Symposium

Symposium 1

Current somatosensory investigation reveals how skin feels the present

(March 21, 8:30～10:00, Room D)

S01-1

Simultaneous observation of skin receptors and central terminations on single primary sensory neuron

Ebara, Satomi[1]; Tonomura, Sotatsu[1]; Uta, Daisuke[2]; Furue, Hidemasa[3]; Furuta, Takahiro[4]; Kuroda, Daichi[1]; Kumamoto, Kenzo[1] (*[1]Dept Anatomy, Meiji Univ Integrative Med, Kyoto, JP; [2]Dept Applied Pharm, Grad Sch Med and Pharm Sci, Toyama Univ, Toyama, JP; [3]Dept Information Physiol, Div Neural Signaling, NIPS, Okazaki, JP; [4]Dept Morphological Brain Sci, Grad Sch of Med, Kyoto Univ, Kyoto, JP*)

Skin is innervated by primary sensory neurons known as pseudo-unipolar cells. Both peripheral and central terminations and the firing characteristics were simultaneously identified and characterized by means of intracellular labeling and recording in the rat trigeminal ganglia in vivo. Well-labeled 32 TG neurons in 32 rats were terminated as single kind of mechanoreceptors including Merkel (13), club-like (10) and lanceolate endings (7). Seven of the club-like-ending neurons never branched in their peripheral branch to the end in the follicle. They indicated relatively shorter duration at base time of action potential and showed higher frequency by air spray than the other type of endings. One of labeled lanceolate neurons distributed a 2×5 mm square receptive field on the upper eyelid. Central processes of all types of labeled neurons extended typically as far as the level of the second cervical segment of the spinal cord while emitting in excess of 20 collaterals to terminate in the trigeminal nuclei. Two samples (Merkel and club-like) showed bifurcation of the trunk axons at the level of the principal nuclei. The both trunk axons alternately gave off collaterals throughout the trigeminal tract. Those simultaneous observations on single primary sensory neurons may provide new aspects of skin sensory system.

(COI: No)

S01-2

Touch activates mechanosensitive ion channels in Merkel cells in vitro

Nakatani, Masashi[1,2]; Nelson, Aislyn M[3]; Lumpkin, Ellen A[1,4] (*[1]Columbia Univ. Med Ctr., New York, USA; [2]Keio University, Yokohama, Japan; [3]Baylor College of Medicine, Houston, TX, USA; [4]Columbia Univ., New York, USA*)

Merkel cell-neurite complexes are gentle touch receptors that mediate slowly adapting type I (SAI) responses. Since Merkel cells have been proposed to be mechanosensory cells that transduce mechanical stimuli into electrical signals that activate somatosensory neurons. Consistent with this model, conditional knockout mice that lack Merkel cells show the loss of touch-evoked SAI responses. Moreover, in vitro studies on cultured Merkel cells report calcium elevation in Merkel cells in response to swelling or membrane stretch. Previous studies support the contribution of Merkel cells to touch sensation, however, the central question of whether Merkel cells are intrinsically touch sensitive is unanswered. To tackle this problem, we performed live-cell imaging and electrophysiological recordings from mouse Merkel cells. Touch-evoked responses were monitored with either ratiometric calcium imaging or tight-seal, whole-cell recordings. Merkel cells displayed reversible calcium responses to focal displacements applied to somata. Moreover, electrophysiological recordings demonstrated mechanically activated inward currents at a negative holding potential. Merkel-cell currents adapted exponentially to sustained stimuli. Quantitative PCR indicated that Merkel cells expressed both Piezo1 and Piezo2 genes. Together, these data demonstrate that Merkel cells are intrinsically mechanosensitive in the absence of other skin cells or somatosensory neurons.

(COI: No)

S01-3

Cortical feedback control of thalamic sensory-evoked recurrent responses in rat vibrissa/barrel system

Hirai, Daichi; Shibata, Ken-ichi; Kaneko, Takeshi; Furuta, Takahiro (*Grad.Sch.Med. Kyoto Univ., Kyoto, Japan*)

Sensory gating is crucial to perception and active sensing. How and where this process takes place in is still longer unknown. Here, we identified cortical feedback could modulate temporal patterns of thalamic responses during awake sensory processing, even though their EPSPs could not directly drive spike discharges in thalamus. We broke barrel field primary somatosensory cortex (S1BF) in rats and assessed the effect on the ventral posterior medial nucleus (VPM) that projections to S1BF. After S1BF lesion, we found significant sensory-evoked rebound responses more than 50ms after stimulus onset with low-threshold spikes (LTS)-like burst in VPM and thalamic reticular nucleus (TRN), whereas smaller proportion of neurons in natural brain showed rebound responses. These results suggest thalamo-reticular circuits innately generate recurrent activity and corticothalamic feedback could suppress this activity. These results shed light on the cortical neurons as active component of sensory system and show the importance of cortical feedback in the fine control of subcortical undesirable activity.

(COI: No)

S01-4

Modulation of spinal sensory synaptic transmission by TRPV1-expressing afferent fiber

Furue, Hidemasa (*Dept Information Physiol, NIPS, Okazaki, Japan*)

Recent studies have shown that the transient receptor potential vanilloid subfamily member TRPV1, is expressed on primary afferent C fibers, and this capsaicin-activated cation channel has been proposed to play an important role in somatosensory thermal or pain signaling. However, relatively little is known about the role of the synaptic inputs from the C-fiber afferents on the spinal components of the modulation of somatic sensory transmission. We examined how capsaicin-sensitive afferent fibers modulate spinal nociceptive transmission by using in vivo and slice patch-clamp recording techniques. Superficial spinal dorsal horn (SSDH) neurons tested received excitatory monosynaptic inputs from A δ and C fibers. Application of capsaicin presynaptically increased the frequency of the miniature excitatory postsynaptic currents (EPSCs) in most of the SSDH neurons. In GABAergic (VGAT-Venus labelled) neurons in the SSDH, capsaicin also increased the frequency of miniature EPSCs, suggesting that SSDH neurons including GABAergic interneurons make an excitatory synaptic contact with TRPV1-expressing fibers. In SSDH neurons in vivo, naturalistic sensory touch stimulation applied to the skin elicited a barrage of inhibitory postsynaptic currents (IPSCs), and the touch-evoked IPSCs were inhibited by blockade of spinal capsaicin-sensitive C fiber excitatory synaptic inputs. These results suggest that tactile cutaneous stimulation may be conveyed by a subpopulation of TRPV1-expressing C fibers, and activate inhibitory GABAergic neurons in the SSDH to reduce noxious transmission.

(COI: No)

Symposium 2

Architecture and molecular mechanisms in sensory systems

(March 21, 8:30～10:00, Room G)

S02-1

Elementary response of olfactory receptor neurons (ORNs) to odorants and its associated signaling

Yau, King-Wai (*Dept Neuroscience, Sch of Med, Johns Hopkins Univ, Baltimore, Maryland, United States of America*)

This talk will summarize some of our past key experiments on olfactory transduction. The sense of smell begins with odorant molecules binding to odorant receptors on the membrane of ORN cilia, thereby activating a G protein, Golf, and the downstream effector enzyme, an adenylyl cyclase (ACIII). As a result, the intracellular cAMP concentration rises and opens a cyclic-nucleotide-gated, non-selective cation channel to depolarize the cell. With repeated, identical weak odorant pulses and variance analysis to study the ensemble of elicited responses, we found in both frog and mouse ORNs that the unitary response was surprisingly constant, despite large variations in the macroscopic sensitivity, across ORNs. We infer that an odorant-binding event has a very low probability of activating sensory transduction at all. Thus, even when successful, the resulting unitary response apparently involves a single active G alpha olf-ACIII molecular complex. This low signal amplification is in contrast to rod phototransduction in vision, where each photoisomerized rhodopsin molecule is known to produce substantial amplification by activating many downstream G protein molecules, hence many effector-enzyme molecules. From the action-potential firing, we estimated that perhaps 20 or so odorant-binding events that successfully triggered transduction in an frog or mouse ORN will lead to signaling to the brain. If time permits, some unpublished recent experiments will also be discussed.

