components of mammalian SWI/SNF complex, wich was shown to contain BCL7 proteins in human, in *bcl-7* and *lin-28* RNAi/mutant worms.

In the future, we will further clarify the relationship between maintaining the undifferentiated state of stem cells and functions of *bcl-7*, *lin-28* and *let-7* genes. (COI: No)

2P-523

Platelet-rich plasma supplementation increase CD34 hematopoietic stem cell proliferation in vitro

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Background: Platelet-rich plasma (PRP) is a potential non-bovine serum replacement supplementation for hematopoietic stem cell culture in vitro. PRP could secrete multiple growth factors to stimulate proliferation and differentiation of stem cells. This study seeks to explore the potentials of platelet-rich plasma to replace ordinary bovine serum for future hematopoietic stem cells (HSCs) propagation.

Methods: Human HSCs were collected from umbilical cord blood (UCB). The CD34+ HSCs were isolated with a positive cells selection based on their surface CD34 antigens using the Mini MACs column. The CD34+ HSCs then cultured, harvested and stained using Giemsa staining. The viable harvested cells were counted using tryphan blue exclusion method.

Result: Our result showed that PRP supplementation increase CD34+ cells proliferation compared with FBS supplementation medium. The terminal differentiated blood cells morphology were found in both PRP and FBS supplemented medium.

In conclusion, PRP supplementation medium potentially surpass FBS for HSCs proliferation in vitro.

***Keywords*:** platelet-rich plasma, CD34+ HSCs, in vitro culture (COI: No)

2P-524

Identifying heterogeneity of ground state pluripotency in mouse embryonic stem cells

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Mouse embryonic stem cells (ESCs) are maintained in pluripotent states in serum free medium in the presence of Mek and Gsk3 inhibitors and LIF (2i/LIF), which is called a ground state culture condition. Core pluripotency transcription factors known to fluctuate under serum/LIF such as *Nanog* are homogenously expressed under 2i/LIF, implicating that ground state pluripotency is static in nature. However, recent reports of single cell transcriptome analyses revealed heterogeneously expressed gene modules in ground state, with the significance of this heterogeneity remains elusive. Here we report the heterogeneity of ground state ESCs was associated with their differentiation potency. A gene trap vector using Venus as a reporter was randomly inserted genome-wide for the trapped gene expression. Thousands of single cell-derived colonies were microscopically observed and clones showing heterogeneous Venus expression at the single cell level were identified. From this screen, a clone showing distinct differentiation potency between Venus-positive and -negative cells was identified. This notion was further supported by RNA-seq analysis, in which distinct gene ontology was enriched between Venus-positive and -negative ESCs. Unexpectedly, Venus-negative cells showed high expression of two-cell stage embryo-specific markers (e.g. *Zscan4* and MERVL). These results demonstrate that ground state is not in a static state but is dynamically fluctuating. (COI: No)

2P-525

Bioactive Ligands-Based Neuronal Reprogramming of Human Dedifferentiated Fat Cells

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Transcription factor (TF)-based reprogramming is a promising approach for neuronal reprogramming. However, the overexpression of ectopic transgenes limits their current clinical treatment. Alternative strategies for neuronal reprogramming of clinically available cells are necessary. Dedifferentiated fat cells (DFATs) are fibroblast-like cells from mature fat cells. We have previously found that bioactive ligands (BL)-based neuronal reprogramming in canine DFATs (cDFATs) using all-trans retinoic acid (ATRA) and basic fibroblast growth factor (bFGF).

In this study, we investigated the neuronal reprogramming of human DFATs (hDFATs) and its characteristics. As previously reported in cDFATs, hDFATs were treated with ATRA and bFGF. However, ATRA and bFGF failed to induce neuronal differentiation in hDFATs. On the other hand, when the BL "X" was added in the medium with ATRA and bFGF, the expression of neuron markers increased. On the study with the neuronal function of the cells using whole-cell patch-clamp analysis, the action potential was recorded. On the Ca^{2+} imaging study, a high concentration of KCl and the Na^{+} channel activator veratridine induced an increase in $[Ca^{2+}]_i$. Acetylcholine and dopamine also provoked an increase in $[Ca^{2+}]_i$.

In conclusion, our results provide new insights into the BL-based neuronal reprogramming of hDFATs without genetic manipulation, which may contribute to the generation of clinically available autologous cell source for neuronal diseases. (COI: No)

2P-526

Withdrawn

2P-527

Grafted hypothalamic Neurons from Mouse ES Cells survived in hypothalamus or pituitary

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Purpose

Recently, we have established the method to purify hypothalamic precursors from mouse embryonic stem cell (mESC) to differentiate the various hypothalamic neurons. To assess if these derived neurons are useful as a source for regenerative medicine, we have conducted both allo- and hetero-graft to various regions in hypothalamus or pituitary.

Methods and Results

EB5, a mESC cell line, was transfected with Rosa26 targeting vector Ai9, and then treated with Cre recombinase to stably express tdTomato (EB-Tomato). EB-Tomato was induced to hypothalamic neurons by floating culture method. The hypothalamic progenitors were purified using the cell-surface antigens. For differentiation, these cells were enzymatically dissociated and cultured on Matrigel-coated cover glass. At day 28, when cells developed to show hypothalamic nature, the cells were dissociated again and transplanted to various regions in hypothalamus or pituitary using the stereotaxic apparatus on either of SCID/NOD mice or DDI/Ddia rat brain. We found that td-Tomato positive neurons survived in the supraoptic nucleus and median eminence of hypothalamus or anterior pituitary over 30 days in both grafts. In addition, some grafted neurons migrated and directly connected to intrinsic vasopressin neurons.

Conclusion

In conclusion, we have successfully transplanted mESC derived hypothalamic neurons to hypothalamus or pituitary. These approaches will promote the medicine for various hypothalamic and pituitary disorders. (COI: No)

2P-528

Effects of beta 3-adrenergic receptor gene Trp64Arg mutation on high-fat sweet food preference

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Several gene mutations influencing metabolism have been uncovered by research on the human genome. Previous studies have shown that basal metabolic expenditure (BME) per day in humans with Trp64Arg (T64A) mutations of the beta 3-adrenergic receptor (B3AR) gene was significantly lower than that in humans with the wild type (WT) gene. However, effects of the T64A mutation on food preference (FP) are largely unknown; thus, we investigated the effects of this mutation on FP among healthy young adults (mean age, 23.9; n = 41). Preference regarding 4 types of foods (sweet, salty, sour, or bitter) was examined using a self-reported questionnaire. Mutation analysis of the B3AR gene was also performed; subjects were subsequently divided into two groups—subjects with WT or mutant B3AR. BME, but not body mass index (BMI), was significantly lower in subjects with the mutant gene than that in subjects with the WT, as previously reported. Moreover, the degree of sweet food (SF) preference in subjects with the mutant gene was significantly higher than that in subjects with the WT. When SFs were further divided into two subgroups of high-fat and low-fat SFs, the degree of FP for high-fat SFs, not low-fat SFs, was significantly different between the two subject groups, suggesting that the T64A mutation may affect the preference degree for high-fat SFs. Thus, understanding the relationship between the B3AR gene T64A mutation and high-fat SF preference will be valuable in obesity prevention. (COI: No)

2P-529(Y-38)

Vitamin D Receptor Polymorphism Fok1 and Chest X-ray in Tuberculosis Patients of Batak Ethnic

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Tuberculosis (TB) is one of the infectious diseases that is still a health problem of the world, especially in developing countries. The polymorphism of the vitamin D receptor gene (VDR) by some studies may affect the workings of vitamin D and may affect the person to become more susceptible to M. tuberculosis infection. This study aims to determine polymorphism of Vitamin D Fok1 Receptor Gene and the Chest X-Ray Result in Patients with Pulmonary Tuberculosis of Batak Ethnic who werw given vitamin D suplementation. A total of 42 patients divided into 2 group (vitamin D and placebo groups). The vitamin D group is given vitamins at a dose of 2.5 mg (100,000 IU). The results show that 26 patients have Ff genotype (62%), 14 patients have FF genotype (33%) and 2 patients have ff genotype (5%). In the calculation of the number of proportions that had improved in the FF polymorphism group were 6 people in the vitamin D group and 2 in the placebo group while the improved number of Ff + ff polymorphism groups were 8 in the vitamin D group and 5 in the placebo group (COI: No)

2P-530

Quick eating elevates blood glucose level, a practice for registered dietitians students

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It is well known that the relationship between how fast (or slow) to eat a meal and blood glucose level. For example, the blood glucose concentration (BG) elevates significantly higher in people who eat fast than slow eater. This is why we educate the patients of type 2 Diabetes Mellitus to eat slowly to prevent BG elevation. The students who study to become the registered dietitian know these facts by reading textbooks, however, they can not realize the relationship as their real experiences.