(COI: No)

S02-2

Respiration rhythm and olfaction

Mori, Kensaku; Manabe, Hiroyuki; Narikiyo, Kimiya (*Dept Physiol, Grad Sch Med, Univ of Tokyo, Japan*)

In land mammals including humans, olfactory perception critically depends on discrete respirations, each consisting of an inhalation phase followed by an exhalation phase. During the inhalation phase, odorants are drawn into the nasal cavity and activate olfactory sensory neurons in the nasal sensory epithelium. Thus the central olfactory system is driven from the external odor stimuli and processes the olfactory sensory information during the inhalation phase. On the contrary, the central olfactory system is temporarily isolated from the external odor world during the exhalation phase. Therefore respiration rhythm plays a key role orchestrating the information processing mode across a number of regions in the central olfactory system, which includes the olfactory bulb and numerous areas of the olfactory cortex. We made electrophysiological recordings of local field potentials and single-unit activities in behaving rats while the respiration pattern of rats was monitored by a thermocouple placed in the nasal cavity. We report dynamic changes in the operation mode of information processing in the central olfactory system during the inhalation-exhalation sequence of respiration.

(COI: No)

S02-3

Does calmodulin modulate the functions of the TMEM16 calcium-activated chloride channels?

Chen, Tsung-Yu (*Center for Neuroscience and Department of Neurology, University of California, Davis, USA*)

TMEM16 gene family members, TMEM16A and TMEM16B, have recently been identified to be the calcium-activated chloride channels (CaCCs) in various sensory and respiratory tissues in the nose and are important for the olfactory functions of vertebrate animals. It has been known that CaCCs play a critical role in amplifying the odorant-induced inward current through the calcium-permeable cyclic nucleotide-gated channels (CNC) in olfactory receptor neurons. In the odorant signal transduction process, modulation of the olfactory CNC by calcium-calmodulin has been known to be critical for olfactory sensory adaptation. Whether calmodulin modulates the TMEM16 family members is, however, controversial. Biochemical experiments from different laboratories showed controversial results regarding calmodulin binding to TMEM16 family members. Functionally, calmodulin was thought by some investigators to be required for the activation of the TMEM16 CaCCs by calcium, although experiments from other laboratories including ours suggested that activation of TMEM16A and TMEM16B channels does not require calmodulin. Finally, calcium-calmodulin was also shown to alter the anion permeability of TMEM16A channel. However, by directly applying calcium-calmodulin to the intracellular side of excised inside-out membrane patches, we are unable to observe this calcium-calmodulin effect on the anion permeation of TMEM16A channel, although the calmodulin used in our laboratory rigorously inhibits the olfactory CNC formed by subunit CNCA2.

(COI: No)

S02-4

Measurement of metabolic activity of single mammalian photoreceptors

Koutalos, Yiannis; Adler, Leopold; Chen, Chunhe (*Department of Ophthalmology, Medical University of South Carolina, Charleston, USA*)

In vertebrate rod photoreceptors, all-trans retinal is released by photoactivated rhodopsin following light excitation. All-trans retinal is then reduced to all-trans retinol, in a reaction that requires NADPH. The extent of conversion of all-trans retinal to all-trans retinol is a measure of the NADPH-generating capacity of the cell. We have used the fluorescence of all-trans retinal and all-trans retinol to monitor their levels in single rod photoreceptor cells with fluorescence imaging. Rod photoreceptors were isolated from the retinas of dark-adapted mice and human donor eyes. All-trans retinal was generated either endogenously by photoactivating rhodopsin with long-wavelength light (longer than 530 nm), or supplied exogenously with bovine serum albumin as carrier. The fluorescence signals of all-trans retinal and all-trans retinol were distinguished on the basis of the large difference in their absorption spectra. Conversion of all-trans retinal to all-trans retinol was measured from the ratio of the fluorescence intensities excited by 340 and 380 nm light (emission longer than 420 nm). Experiments were carried out at 37 ^{0}C. We find that NADPH generation requires the presence of extracellular metabolic substrates, with glutamine supporting NADPH generation to levels comparable to those of glucose. The results suggest that in rod photoreceptors mitochondria-linked pathways can generate substantial amounts of NADPH. This allows the cells to utilize a variety of metabolic substrates to maintain viability under transient nutrient shortages.

(COI: No)

S02-5

Spatial structure of actin cytoskeletons in retinal pigment epithelial and photoreceptor cells

Usukura, Jiro (*Grad.Sch.Sci.Nagoya Univ., Nagoya, Japan*)

The spatial organization of cytoskeletal actin filaments in retinal pigments epithelial and photoreceptor cells were studied by high voltage TEM (1000 KV), high resolution SEM and freeze etching method. Actin cytoskeletons have been investigated so far exclusively with fluorescent light microscopy by Phalloidin staining or GFP tag method using culture cells. Therefore, actin filaments in cytoplasm, in particular, peri-nuclear region in real tissue cells were not observed enough yet. In order to detect real spatial structure of actin cytoskeleton, unroofed whole pigment epithelial cells cultured from monkey eye were applied to 1000 KV TEM. Our innovative methods detected incredibly more abundant actin filaments than in fluorescent microscopy. Interestingly, no stress fibers were found in spite of culture cells. Remarkable amount of actin filaments occupying the entire cytoplasm extended in all directions with aggregation and dispersion to form meshwork, and eventually divided cytoplasmic space into several domains. These actin filaments contained specific anti-myosin II antibody binding site. However, myosin filaments were not recognized on actin filaments under freeze-etching electron microscopy. Therefore, myosin II might attach to actin filaments as a single molecule or non-detectable short filaments consisting of a few molecules. In photoreceptor cells, actin filaments were observed widely in inner segments from ellipsoid region to synaptic area, though actin filaments in the outer segment were found only in tip of connecting cilium.

(COI: No)

Symposium 3

Frontiers in mitochondrial dynamics and pathophysiology

(March 21, 8:30～10:00, Room H)

S03-1

Physiological roles of mitochondrial fusion and fission in mice development

Ishihara, Naotada (*Inst. Life Sci., Kurume Univ., Kurume, Japan*)

Mitochondria are highly dynamic organelles that change their morphology during cellular signaling, differentiation and pathogenic condition. Several types of GTPase proteins regulate dynamic morphogenesis of mitochondria, although their physiology have been poorly understood. To assess the physiological role of mitochondrial fission, we generated knock-out (KO) mice of mitochondrial fission factor dynamin-related protein (Drp)1 by using Cre-loxP system. Mice lacking the mitochondrial fission GTPase Drp1 have developmental abnormalities, and die after embryonic day 12.5. Neural cell-specific Drp1-deficient mice die shortly after birth due to brain hypoplasia with apoptosis, due to failed proper distribution of mitochondria. In various developmental stages and pathogenic conditions, mitochondrial morphology is highly changed, and the regulated mitochondrial fission might have important roles in tissue differentiation. We also found a novel role of mitochondrial fission in distribution of mtDNA. Mammalian cells typically contain thousands of copies of mtDNA assembled into hundreds of nucleoid structures. We analyzed the dynamic features of the nucleoids in terms of mitochondrial membrane dynamics, and found that nucleoids in Drp1-deficint cells were enlarged by their clustering within hyperfused mitochondria. The dynamics of nucleoid structures regulated by mitochondrial fission contributed to cristae reformation, proapoptotic status of mitochondria, and thus the tissue differentiation in vivo.

(COI: No)

S03-2

Role of mitochondrial ubiquitin ligase MITOL in mitochondrial dynamics and diseases

Yanagi, Shigeru (*Tokyo Univ. Pharm. Life Sci., Tokyo, Japan*)

We have previously identified mitochondrial ubiquitin ligase, MITOL (also known as March5), which regulates mitochondrial dynamics through the ubiquitination of mitochondrial fission factor Drp1. Subsequently, we reported that MITOL ubiquitinated and attenuated cell toxicity of unfolded proteins accumulated in mitochondria such as mutant SOD1 and expanded polyglutamine proteins which cause neurodegenerative disorders, suggesting the involvement of MITOL in mitochondrial quality control and pathogenesis of neurodegenerative diseases. To further understand the role of MITOL in mitochondria, we searched for physiological substrates for MITOL and succeeded to identify microtubule-associated protein 1B-light chain 1 (MAP1B-LC1) and mitofusin2 (Mfn2). Recently, we report that MITOL plays a protective role against nitrosative stress-induced mitochondrial dysfunction mediated by MAP1B-LC1 in neuronal cells, and that MITOL is required for ER-mitochondria interaction via Mfn2 activation. In the symposium, I will show several unpublished data obtained from analyses of MITOL-deficient MEFs and mice, and discuss the role of MITOL in mitochondrial dynamics and diseases.

(COI: No)

S03-3

Mitochondrial dynamics in damaged neurons

Kiryu-Seo, Sumiko; Kiyama, Hiroshi (*Grad.Sch.Med.Nagoya Univ., Nagoya, Japan*)

The physiological relevance of mitochondrial fission in damaged neurons remained to be determined, although numerous studies observe fragmented mitochondria in damaged neurons of neurodegenerative disease and traumatic injury models. To address this issue, attempts have been made to elucidate the functional consequences of mitochondrial fission under physiological and pathological conditions in vivo. In this symposium, we will introduce the recently established unique bacterial artificial chromosome transgenic (BAC Tg) mice, in which mitochondria are labeled with GFP and cre recombinase is expressed simultaneously in injury specific manner, and will discuss the critical role of mitochondrial fission in damaged neurons in vivo. The BAC Tg mice demonstrate that GFP-labeled shorter mitochondria are actively transported to replace pre-existing GFP-negative longer mitochondria in regenerative injured motor axons, suggesting the enhanced activity of mitochondrial fission after nerve injury. Crossing the BAC Tg mice with the dynamin-related protein 1 (Drp1) floxed mice succeeds in the ablation of mitochondrial fission specifically in injured motor neurons. The injury-inducible Drp1 knockout mice show the microglial activation in the proximity of injured neurons from earlier stage and the elongated or gigantic mitochondria with lower quality, prior to neuronal death and axonal degeneration. Thus, mitochondrial fission could be an acute defensive response for injured neurons to satisfy huge amounts of energy demands and to maintain mitochondrial and neuronal integrity.

(COI: No)

S03-4

How dysfunction of mitochondrial quality control causes Parkinson's disease

Matsuda, Noriyuki (*Protein Metabo Pro, Tokyo Metro Inst of Med Sci, Japan*)

PINK1 and PARKIN have been identified as the causal genes responsible for hereditary Parkinson's disease (Kitada et al., 1998). To date, there is significant evidence supporting a functional link between PINK1, Parkin and mitochondrial quality control. PINK1 is a serine/threonine kinase that specifically accumulates on and is activated by mitochondria with a decreased membrane potential. PINK1 then activates the latent ubiquitin ligase (E3) activity of Parkin and recruits it to depolarized mitochondria (Matsuda et al., 2010; Narendra et al., 2010). Parkin catalyzes ubiquitin transfer to various substrates on depolarized mitochondria. As a consequence, inferior mitochondria with low membrane potential are quarantined and degraded via the proteasome and autophagy.

We and other groups have revealed the mechanistic insights into PINK1-mediated Parkin activation recently. Parkin is an intramolecular auto-inhibitory E3 that usually has its catalytic Cys431 core occluded by a RING0 domain. PINK1 phosphorylation of Ser65 in the ubiquitin-like domain of both Parkin and ubiquitin triggers removal of Parkin autoinhibition by phosphorylated ubiquitin, which then results in the conversion of phosphorylated Parkin to the fully active form (Kane et al., 2014; Koyano et al., 2014). On the other hand, while various models for the recruitment process have been proposed, all inadequately explain the accumulated data, thus the molecular basis for PINK1 recruitment of Parkin remains to be fully elucidated. We are now revealing the molecular mechanism of Parkin recruitment, and the newest results will be presented.

(COI: No)

Symposium 4

Dynamic aspects of microscopic localization, stoichiometry and function of membrane protein complexes

(March 21, 8:30～10:00, Room I)

S04-1

Quantitative localization of bio-molecules in the neuronal plasma membrane by immuno-electron microscopy

Fukazawa, Yugo[1]; Shigemoto, Ryuuichi[2] (*[1]Div Cell Biol and Neurosci, Sch Med, Univ Fukui, Fukui, Japan; [2]IST Austria*)

The dendrites of neurons, major receiving part of synaptic input, plays roles in more than integrating and passing received postsynaptic potential down to the soma and axon initial segment at where the action potential is generated. They have ability to shape and integrate individual potentials thereby involved in regulation of neurons behavior. These abilities are achieved by orchestrated actions of transmitter receptor, voltage-gated ion channels and ion pumps expressed in the dendritic plasma membrane (PM). Thus, to understand mechanisms underlying neuronal excitability, it is crucial to identify molecular species expressed in a target neuron and quantity (density) and their distribution of each molecule in the PM. Toward this goal, we have been investigating distribution of several key molecules such as ionotropic receptors and ion channels in pyramidal cells in the hippocampus by means of quantitative immuno-electron microscopic approaches. We have just started to analyze distribution of neuron specific Na/K ATPase which is responsible for maintenance of resting membrane potential. In this presentation, we will introduce our recent results including these from other ongoing analysis.

(COI: No)

S04-2

Spatial Regulation of $GABA_AR$ Synaptic Structure by Glutamate and Calcium

Bannai, Hiroko[1,2]; Niwa, Fumihiro[2]; Triller, Antoine[3]; Mikoshiba, Katsuhiko[2] (*[1]Div Biol Sci, Grad Sch Sci, Nagoya Univ, Nagoya, Japan; [2]RIKEN BSI, Wako, Japan; [3]IBENS, Paris, France*)

GABAergic synaptic transmission regulates brain function by establishing the appropriate excitation-inhibition (E/I) balance in neural circuits. The structure and function of GABAergic synapses are sensitive to destabilization by impinging neurotransmitters. However, signaling mechanisms that promote the restorative homeostatic stabilization of GABAergic synapses remain unknown. Here, we characterized a signaling pathway that promotes the stability of $GABA_A$ receptor ($GABA_AR$) postsynaptic organization by quantum dot-single particle tracking. Slow metabotropic glutamate receptor signaling activated IP_3 receptor-dependent calcium release and protein kinase C phosphorylation to promote $GABA_AR$ clustering and GABAergic transmission. This $GABA_AR$ stabilization pathway counteracted the rapid cluster dispersion caused by glutamate-driven NMDA receptor-dependent calcium influx and calcineurin dephosphorylation, including in conditions of pathological glutamate toxicity. These findings show that glutamate activates distinct receptors and spatiotemporal patterns of calcium signaling for opposing control of GABAergic synapses. This mechanism of inhibitory synaptic stabilization will enable therapies to restore E/I imbalance in major brain diseases.