In this practice, we examine the relationship between the time to eat a fried chicken bento and pre- and post-prandial BG up to 180 min. Eighteen students divided into two groups. F group (n=9) took 10 min to eat the bento, while S group (n=9) took 20 min to finish the bento. There is no significant difference in pre-prandial BG between F and S groups. Both groups showed a significant increase in BG from 30 min. The post-prandial BG was significantly higher at 30 min and 60 min in the F group when compared with the S group. We also examined plasma triglyceride (TG) and total cholesterol (TC) level. TG elevated after 90 min while TC showed no significant change during the examination. After the experiment, students made and presented posters to discuss the results aiming active learning.

Throughout the practice, the students can understand and realize eating slowly has the beneficial effect to prevent quick BG elevation. They also learned the time course of increment BG, TG, and TC. (COI: Properly Declared)

2P-531

Design and Application of Blended Learning in the Teaching Reform of Medical Functional Experiments

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Medical Functional Experiments is an integrated practical course, which includes physiological, pathophysiological and pharmacological experiments. This curriculum is essential and vital for medical students in their basic medical learning stage. In order to improve teaching efficiency and self-learning ability, a teaching reform with blended teaching (guiding) + learning was designed, applied and assessed. It included three steps: ① online autonomous learning on the digital education resource platform; ② face-to-face teaching (guiding) +practical learning; ③collaborative experimental phase of teamwork. Through this three-stage blended teaching mode, students can be provided with the opportunity and practice of "active inquiry", which cultivate on their consciousness of independent learning and inspire their innovative and cooperative side.

Key words: Medical functional experiment; Blended learning; Practice teaching; Physiology; Pharmacology

Funds: Supported by the higher education classroom teaching reform project of Zhejiang Province (kg20160242) and the construction project of blended teaching demonstration course of Wenzhou Medical University.

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2P-532

Active learning on topics related to physiology by the first year medical students

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Seminar series were organized for a small group of the 1st year students at Osaka medical college. The aim is to encourage active learning on physiology topics. Students are instructed to research on the methods, perform experiments, and discuss the results. Topics included: measurement of vital capacity, recording of electro cardiogram and measurement of blood pressure in conditions chosen by the students, estimation of glomerular filtration rate, and epithelial transport of ions. The response of students was generally positive, thanks to their very first exposure to physiology and the introduction to the use of information tools. The cooperation among students was more intimate, presumably due to the smaller size of the group compared to the physiology lab course in the 2nd year. On the other hand, discussion tended to lack depth, due to their lack of knowledge, which can be improved in the future. (COI: No)

2P-533

Do 1st-year medical students' knowledge, attitudes&physical activity affect their physical fitness?

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WHO warns physical inactivity a leading cause of disease and disability. Technology development increases the number of physical inactivities mainly in adolescence. Medical students are the backbone of the future of the nation, health model role to their patients in the future. This study aims to find the relationship between knowledge, attitude, and practice of physical activity with the physical fitness level of 1st-year medical student in Faculty of Medicine Universitas Brawijaya.WHO questionnaire GPAQ used to measure the level of physical activity and self-developed questionnaire to measure knowledge and attitude toward physical activity and their physical fitness level obtained from secondary data of practical work during basic physiology courses using Cooper 12 minutes run test.Most participant (n=172) have high level of knowledge (95.93%, n= 165), positive attitude (53.49%, n=92) but moderate level of physical activity (62.79%, n=108) and poor to very poor physical fitness level (77.91%, n=134). Using the Spearman correlation test, there was no relationship between their high-level knowledge, positive attitude with their poor to very poor physical fitness (p=0.92;p=0.64). There was a correlation between their moderate level of physical activity and physical fitness (r=0.17; p=0.01*). The 1st-year medical student needs to learn holistically on basic physiology science and internalized it. So that they will be able to implement in practice an asset to be a good doctor.

(COI: No)

2P-534

Multiple intelligence and its relationship with academic achievements of medical students

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Purpose: Knowing the profile of multiple intelligence of students, it facilitates teachers to plan and design teaching strategies; and learners know their potentials and weaknesses. We aimed to record the profile of multiple intelligence and its relationship with the academic achievements of a medical students.

Methods: Consenting MBBS first and second years' students (n=231) were enrolled by census method. Multiple intelligence questionnaire was distributed. Their score was correlated with the marks obtained by these students in two consecutive Internal Assessments using Pearson coefficient correlation.

Results: Students rated most in intrapersonal, existential and visual-spatial intelligences (scores: 8.11±1.65; 7.32±1.89; and 7.22±2.07). They rated least in verbal-linguistic and interpersonal intelligences (scores: 5.63±1.98; and 5.64±2.10). No significant correlation was found in any domain of the multiple intelligence with the marks scored by them (intrapersonal intelligence: theory, r= 0.002, p=0.98; practical, r=0.12, p=0.06; grand total, r=0.10, p=0.14; existential intelligence: theory, r= 0.088, p=0.18; practical, r=0.088, p=0.182; grand total, r=0.082, p=0.22, etc.).

Conclusions: Dominant intelligence of medical students were intrapersonal, existential and visual-spatial; and non-dominant were verbal-linguistic and interpersonal intelligences. Our study showed no association between the multiple intelligence and academic achievements of the medical students. (COI: No)

2P-535(Y-39)

Flipped classroom in Faculty of Medicine Universitas Indonesia: a personal experience

Sophie Yolanda (*Department of Medical Physiology, Faculty of Medicine Universitas Indonesia, Indonesia*)

Flipped classroom has been gaining popularity in higher education. A successful flipped classroom should allow students to be critical thinkers, fully engaging, and stimulate a deep understanding. Flipped classroom in Faculty of Medicine Universitas Indonesia has been encouraged and implemented in several lectures, including physiology overview of the body systems lecture in basic biomedical science module since 2016.

Planning started in November 2016, with the support of the university's center for learning resources (CLR) for video production and distribution via e-learning. A 45-minute video was produced, at the end of the video the students were given an assignment to be uploaded via e-learning before the lecture. The lecture was conducted for 60 minutes in December 2016 and December 2017. The lecture's structure consists of clarification of video content, discussion of assignment, in-class group work, and discussion of group work.

In both years, all students submitted assignments before the lecture. The lecture was chosen as one of the top three best lectures of the module for two years in a row. Students in general gave positive comments, especially in engaging their participation and promoting deeper understanding. Whether flipped classroom improves learning assessment and retention needs further study.

In conclusions, when conducted properly, flipped classroom can enhance critical thinking, student engagement, and comprehension. (COI: No)

2P-536

Withdrawn

2P-537

Across-instructor divergence in scoring on practice reports in the orthoptics education with rubrics

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In orthoptic education, as well as in other medical-related fields, a number of instructors have to participate to accomplish practical training, which includes a variety of topics over the ophthalmological examinations and the basic visual sciences. One of the problems in such practical training is divergence among the instructors in scoring the students' achievement. In the U.S., rubric assessments have been used widely from elementary to higher educational levels. Because a rubric is a set of the explicit and descriptive criteria for scoring, assessments with rubric are expected to level out the divergence among instructors. To investigate divergence among the instructors, four instructors scored the reports on the five practical subjects written by students (n=56) with the same 6-term rubrics. Three instructors had 16, 5, and 1-year careers as certified orthoptists (COs) and one was a Ph.D. in Engineering (not CO). Four-way ANOVA revealed significant differences among instructors, subjects, and rubric terms; the most experienced instructor gave the highest scores for the same reports. The student x instructor Interaction was not significant. The result indicates rubric-based formative assessments in orthoptic practices *per se* does not guarantee across-instructor equalization of scoring the student reports. Reconcilements among instructors would be required even with rubrics. (COI: No)

2P-538

The relationship between anemia, dietary habits and subjective symptoms of females

Noriko Takahashi (*Showagakuin Junior College, Japan*)

[Aims]WHO published the Global Nutrition Targets 2025. Further actions are required to reach the World Health Assembly target of a 50% reduction of anemia in women of reproductive age. In Japan, it has not reached yet. Our primary objective was to identify the main problems and use them to create resources for health promotion.[Methods]Subjects were students at a junior college and a nursing school. The nutritional intake status survey involved the use of a BDHQ (brief-type self-administered diet history questionnaire). Estimated hemoglobin level in blood was measured by using the health-monitoring Analyzer ASTRIM FIT(Sysmex Corporation).The statistical analysis was performed using SPSS Statistics16(IBM).Statistical significance was set at less than 5%.[Results]In response to the question of "whether you think you are anemia", there were 50 students (61.7%) at the nursing school and 44 (65.7%) at the junior college who answered "I do not think". Students with the possibility of being anemia were seen 36% at nursing school and 50% junior colleges. There was a significant difference in awareness of anemia and hemoglobin estimate ($p<0.01$).Nutrition intake of protein and iron was a significant difference($p<0.05$). [Conclusion]The fact that there are females with low hemoglobin estimates and poor nutritional intake status and their lack of subjective symptoms, It demonstrates the necessity of nutrition and health education. (COI: No)