(COI: Properly Declared)

S04-3

Impact of transient homodimers: the basic units for signaling and domain formation found for both GPCRs and GPI-anchored receptors

Kusumi, Akihiro (*WPI-iCeMS and Inst. for Frontier Med. Sci. Kyoto Univ.*)

Single-molecule tracking techniques applicable to live cells are now providing researchers with the unprecedented ability to directly observe molecular behaviors in the plasma membrane (PM) of live cells. Using ultra-speed simultaneous two-color single-molecule colocalization and single-molecule FRET, we found that class-A G-protein-coupled receptors (GPCRs) and GPI-anchored receptors (GPI-AR) form transient homodimers with lifetimes on the order of 0.1 s and that these transient dimers are critical for triggering some of the signaling pathways.
(1) We fully determined the dynamic monomer-dimer equilibrium of prototypical GPCRs, N-formyl peptide receptor and adrenergic receptor, I,e, the equilibrium constant and dimer formation-dissociation rate constants, indicating that, under physiological expression conditions at 37 degrees, 42 and 95% of the molecules, respectively, exist as dimers in the live-cell PM at any moment These transient dimers triggered the steady-state signals characteristic of GPCRs.
(2) Meanwhile, GPI-ARs continually form transient homo-dimers through ectodomain protein interactions, stabilized by raft-lipid interactions (termed homo-dimer rafts). When CD59 was ligated, a few homo-dimer rafts turned into a stable oligomer rafts, which triggered intracellular Ca2+ responses. Transient homo-dimer rafts are most likely one of the basic units for organization and function of raft domains containing GPI-ARs.
In conclusion, these results suggest that taking advantage of transient homo-dimers might be a basic strategy for the receptor-based signal transduction in the PM.

(COI: No)

S04-4

Expression density dependent changes of the stoichiometry and function of ion channel complexes

Kubo, Yoshihiro[1,2]; Kitazawa, Masahiro[1,2]; Nakajo, Koichi[1,2] (*[1]Div Biophys & Neurobiol, Natl Inst Physiol Sci, Okazaki, Japan; [2]Physiol Sci, SOKENDAI, Hayama, Japan*)

It has been known that many ion channels do not stand alone but function forming molecular complex with other accessary subunits. Biochemical analyses provide us with information as to the molecular identity in the complex as well as the bulk average of the stoichiometry. However, the detail of the stoichiometry had not been analyzed. Ulbrich et al (Nat Methods, 2007) applied a single molecule imaging technique to determine the stoichiometry of ion channel complexes. It is possible to evaluate the number of subunits in the complex by counting the number of bleaching steps of the fluorescent protein tagged to the subunits by single molecule imaging. In this presentation, two examples from our achievements are introduced. (1) KCNQ1/ KCNE1 K^+ channel complex plays important roles in the cardiac rhythmic beating, and it had been generally accepted that the stoichiometry is 4:2. We applied single molecule subunit counting technique and demonstrated the presence of 4:4 complex. Furthermore, we observed that the stoichiometry varies depending on the relative expression density (Nakajo et al, PNAS 2010). (2) Kv4 K^+ channel plays roles in the neuronal and cardiac functions. It is well accepted to form a molecular complex with accessary subunits such as KChIP and DPP. We analyzed Kv4.2/KChIP4 complex and observed that the stoichiometry changes with the increase in the relative expression level of KChIP4, as if KChIP4 binds to the 4 independent sites with no preferred stoichiometry (Kitazawa et al, JBC 2014).

(COI: No)

Symposium 5

The strategies aimed at maintenance of tissue perfusion ～Regulation of cardiomyocyte apoptosis and angiogenesis～

(March 21, 8:30～10:00, Room J)

S05-1

TCTP expression level may be critical for protection against apoptosis of cardiomyocytes and development of cardiac dysfunction

Fujita, Takayuki; Cai, Wenqian; Hidaka, Yuko; Jin, Hui-lin; Hasegawa, Nozomi; Suita, Kenji; Ishikawa, Yoshihiro (*Cardiovascular Research Institute, Yokohama City Univ. Yokohama, Japan*)

Translationally Controlled Tumor Protein (TCTP), one of the anti-apoptotic proteins, is ubiquitously expressed in human tissues. TCTP is reported to exert anti-apoptotic effect through direct interaction with p53 and MCL-1. TCTP works as a p53 inhibitor. In addition, TCTP inhibits degradation of MCL-1, an anti-apoptotic protein, thereby maintaining its expression level.Doxorubicin (DOX) is a widely used chemotherapeutic agent for cancer therapy. However, its clinical usage has been limited by its serious cardiotoxicity. We found that after treatment of DOX, protein expression of TCTP was significantly decreased both in cultured rat ventricular cardiomyocytes and mouse heart. Moreover, down-regulation of TCTP by siRNA induced apoptosis of cardiomyocytes. In addition, our recent studies showed that Dihydroartemisinin (DHA), a TCTP down-regulating agent, induces apoptosis of cardiomyocytes and cardiac dysfunction. Consistently, cardiac specific overexpression of TCTP prevents both of doxorubicin-induced and DHA-induced apoptosis of cardiomyocytes and cardiac dysfunction. These findings indicate that TCTP expression level may be critical for protection against apoptosis of cardiomyocytes and development of cardiac dysfunction. TCTP may be a novel potent therapeutic target for heart failure.

(COI: No)

S05-2

Disruption of Epac1 decreases phosphorylation of phospholamban and protects the heart against chronic catecholamine stress

Okumura, Satoshi (*Department of Physiology, Tsurumi University, Japan*)

Protein kinase A (PKA) phosphorylates multiple molecules involved in calcium (Ca^{2+}) handling in cardiac myocytes, and is considered to regulate β-adrenergic receptor-mediated enhancement of cardiac contractility. However, this paradigm has been challenged by the recent identification of Epac (exchange protein activated by cAMP), which is activated by cAMP independently of PKA. Epac1-null mice (Epac1KO) showed decreased cardiac contractility with decreased phospholamban (PLN) phosphorylation at serine-16, the major PKA-mediated phosphorylation site. Intracellular storage of Ca^{2+} was decreased in Epac1KO. However, PKA expression remained unchanged and isoproterenol improved cardiac contractility. In contrast, direct activation of Epac led to increased phospholamban phosphorylation at serine-16 in cardiomyocytes and this phosphorylation is considered to involve Epac1/PLC/PKC. More importantly, chronic isoproterenol infusion (60mg/kg/day for 7days) induced a similar degree of cardiac hypertrophy in Epac1KO and WT, but subsequent cardiac dysfunction was prevented in Epac1KO, in association with decreased cardiac myocyte apoptosis and fibrosis. Epac1 is an important regulator of phospholamban phosphorylation, independently of PKA, and appears to regulate cardiac responsiveness to chronic catecholamine stress.

(COI: No)

S05-3

Visualization of Angiogenesis

Morikawa, Shunichi (*Tokyo Women's Med. Univ., Tokyo, Japan*)

Angiogenesis is an important event not only in normal developmental process, but also in abnormal pathological processes such as malignant tumors. In tumors, angiogenesis is a serious problem since it helps proliferation of tumor cells by supplying them oxygen and nutrients and also helps metastatic spread of tumor cells via blood stream. Therefore, in tumor therapy, suppression of the angiogenesis need to be taken in account. On the contrary, in the therapy of ischemic diseases such as myocardial infarction, re-perfusion of blood in ischemic area is necessary; promotion of angiogenesis is conversely important in this case. To cope with the diseases, we need to take a closer look into the actual scene of angiogenesis and study the cellular mechanism of it. For this purpose, clear and detailed visualization of angiogenesis is required in the first place. In the session, representative images of the actual scene of angiogenesis will be presented; they include fine-structural images of newly forming vessels revealed by ultrathin sections, or three-dimensional (3D) images that have been re-constructed from serial cross sections. By the 3D images, we can see the whole appearance of newly forming vessels that we cannot grasp by thin sections. From these images, we can assess how endothelial cells proliferate and grow, and how pericytes behave during angiogenesis. We can also see an increased permeability and abnormal coverage of basement membrane of newly forming vessels.

(COI: No)

S05-4

Novel mechanisms involved in the endothelial differentiation of arteries and veins

Saito, Erina[1,2]; Isogai, Sumio[1]; Kimura, Eiji[1]; Shimoda, Hiroshi[2]; Hitomi, Jiro[1] ([1]*Iwate Med Univ., Iwate, Japan;* [2]*Grad.Sch.Med.Hirosaki Univ., Aomori, Japan*)

It had been long believed that flow dynamics play crucial role in the capillary bed to determine the arterial venous identity, but recent studies revealed that genetic cues induce the cell fate before blood flow initiates. Since the findings, vascular biologists focused their attention intensely upon the expression of artery and vein specific genes in the vascular development. It was inevitable to detect when and how the molecular differences reflect the phenotypic differences. These molecular evidences forced us to reconsider the biological mechanisms involved in the differentiation of endothelial cells from the mesoderm, acquisition of arterial or venous identity and tube formation. To verify the differentiation of endothelium from the mesoderm, we captured the time lapse movies following whole the morphogenetic process of the primary vascular system for the brain in vivo using fli1 EGFP transgenic zebrafish. The arterial angioblasts coalesced into the small luminized cluster, and the tightly adhered angioblasts generated seamless major artery by so called cord hollowing mechanism. On the contrary, the venous angioblasts never coalesced, but taking a leaf-like shape individually, encircle a wide lumen. This patchwork like formation manner and a larger number of cell participated with the formation of vein allowed to form a wide seamed vessel. Our results suggest that endothelial precursors of major cerebral arteries and veins have completed each individual morphogenesis using different mechanism through vasculogenesis and angiogenesis.

(COI: No)

S05-5

Vasohibin-2 modulates tumor onset by normalizing tumor angiogenesis

Kitahara, Shuji (*Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, USA*)

Vasohibin-2 (VASH2) has been identified as an extrinsic and vascular endothelial growth factor independent angiogenic factor that is highly expressed in tumor cells. In the present study, we aimed to find whether pre-existing vascular changes can be used to predict tumor transformation as benign or malignant. We sought to characterize the microvascular changes and tumor development in the intestinal tract of $Apc^{Min/+}$ mice and the $Apc^{Min/+}/Vash2^{-/-}$ mice. $Apc^{Min/+}$ mice provide a unique orthotopic model for the development of spontaneous adenomatous polyposis and subsequent carcinomas-termed the adenoma-carcinoma sequence. $Apc^{Min/+}$ mice were mated with $Vash2^{-/-}$ mice with a mixed C57BL/6 background and the resulting pups were screened for the Min mutation and for the $Vash2^{-/-}$ gene by PCR. The intestinal tumors of $Apc^{Min/+}$ mice and the $Apc^{Min/+}/Vash2^{-/-}$ mice were removed and either frozen or epon-embedded for subsequent analyses. $Apc^{Min/+}/Vash2^{-/-}$ mice showed a significant decrease in the number of polyps in the small intestine. Furthermore, functional tumor blood vessels were decreased, and pericyte coverage tumor blood vessels were increased. We may propose that VASH2 modulates the onset of tumors in the gastrointestinal tract by regulating tumor angiogenesis.

(COI: No)

S05-6

The function of platelet factor CLEC-2 in the hematopoietic stem cell niche

Ishizu, Ayako; Suda, Toshio (*National University of Singapore, Singapore*)

The bone marrow (BM) niche governs the integrity of hematopoietic stem cells (HSCs). While vascular and perivascular cells constitute potent niches, we recently identified mature megakaryocytes (Mk) as an independent niche component. Ontogenetically, the vascular and hematopoietic system development intertwine; molecular defects in platelets result in blood and lymphatic vessel dis-segregation and hematopoietic abnormalities. We explored the function of one of these markers, platelet activation receptor C-type lectin like receptor-2 (CLEC-2) in hematopoiesis. Specific deletion of CLEC-2 on Mk lineages (*PF4Cre:CLEC-2$^{floxed/floxed}$*; *Clec2$^{Mk\delta/\delta}$*) confined the function of BM HSCs; *Clec2$^{Mk\delta/\delta}$* HSCs showed reduced cell cycle quiescence and lower post-transplantation chimerisms in competitive BM transplantation assay. *Clec2$^{Mk\delta/\delta}$* mice exhibited abnormal HSC mobilization to the peripheral blood and splenomegaly due to extramedullary hematopoiesis reflecting a failure of *Clec2$^{Mk\delta/\delta}$* BMs to sustain HSCs. Although *Clec2$^{Mk\delta/\delta}$* Mk progenitors matured without arrest in normal ploidy, sorted *Clec2$^{Mk\delta/\delta}$* Mks failed to maintain HSC populations in vitro. CLEC-2 deficient Mks exhibited decreased production of various niche factors including Thrombopoietin (Thpo). Furthermore, despite presenting thrombocytopenia, serum and BM Thpo levels were remarkably low in *Clec2$^{Mk\delta/\delta}$* mice. We identify CLEC-2 as an Mk specific molecule that formulates a niche for the maintenance of HSCs through the modulation of Thpo production. Our study provides insight into the close relation between the vascular system and Mks regarding hematopoiesis.

(COI: No)

Symposium 6

Front in progress on aerospace medicine and biology

(March 21, 14:00～15:30, Room B)

S06-1

Response of osteoclasts in the regenerating scales of goldfish under microgravity during space flight

Ikegame, Mika[1]; Tabata, Makoto[2]; Hattori, Atsuhiko[3]; Suzuki, Nobuo[4] ([1]*Dept.Oral Morph., Okayama Univ.Grad.Sch.Med.Dent.Pharm., Okayama, Japan;* [2]*Grad.Sch. Tokyo Med.Dent.Univ., Tokyo, Japan;* [3]*Coll.of Lib.Arts Sci., Tokyo Med.Dent.Univ., Chiba, Japan;* [4]*Inst.Nat.Environ.Technol., Kanazawa Univ., Ishikawa, Japan*)

Microgravity during space flight leads to rapid bone loss. However, the cellular mechanisms underlying this phenomenon remain unclear. Teleost scale is a calcified tissue which is similar to mammalian bone tissue in many aspects. We investigated the response of osteoclasts during space flight using goldfish scales. Regenerating scales were incubated for 86 h under microgravity (F-μg) or artificial 1 g (F-1g) at the International Space Station. We also performed three-dimensional clinostat experiments to examine immunohistochemical localization of receptor activator of nuclear factor kappa-B ligand (RANKL), which is a crucial factor for the development and activation of osteoclasts, in the scales under modeled microgravity. A significant increase in osteoclast's nucleus number and the size of actin-ring was observed in F-μg group compared to F-1g group. Real-time PCR analysis demonstrated the up-regulation of RANKL gene expression in F-μg group. Furthermore, the immunohistochemical localization of RANKL was increased under the modeled microgravity. These results suggest that one of the mechanisms of bone loss during space flight is the stimulated activity of osteoclasts via up-regulation of RANKL expression.

(COI: No)

S06-2

Bone formation and resorption under microgravity in medaka rearing in International Space Station (ISS)

Takano, Yoshiro (*Grad.Sch.Med.Dent., TMDU.Tokyo, Japan*)

Molecular mechanisms underlining loss of bone mineral density (BMD) during space flight still remain unclear. With strong supports from JAXA and JSF, we were fortunate to have an opportunity to examine structure and function of bone-related cells in medaka rearing under microgravity in the ISS up to 2 months. We focused on the pharyngeal bone, which is the site of dynamic bone remodeling.

Method: To investigate the activity of osteoclasts (OC) and osteoblasts (OB), we established the TRAP promoter-GFP/Osterix promoter-DsRed double Tg medaka and reared in the aquatic habitat at "Kibo", Japanese experimental module in the ISS. Fish were either fixed with 4 % PFA at day 16 and day 58, or preserved in RNAlater at day 2 and day 62. BMD was measured by Soft X-ray and μCT and the OC and OB activities by confocal microscopy and histological methods. The gene expression level was examined by the whole transcriptome analysis using HiSeq.

Results and Discussion: In 16 days-rearing group, the total volume of GFP and DsRed expressing cells decreased respectively by 49% and 68% ($p<0.05$) while the OC/OB volume ratio elevated 1.45 folds compared to 1G control ($p<0.05$). In 58 days group, BMD decreased by 30% compared to time-matched 1G control without notable changes in enzymatic activities and ultrastructure in OC and OB cells. These data indicate that, under microgravity, while OC and OB become less active in 16 days, the OC/OB ratio increases and leads to decreased BMD in later periods.