2P-539

Comparison of two models which explain negative feedback at a junior college

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Understanding negative feedback (neg FB) is vital in physiology. Two neg FB models were developed: 1) linear model and 2) on-off model. Model 1: the engine revolution (the 'cause') enhances the car speed (the 'result'); the uphill/downhill ('inhibiting noise'/'enhancing noise') decreases/increases the speed; when the speed is less/more than the 'set point', neg FB increases/decreases the engine revolution, increasing/decreasing the speed. Model 2 "on": the heater/cooler and winter/summer have the opposite effect on room temperature. Neg FB turns on the heater/cooler, decreasing the coldness/heat. Model 2 "off": the heater/cooler and summer/winter have the same effect on room temperature. Neg FB turns off the heater/cooler, decreasing the heat/coldness. Model 1/Model 2 would be more complex/basic, and the use of/no use of the set point concept would be more/less accurate. However, to explain the increase in insulin secretion after a meal, in the linear model, the 'result' must be worded as 'decrease in blood sugar', and the meal must be the 'inhibiting noise' making it necessary to use double negatives in the verbal explanation. Whereas, in the on-off model, cooler/summer/room temperature were simply replaced with insulin/meal/blood sugar. In a health science junior college, the on-off model was more favored than the linear model by the students. This indicates that more accurate and complex models are not necessarily more effective in certain classrooms. (COI: No)

2P-540

A new criterion for inclusion/exclusion from acupuncture treatment with blood pressure balance

Mayumi Watanabe; Zaigen Oh (*Faculty of Health Sciences, Kansai University of Health Science, Japan*)

Objective: Cerebral infarction (CI), is one of the major causes of death among middle-aged and older people. These people also often receive home acupuncture therapy. Most patients are unaware of CI and strokes occur without advance warnings. Modern medicine can be very helpful in cases of acute CI, as long as treatment begins within a few hours of onset. Although home acupuncturists consider that early detection of CI would be very useful, pre-stroke studies are rare.
Methods: In this retrospective study, vital signs that home acupuncturists often measure, such as body temperature, pulse, and blood pressure (BP), were simultaneously obtained from the right and left arms of patients who received weekly acupuncture treatment. We focused on the difference in values between the right and left arms (the balance) and compared them between patients who developed CI [CI(+)] (n = 18) and age-/gender-matched patients who did not [CI(−)] (n = 18).
Results: Results showed that left/right BP was significantly more unbalanced in the CI(+) group than in the CI(−) group, especially immediately before the onset of CI. Moreover, all CIs occurred on the side in which BP was lower.
Conclusion: If BP balance is useful for early detection of CI, we may also use it to determine whether acupuncture treatment will likely be effective or not. This may provide a seamless transition from traditional to modern medicine. In this way, acupuncture application may help maintain patient quality of life. (COI: No)

2P-541(Y-40)

The Anti-depressive and the Involvement of ERK Pathway of Electroacupuncture on Depression Model

Shao-Yuan Li[1]; Pei-Jing Rong[1,2]; Xiao Guo[1] ([1]*Institute of Acu.-Moxi., China Academy of Chinese Medical Sciences, China*; [2]*Guangzhou University of Chinese Medicine*)

Objective To observe the anti-depressive effect of electroacupuncture on auricular region (transcutaneous auricular vagus nerve stimulation, taVNS) and the involvement of extracellular signal-regulated kinase (ERK) signal pathway on chronic unpredictable mild stress (CUMS) rats. **Methods** 25 Sprague Dawley (SD) rats were randomized into control, model, taVNS, PD98059, PD98059/taVNS group. 7 kinds of chronic unpredictable stimulation for 21 days were administrated for depression model. taVNS was administrated 30 min daily for 28 days. Rats were sacrificed after experiment and hippocampus were collected to detect the expression of Raf, p-Raf, ERK1/2, p-ERK1/2, CREB, p-CREB by western blot. **Results** Compared with control group, the expression of Raf, p-ERK, CREB and p-CREB decreased significantly in model, PD98059, PD98059/taVNS group. Except for PD98059/taVNS group, compared with control group, p-Raf and ERK expression decreased significantly in the other groups. Compared with model group, the expression of Raf, p-Raf, ERK, p-ERK, CREB, p-CREB increased significantly in taVNS group. Compared with control group, Raf, p-Raf and ERK expression increased in PD98059/taVNS group, and p-ERK decreased in PD98059 group. Compared with taVNS group, Raf, p-Raf, p-ERK, CREB and p-CREB expression decreased significantly in PD98059. **Conclusion** The anti-depressive effect of taVNS may be related to the activity of the upstream and downstream proteins of ERK signaling pathway. (COI: No)