Conclusion: Taken together, we conclude that microgravity exerts a condition similar to the low bone-metabolic rotation osteoporosis.

(COI: No)

S06-3

Cardiovascular responses to acceleration in rats

Maruyama, Satoshi[1]; Nishida, Yasuhiro[2] ([1]*Aeromedical Laboratory, Japan Air Self-Defense Force;* [2]*Department of Physiology, National Defense Medical College*)

During flight, various aircraft maneuvers often produce sustained acceleration (+Gz stress) during pitch and banked turns. Acceleration of +4Gz or greater values can cause deficiency in retinal and cerebral perfusion and results in visual field abnormality such as gray out, black-out or loss of consciousness. Peripheral and central vision disturbances are caused by a reduction in peripheral retinal perfusion. These visual disturbances precede reduced brain perfusion and are followed by a loss of consciousness. Understanding of cardiovascular and autonomic responses to +Gz stress is very important to secure flight safety. We have investigated the effects of +Gz on the cardiovascular system using rats and found some important results: (1) +5 Gz stress may suppress baroreflex response and subsequently cause increased hypotension; (2) brain blood flow response to Gz exposure depends on the brain loci that are affected; and (3) repetitive lower acceleration (+1.5Gz) exposures eliminate in decrease of cerebral arterial pressure and reduction of cortical oxygen concentration. We also describe some basic physiological phenomenon affected by sustained acceleration.

(COI: No)

S06-4

Hypoperfusion-reperfusion injury and reactive oxygen species: spin resonance analyses

Tokumaru, Osamu[1]; Ogata, Kazue[1]; Kitano, Takaaki[2]; Yokoi, Isao[1] ([1]*Dept Neurophysiol, Oita Univ Fac Med, Oita, Japan;* [2]*Dept Anesthesiol, Oita Univ Fac Med, Oita, Japan*)

Under high Gz acceleration environment (head-to-foot inertial force), fighter pilots are subject to hypoperfusion in brain. Decrease in tissue oxygen concentration leads to breakdown of ATP to hypoxanthine and activation of xanthine oxidase. On reperfusion, resupply of oxygen leads to production of superoxide anion radicals, from which many kinds of free radical species are produced in a series of chain reactions, resulting in tissue damage.By phosphorous nuclear magnetic resonance (NMR) spectroscopy, it is possible to measure high-energy phosphates, phosphocreatine (PCr) and ATP in rat brain slices. The observation revealed decrease in PCr and ATP during ischemia and delayed recovery of energy metabolic status compared with those of oxygen and glucose. Free radicals can be directly observed by electron spin resonance (ESR) spectroscopy. Using ESR, we have evaluated direct scavenging activity of antioxidants against multiple kinds of free radicals. Since the life time of free radicals are short (μs-ms), it is difficult to observe free radical production in living tissue. We have tried to detect it only in vain. In clinical settings, decrease in vitamin C radicals in serum is observed in post-operative patients. Application of those "spin resonance analyses" will make it possible to better understand the pathophysiology including change in energy metabolism and production of reactive oxygen species in rat brain exposed to high Gz environment, contributing to the improvement of the countermeasures and flight safety.

(COI: No)

Symposium 7

Recent advances in the research on the trigeminal ganglion

(March 21, 14:00～15:30, Room D)

S07-1

Pharmacological Action of Eugenol: Go beyond Dental Clinic

Oh, Seog Bae (*Dept Neurobiol and Physiol, Sch Dent, Seoul National Univ, Seoul, South Korea*)

Eugenol, an active ingredient of essential oil extracted from cloves and other herbs, is used extensively in dentistry for the analgesic purpose. However, the molecular mechanism underlying the analgesic activity of eugenol is mostly unknown. A series of investigation in our lab have revealed that eugenol modulates various ion channels that are responsible for nociception, generation of neuronal spikes, and synaptic transmission in the trigeminal system. Pharmacological action of eugenol includes inhibition of action potential firing by reducing voltage-gated sodium, calcium and potassium channels in the trigeminal ganglion neurons. In addition, we found eugenol inhibits hyperpolarization-activated cyclic nucleotide-gated (HCN) channels that play a crucial role in mechanical allodynia in neuropathic pain state. Concurrently, eugenol successfully reversed mechanical allodynia in experimental trigeminal neuropathic pain animal, at much less dose than it blocks sodium channels. Modulation of $P2X_3$ receptor, an ionotropic ATP receptor, might be another mechanism by which eugenol exerts its analgesic activity. Activation of TRPV1 and TRPA1 by eugenol might also contribute to the analgesic effect, since pharmacological activation of TRPV1 and TRPA1 has been shown to produce biphasic action of initial pungent and sustained inhibition of nociception. In conclusion, eugenol is a natural compound that displays a number of pharmacological properties with therapeutic potential for versatile analgesic applications.

(COI: No)

S07-2

Modulatory mechanisms of inflammatory nociceptive signals in the trigeminal ganglia

Takeda, Mamoru (*Lab Food Physiol Sci, Sch life Enviro Sci, Azabu Univ, Kanagawa, Japan*)

Peripheral tissue inflammation can alter the properties of somatic sensory pathways, causing behavioral hypersensitivity and resulting in increased responses to pain caused by noxious stimulation (hyperalgesia) and normally innocuous stimulation (allodynia). Although no synaptic transmission has been found in the primary sensory ganglia, it has been discovered that the activity of neighboring neurons elicits a functional cross-excitation in the somata of affected sensory neurons under normal conditions, indicating that non-synaptically released diffusible chemical messengers modify the neuronal excitability of the sensory ganglia (Amir and Devor 2000). Non-synaptically released chemical mediators were derived from both the neurons and the satellite glia (e.g. neuron-neuron and neuron-glia interactions). More recent studies have suggested that modulation of neuronal excitability within sensory ganglia, including trigeminal ganglia may trigger chronic pain via the autocrine/paracrine mechanism, and this augmented excitability of the primary afferent neurons may also cause the development of changes in the central pain-signaling neurons (central sensitization).Therefore, the present talk focuses on the modulation of the neuronal signal by cross-talk in the trigeminal ganglia, particularly with regard to its contribution to inflammatory pain, and discusses the potential therapeutic target for the prevention of hyperalgesia/allodynia.

(COI: No)

S07-3

Involvement of intra-trigeminal ganglionic communication in ectopic orofacial pain

Shinoda, Masamichi; Iwata, Koichi (*Dept Physiol, Sch Dent, Nihon Univ, Tokyo, Japan*)

Pathological orofacial pain which spreads to a wide area in the trigeminal territory occurs with orofacial inflammation or trigeminal nerve injury. However, the peripheral mechanisms underlying such ectopic orofacial pain remain unclear. We investigated the involvement of intra-trigeminal ganglionic communication in ectopic orofacial pain via nitric oxide (NO), nerve growth factor (NGF) or calcitonin gene-related peptide (CGRP) following orofacial inflammation or trigeminal nerve injury. Heat or mechanical hypersensitivity was induced in the ipsilateral whisker pad skin following inferior alveolar nerve transection (IANX) or lower lip inflammation, and suppressed by transient receptor potential vanilloid 1 (TRPV1) or $P2X_3$ receptor ($P2X_3R$) antagonism, respectively. Neuronal nitric oxide synthase (nNOS), NGF and CGRP expression in the trigeminal ganglion (TG) was increased following lower lip inflammation. Moreover, intra-trigeminal administration of tyrosine kinase receptor or nNOS inhibitor diminished the heat or mechanical hypersensitivity. The lower lip inflammation increased the number of $P2X_3R$- and TRPV1-positive TG neurons that innervate the whisker pad skin, which was annulled by anti-NGF intra-trigeminal ganglionic administration. The present findings suggest that intra-trigeminal ganglionic communication via NO, NGF or CGRP signaling resulted in upregulation and/or sensitization of TRPV1 or $P2X_3R$ in TG neurons following orofacial inflammation or trigeminal nerve injury, which may develop ectopic orofacial pain.

(COI: No)

S07-4

alpha-2/delta-1 subunit of dihydropyridine receptor in the trigeminal ganglion

Sato, Tadasu; Tachiya, Daisuke; Ichikawa, Hiroyuki (*Grad. Sch. Dent. Tohoku Univ., Sendai, Japan*)

Immunohistochemistry for alpha-2/delta-1 subunit of L-type calcium channel was performed on the rat trigeminal ganglion (TG). The immunoreactivity (IR) was detected in one third of TG neurons (32.8 %). These neurons were mostly small or medium-sized. A double immunofluorescence method revealed that half of alpha-2/delta-1-immunoreactive (IR) neurons were also immunoreactive for calcitonin gene-related peptide (54%). In addition, 41 % of alpha-2/delta-1-IR DRG and TG neurons contained vanilloid receptor subtype 1. However, co-expression of alpha-2/delta-1 with vanilloid receptor subtype 2 was infrequent (6%). A retrograde tracing method also demonstrated that alpha-2/delta-1-IR was common among cutaneous TG neurons (48.1 %) and relatively rare among tooth pulp TG neurons (24.4 %). Transection of the infraorbital nerve dramatically increased the number of alpha-2/delta-1 subunit-IR neurons in the TG. These findings indicated that small to medium-sized nociceptors with unmyelinated axons contain alpha-2/delta-1subunit of L-type calcium channel in the TG. The subunit in TG neurons may be associated with nociceptive transmission from oro-facial regions.

(COI: No)

S07-5

Vesicular Nucleotide Transporter (VNUT) regulates ATP signaling in Trigeminal Ganglion

Gunjigake, Kaori[1]; Goto, Tetsuya[2] ([1]*Kyushu Dental Univ., Kitakyushu, Japan;* [2]*Kagoshima Univ., Kagoshima, Japan*)

By oral nociceptive stimulation, neurons in trigeminal ganglion (TG) produce various neurotransmitters that communicate with other TG neurons. Though neurons in TG are surrounded by satellite glial cells (SGCs), little is known about the interactions between SGCs and TG neurons. We have focused on adenosine-5'-triphosphate (ATP) as a neurotransmitter, and investigated the association of the vesicular nucleotide transporter (VNUT); ATP transporter, with neurons and SGCs in TG using immunocytochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR), and in situ hybridization (ISH) in rats after rat upper molar extraction. After extraction, ATF3-immunoreactive (IR) neurons appeared in the maxillary nerve region. The ATF3-IR, damaged, neurons were surrounded by GFAP-IR, active, SGCs. Interestingly, ATF3 immunonegative neurons were also surrounded by GFAP-IR SGCs. The number of GFAP-IR SGCs and $P2X_3$ receptor-IR neurons was increased after extraction in a time-dependent manner. RT-PCR and in situ hybridization confirmed the increase of VNUT mRNA expression in neurons and SGCs in TG. Our results suggest that peripheral nerve injury induced the mutual activation of TG neuron and SGCs, possibly by VNUT-mediated ATP release.

(COI: No)

Symposium 8

Neural regulation of vascular function - Integration of anatomical and physiological evidence

(March 21, 14:00～15:30, Room E)

S08-1

Control of skin and skeletal muscle blood flow by sympathetic nerve activities in humans

Kamijo, Yoshi-ichiro[1,2]; Ogawa, Yu[2]; Nose, Hiroshi[1,2] (*[1]IBS-ICCER Shinshu Univ., Matsumoto, Japan; [2]Dept. of Sports Med. Sci., Shinshu Univ. Grad. Sch. of Med., Matsumoto, Japan*)

In humans, skin blood flow (SkBF) and sweat rate increase in hyperthermia; however, blood pooling in the cutaneous veins in an upright position and hypovolemia due to sweat loss decrease the venous return to the heart. If not compensated, they would threaten the maintenance of arterial pressure. To prevent this, cutaneous vasodilation is suppressed through baroreflexes; however, the efferent path has not been identified. Recently, we have reported that a component of skin sympathetic nerve activity (SSNA) synchronized with cardiac cycle might be involved in the mechanisms; however, the component might contain muscle sympathetic nerve activity (MANA). Here, we tested whether head-up tilt (HUT) would suppress the SSNA component while enhance MSNA in hyperthermia. In 12 men (22-24yr), wearing a perfusion suit, we measured right atrial volume (RAV), carotid artery diameter (CAD; echocardiography), esophageal temperature (T_{es}), cutaneous vascular conductance (CVC = SkBF/ mean arterial pressure), SSNA and MSNA (microneurography; the peroneal nerve; N=6) during supine and 30° HUT. SSNA component and CVC increased as T_{es} increased by 0.7℃ with 47-℃ water perfusion into the suit. We found that HUT suppressed the increases while enhanced MSNA and HR with reduced RAV and CAD. These results suggest that the SSNA component does not contain MSNA and both activities significantly contributes to the maintenance of arterial pressure by baroreflexes in hyperthermic humans in an upright position.

(COI: No)

S08-2

Neural vasodilator mechanisms contribute to increased blood flow to non-contracting muscle during one-legged cycling in humans

Matsukawa, Kanji; Ishii, Kei; Liang, Nan; Endo, Kana (*Dept Integrative Physiol, Grad Sch Biomed and Health Sci, Hiroshima Univ, Hiroshima, Japan*)

Whether neurally-mediated vasodilatation may contribute to exercise hyperemia has not been completely understood. Bülbring and Burn (1935) found for the first time the existence of sympathetic cholinergic nerve to skeletal muscle contributing to vasodilatation in animals. Blair et al. (1959) reported that atropine-sensitive vasodilatation in skeletal muscle appeared during mental stress in humans. However, such sympathetic vasodilator mechanism for muscle vascular bed in humans is generally denied at present, because surgical sympathectomy, autonomic blockade, and local anesthesia of sympathetic nerves cause no substantial influence on vasodilatation in muscle not only during mental stress but also during exercise. On the other hand, neural mechanisms may play an important role in regulating blood flow to non-contracting muscle. Relative changes in oxygenated-hemoglobin concentration (Oxy-Hb) of the contralateral vastus lateralis muscle, as index of tissue blood flow, were measured during 1-min one-legged cycling. The Oxy-Hb increased at the early period of exercise and the increase was sustained throughout exercise. Propranolol (0.1mg/kg iv) failed to affect the initial Oxy-Hb increase, whereas atropine (0.01-0.015mg/kg iv) abolished the initial increase. Both drugs blunted the later component of the Oxy-Hb increase during the exercise. Thus the rapid cholinergic and delayed β-adrenergic vasodilator mechanisms may contribute to increase muscle blood flow to non-contracting muscle during exercise.

(COI: No)

S08-3

Regulation mechanisms of blood flow examined by histochemistry

Kawamata, Seiichi; Kurose, Tomoyuki (*Inst.Biomed.HealthSci., Hiroshima Univ., Hiroshima, Japan*)

For better understanding of microvascular circulation, this study examined the proportion of open and functioning capillaries in the leg muscles (20, 30, 37 and 40℃), pancreas and small intestine of anesthetized rats. FITC-labeled *Lycopersicon esculentum* lectin was injected into the heart, mixed with blood and allowed to circulate for 3 min in the whole body. Open and functioning blood vessels were detected by immunostaining for this lectin bound to endothelial cells, whereas closed capillaries without blood flow were unstained. To detect all capillaries, sections were stained for PECAM-1 (CD31). The proportion of open and functioning capillaries in rat leg muscles was high in a period of 3 min at 37℃. Based on histochemical results, it was concluded that the blood flow of each capillary considerably decreased at 20 and 30℃ and probably increased at 40℃, whereas the proportion of open and functioning capillaries was essentially unchanged in the range of 20 to 40℃. In addition, the proportions of open and functioning capillaries are high and similar among the leg muscles, pancreas and small intestine in spite of their structural and functional differences. Therefore, microvascular systems of tissues and organs seem to control microvascular blood flow by changing the blood flow of each capillary, whereas the proportion of open and functioning capillaries is essentially unchanged. The blood flow considerably changes depending on the blood flow velocity and the size of blood vessels. These factors are probably regulated by the nerve activity, vasoactive substances, blood pressure and so on.