2P-542

Vasorelaxant induced by cucurbitacin B 3-oxime 22,24-dihydroisoxazole in rat thoracic aorta

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In this study, we investigated the effect of the Cucurbitacin B 3-oxime 22,24-dihydroisoxazole, cucurbitacin B analog on isolated rat thoracic aorta as well as its vasorelaxant mechanism. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the Faculty of Medicine, Chiang Mai University, Thailand. The aortic rings with or without endothelium were prepared from rats and suspended in organ chambers, and pre-contracted by phenylephrine (PE, 10 μM) before adding the cucurbitacin B analog (10^{-15}–$10^{-3.5}$M) cumulatively. The cucurbitacin B analog (10^{-15}–$10^{-3.5}$ M) relaxed the isolated aortic rings with and without endothelium, pre-contracted with PE in a concentration-dependent manner. Mechanical removal of endothelium did not significantly change the cucurbitacin B analog-induced relaxation. In the Ca^{2+}-free solution, extracellular Ca^{2+}-induced concentration was markedly inhibited in high K^+ and pre-contracted PE, and the transient contraction induced by PE and caffeine was suppressed. This study suggests that cucurbitacin B analog directly acts as a vasorelaxant to smooth muscle cells, and it involves inhibition of voltage-operated Ca^{2+} channels (VOCCs) and receptor-operated Ca^{2+} channels (ROCCs).
There is no actual or potential conflict of interest in relation to this presentation. (COI: Properly Declared)

2P-543

Withdrawn

2P-544

Ex-vivo investigation on the anti-coagulation effect of a Chinese medicinal herb

Ellie Sm Chu; Ly Ho; Ricky Wk Wu (*School of Medical and Health Sciences, Tung Wah College, China*)

Background
Rhizoma Atractylodis Lanceae (RAL) is a perennial herb belongs to the family of *Asteraceae* that distributed in China, Japan and Korea. This herb has been widely applied in Chinese medicinal decoctions for rheumatic disease, edema and digestive disorders. Studies reported that its active components are effective in anti-inflammation and anti-platelet aggregation. However, in depth mechanism of this herb on anti-coagulation effect are limited and remain to be explored.
Methodology
Human consented venous blood samples from normal healthy subjects were collected in sodium citrate collecting tubes. The plasma were isolated and pre-treated with RAL *ex-vivo* at 37°C from 0-2 hours incubation followed by testing the coagulation time of prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) using the semi-quantitation assays (Sysmex).
Results and Discussion
Results demonstrated that RAL prolonged PT, APTT and TT at 2 hours incubation. The prolonged results of PT and APTT indicating that the common pathway of coagulation cascade were affected by the treatment of RAL; whereas the prolonged TT results showing the RAL was involved in the conversion of fibrinogen to fibrin.
Conclusion
This *ex-vivo* study determined that *Rhizoma Atractylodis Lanceae* might significantly involve in coagulation cascades. More in depth studies in evaluating the anti-coagulation effect through different coagulation factors paves the way to be elucidated. (COI: Properly Declared)

2P-545

Nutmeg Extract Increases Skeletal Muscle Mass in Ageing Rats and Inhibition of Autophagy

Yuni Susanti Pratiwi[1,2]; Ronny Lesmana[1,2]; Hanna Goenawan[1,2]; Nova Sylviana[1,2]; Setiawan Setiawan[1,2]; Vita Murniati Tarawan[1,2]; Keri Lestari[3]; Rizky Abdullah[3]; Lazuardhi Dwipa[5]; Ambrosius Purba[1,2]; Unang Supratman[2,4] (*[1]Division of Physiology, Department Basic Medical Science, Faculty of Medicine Universitas Padjadjaran, Indonesia*; *[2]Central Laboratory, Universitas Padjadjaran*; *[3]Faculty of Pharmacy, Universitas Padjadjaran*; *[4]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran*; *[5]Department of Internal Medicine, Faculty of Medicine-Hasan Sadikin Hospital, Universitas Padjadjaran*)

Sarcopenia is still a major challenge to healthy ageing. Interestingly, nutmeg has been known to stimulate PPAR gamma which may contribute to myogenesis process in cardiac muscle. In the present study, we want to explore the potential effect of nutmeg inpreservingskeletal muscle mass of ageing rats.

The nutmeg extract used in this study is free safrole and myristicin nutmeg extract. Twenty male Wistar male rats, 80 weeks old with weight around 450 grams were randomly divided into control and treatment group. Nutmeg extract was given to the treatment group for 12 weeks, and water was given to the control group by gavage. The soleus and gastrocnemius muscle were removed, weighed, and rapidly frozen in liquid nitrogen and stored at -80°C until use. We thus examined gene expression of MyoD, Pax7, myogenin, MHC-1, IGF-1) and markers related to protein synthesis and Akt-mTOR autophagy pathway.

We found that nutmeg increased increase significantly in soleus muscle weight. Furthermore, nutmeg extract increased MyoD, Pax7, Myogenin, MHC Iand IGF1 gene expression significantly in soleus muscle. Furthermore, nutmeg increased Akt protein levels and activation of mTOR. This signalling process inhibit autophagy activity and stimulate or at least preserve muscle mass.

Nutmeg extract increases soleus muscle weight by partly stimulatemyogenesis, regeneration processand preserve muscle mass via IGF-AKT-mTOR pathwaylead to inhibition of autophagy activity in ageing skeletal muscle. (COI: No)

2P-546

Analgesic effect of isoliquiritigenin on oral ulcer-induced pain by blocking of Na_v channels

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The traditional Japanese herbal medicine (Kampo) hangeshashinto has analgesic effect on oral ulcer-induced pain. Previously, we found that isoliquiritigenin (ILG), an ingredient in hangeshashinto, showed antagonistic effect on voltage-dependent sodium channel (Na_v)1.8. However, other studies have reported that ILG is a potent inhibitor of voltage-dependent potassium channels. So, it is unclear whether ILG has analgesic effect on pain. In this study, to clarify effect of ILG on nociception, we investigated effects of ILG on pain-related behaviors in vivo and on voltage-dependent ion channels in vitro. In addition, we simulated firing properties of neuron by analyzing the behavior of H-H model in NEURON software. On oral ulcer model rats, swab application of ILG inhibited pain-related behaviors. Pain-related behaviors following subcutaneous injections of TRPV1, TRPA1 and TRPV4 agonists into the hind paw were suppressed by co-injection of ILG. ILG exhibited antagonistic effects on $Na_v1.1$, $Na_v1.3$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$ and $Ca_v2.2$ in automated patch-clamp recording. In trigeminal ganglion neurons, ILG suppressed inward and outward currents and action potential generation. Decreasing sodium and potassium conductance in H-H model, the number of spikes was lower than control in computer simulation. These findings suggest that ILG suppress sensory neuronal activity through blocking Nav, resulting in oral ulcer-induced pain. (COI: No)

2P-547

Withdrawn

2P-549(Y-41)

Malaysian Tualang Honey Protects Endothelial Barrier Integrity from Insults by Hydrogen Peroxide

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Malaysian Tualang Honey (MTH) contains high phenolic content with high antioxidant capacity and reported with anti-inflammatory activities. Whether MTH can be used as vascular protective agent to mitigate oxidative insults is still unclear. Thus, this study aimed to evaluate the effects of MTH on vascular protection and its underlying mechanism in response to hydrogen peroxide (H2O2). The effect of MTH on endothelial function was assessed by using in vitro vascular permeability assay kit and in vivo Miles assay after exposed to H2O2. DCFDA dye was used to determine the reactive oxygen species (ROS) scavenging capability in MTH. Changes in both calcium and cAMP signaling in endothelial cells were studied. Endothelial cell pretreated with MTH (0.01-1.0%) significantly reduced FITC-dextran permeability through the endothelial barrier in transwell in the presence of H2O2($p<0.05$). Evans blue dye leakage from small vessels in mice was also found to improve with MTH treatment ($p<0.05$). MTH at 1.0% significantly ($p<0.05$) decreased ROS production with the highest inhibition rate 75.1%. MTH, at 0.1%, also inhibited H2O2-induced calcium signaling by 98%, and significantly enhanced cAMP level in endothelial cells ($p<0.05$). In conclusion, our data suggests that MTH is capable of protecting endothelial barrier from oxidative insults, through increased cAMP production and reduced ROS and calcium level, thus proves MTH a potential therapeutic adjuvant in treating vascular dysfunction. (COI: No)

2P-550

Acetophenone dimers from *Acronychia pendunculatainduce* an apoptotic effect on human leukaemia cells

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Purpose We investigated the apoptotic activities of acrofolione A (**1**) and B (**2**) isolated from *Acronychia pedunculata*against a human pre-B cell leukaemia cell line (NALM-6) to explore the apoptosis-related signalling molecules targeted by **1** and **2**.

Methods The apoptosis effects of **1** and **2** in NALM-6 cells were investigated by TUNEL staining, annexin V, mitochondria membrane potential, and caspase 3/7 activity. We carried out a protein array to explore the signalling molecules involved in apoptosis comprehensively.

Results Acrofolione A (**1**) suppressed the growth of NALM-6, K562, and HPB-ALL cells (IC_{50} 16.7 ± 1.9, 17.9 ± 0.3, and 10.1 ± 0.2 mM, respectively) more effectively than acrofolione B (**2**). Both compounds time-dependently increased the number of NALM-6 cells with abnormal nuclei, and increased the number of annexin V-positive cells and decreased the mitochondrial membrane potential of NALM-6 cells. Acrofolione A (**1**) markedly elevated caspase 3/7 activity and increased the number of TUNEL-positive cells. Cells treated with either compound showed enhanced expression of cleaved PARP and cleaved caspase 3 and 7, and reduced survivin protein levels.

Conclusions Acrofolione A (**1**) and B (**2**) may be useful in the treatment of various types of leukaemia. (COI: No)

2P-551

Purple rice husk extract preserves mitochondrial integrity and reduces diabetic kidney injury

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This study examined the benefit of purple rice husks, the leftovers from milling process, on diabetic nephropathy. High-fat/streptozotocin diabetic rats (DM) were given vehicle, husk extract (PRHE), and metformin, respectively, for 12 weeks, while control rats received standard diet. Diabetic nephropathy was successfully induced as shown by the presence of metabolic alterations (increased blood glucose, insulin, HOMA IR, triglyceride, cholesterol) and renal abnormalities (podocyte and tubular injuries, albuminuria, increased serum creatinine, decreased creatinine clearance). Metformin and PRHE were equally effective in diminishing all these changes, which were associated with improved mitochondrial integrity (decreased swelling, reactive oxygen species production and membrane potential changes) with subsequent reduced renal oxidative damages (decreased lipid peroxidation but increased antioxidant glutathione, superoxide dismutase, glutathione peroxidase). HPLC analysis showed that cyanidin-3-glucoside is the key component of PRHE and may underlie its effects. These data reveals the efficacy of PRHE to protect against the development and progression of diabetic nephropathy, which is potentially due to its abilities to retain mitochondrial integrity and oxidative equilibrium within the kidney. The findings not simply disclose the benefits of this agricultural waste, but create an opportunity to increase its value through the development as a useful health product in the future. (COI: No)

2P-552

Addition of hexachlorocyclohexane provokes insulin resistance in 3T3-L1 mature adipocytes

Amire Alimu; Junetsu Ogasawara; Takahiko Yoshida (*Department of Hygiene, Asahikawa Medical University, School of Medicine, Japan*)

[Purpose]
The growing bodies of evidences have shown that intake of pesticides evoke obesity-induced type2 diabetes in human body. Indeed, previous study has reported that addition of organochlorine insecticide to differentiating 3T3-L1 adipocytes accelerate cell hypertrophy via activation of adipogenic pathway. However, little is known that the effect of insecticide on physiological response in mature adipocytes, which are main component of mammalian adipose tissue. The present experiment was designed to investigate the effect of hexachlorocyclohexane (HCH), an organochlorine insecticide, on cellular response in mature adipocytes.
[Method]
The 3T3-L1 preadipocytes were cultured with differentiation medium for 7 days. After that, obtained mature 3T3-L1 adipocytes were divided into two groups: Control and HCH. For HCH, cells were incubated with HCH (50μM) at 37℃ for 7days. Thereafter, cells were harvested and used for each assay via sample preparation.
[Result]
Addition of HCH was significantly suppressed glucose uptake in mature 3T3-L1 adipocytes. Under this condition, phosphorylation of AKT, a regulatory molecule of glucose uptake, was significantly decreased in HCH compared with Control. Moreover, treatment of HCH significantly augmented production of intracellular reactive oxygen spices and its marker molecules.
[Conclusions]
These results suggest that HCH suppresses insulin-stimulated glucose uptake through reduction of phosphorylated AKT in 3T3-L1 mature adipocyte. (COI: No)

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