(COI: No)

S08-4

Neural control of pulmonary blood vessels in health and disease

Schwenke, Daryl O.[1]; Tsuchimochi, Hirotsugu[2]; Nagai, Hisashi[3]; Sonobe, Takashi[2]; Fujii, Yutaka[2]; Umetani, Keiji[4]; Shirai, Mikiyasu[2] (*[1]Dept of Physiol, University of Otago, Dunedin, New Zealand; [2]Dept of Cardiac Physiology, National Cerebral and Cardiovascular Research Institute, Osaka, Japan; [3]Dept of Forensic Medicine, University of Tokyo, Japan; [4]Japan Synchrotron Radiation Research Institute, Hyogo, Japan*)

Chronic intermittent hypoxia (IH) provokes a centrally-mediated increase in sympathetic nerve activity (SNA). The effect of this sympathetic hyper-excitation on the pulmonary vasculature remains unclear. We aimed to assess the effect of sympathetic excitation in modulating acute hypoxia pulmonary vasoconstriction (HPV), and the central β-adrenergic signalling pathway for facilitating the increase in SNA. Sprague-Dawley rats were exposed to IH for 8 hours/day for 6 weeks. Subsequently, pulmonary SNA was recorded in rats, and the pulmonary vasculature was visualized using microangiography. Pulmonary responses to acute hypoxia were assessed before and after central β-adrenergic receptor blockade (Metoprolol, 200 nmol). Chronic IH increased baseline SNA (110% increase), and exacerbated the sympathetic response to acute hypoxia. Moreover, the magnitude of HPV in IH-rats was blunted compared to control-rats (10% and 20% vasoconstriction, respectively). In only the IH rats, β-receptor blockade attenuated the hypoxia-induced increase in pSNA and exacerbated acute HPV, so that both sympathetic and HPV responses were similar to that of control-rats. These results provide compelling evidence that the centrally-mediated increase in SNA following IH acts to blunt the local pulmonary vasoconstrictor effect of acute hypoxia.

(COI: No)

S08-5

Neural control of cerebral cortical blood flow

Uchida, Sae (*Dept Auton Neurosci, Tokyo Metropol Inst Gerontol, Tokyo, Japan*)

The neural vasodilative system, consisting of cholinergic fibers projecting from the basal forebrain to the cerebral cortex, was found by Sato et al. in 1989. This finding has been confirmed by several other investigators and the underlying vasodilative mechanisms have been examined. At this symposium, our extensive research on the cholinergic vasodilative system will be introduced.

1. Decline of the cholinergic vasodilative system in very old rats and the underlying mechanism

The cholinergic cortical vasodilation in response to stimulation of nucleus basalis of Meynert (NBM) is maintained in old rats (2 years old), but it declines in very old rats (3 years old). The decline of the vasodilation in very old rats is mainly due to age-related decline of nicotinic acetylcholine receptor (nAChR) activity. The subtype of nAChRs that mediates the cortical vasodilation is $\alpha 4\beta 2$, but not $\alpha 7$.

2. Effect of acupuncture on the cholinergic vasodilative system and their relationship with aging

Acupuncture-like stimulation of a forepaw, the back, and a hindpaw increases cortical ACh release. Cortical blood flow (CBF) is increased by the stimulation of paws but it is not affected by stimulation of the back. The stimulation of paws increases blood pressure (BP), whereas that of the back decreases BP. Thus, an increase in ACh release is independent of BP changes, whereas responses in CBF are influenced by BP changes. In a rat model, where BP is not changed by forepaw stimulation, an increase in CBF is still elicited via the cholinergic vasodilative system. Acupuncture-induced activation of the cholinergic vasodilative system is observed even in very old rats.

(COI: No)

Symposium 9

Regulatory mechanisms of sperm properties toward fertilization success

(March 21, 14:00～15:30, Room F)

S09-1

ADAM3 and sperm fertilizing ability

Ikawa, Masahito (*Res Inst Micobial Dis, Osaka Univ, Osaka, Japan*)

Sperm binding to zona pellucida (ZP) has been believed to be prerequisite for the physiological acrosome reaction and the subsequent fertilization process in mammals. In 1997, we reported that the testis specific endoplasmic reticulum chaperone, calmegin (Clgn), is required for sperm binding ability to ZP1. To date, more than 10 knockout mouse lines (Ace, Adam1a, Adam2, Adam3, Calr3, Clgn, Pdilt, Pmis2, Rnase10, Tex101, Tpst2) were reported to be infertile and share the similar phenotype. Strikingly, all the mutant mice showed the defective ADAM3 localization in the mature spermatozoa. Thus sperm ADAM3 was considered to play indispensable role during fertilization by mediating sperm-ZP binding. However we showed that the mutant spermatozoa lacking ADAM3 successfully fertilize ZP intact eggs if they were surrounded in cumulus cells2. The data suggest that the presence of numerous acrosome intact sperm binding to ZP surface of cumulus free eggs is less important than previously supposed. The idea is supported by the report that most fertilizing mouse spermatozoa begin their acrosome reaction during cumulus penetration before contact with the ZP3. More importantly, we found that the spermatozoa lacking ADAM3 are unable to migrate through utero-tubal junction (UTJ) in the female reproductive tract. Therefore the mechanism of sperm migration through UTJ should be investigated in the future study.

(COI: No)

S09-2

The non-genomic regulation of sperm hyperactivation by steroids

Fujinoki, Masakatsu (*Dept Physiol, Sch Med, Dokkyo Med Univ, Mibu, Tochigi, Japan*)

During capacitation, mammalian spermatozoa are hyperactivated. Hyperactivation is a modification of flagellar movement to create the driving force for penetrating the zona pellucida. Recently, it has been suggested that several hormones regulated sperm hyperactivation. In hamster, progesterone enhances sperm hyperactivation, and estradiol suppresses progesterone-enhanced hyperactivation. Both steroids dose-dependently affect sperm hyperactivation. When progesterone enhances sperm hyperactivation, progesterone binds to membrane progesterone receptor and activates phospholipase C and protein kinases. Finally, tyrosine phosphorylations of sperm proteins are increased and/or enhanced together with enhancement of sperm hyperactivation. Estradiol also binds to membrane estrogen receptor when estradiol suppresses progesterone-enhanced hyperactivation. Although detailed signals are not clear, many tyrosine phosphorylations of sperm proteins are inhibited by estradiol together with suppression of progesterone-enhanced hyperactivation. Interestingly, regulation of sperm hyperactivation by steroids was disrupted by diethylstilbestrol.

(COI: No)

S09-3

Ever-changing fertilization paradigms-in a case of mammalian sperm acrosome reaction

Hirohashi, Noritaka (*Oki Marine Station, Shimane Univ., Japan*)

Science is interesting because search for truth never ends. The sperm acrosome reaction (AR), first discovered by Jane Clark Dan in echinoderms, is believed to be essential for sperm-egg fusion to occur in the protostomes and the Deuterostomes. In eutherian mammals, the AR is thought to be prerequisite for the sperm to pass through the extracellular investment of the oocyte as referred to the zona pellucida (ZP). A long-standing hypothesis in the commitment of the AR states that the ZP induces the AR. This has been reinforced by the following empirical data using acid-solubilized ZP glycoproteins in different mammalian species. Research moved into molecular details and the technology also moved from biochemistry to genetic engineering. The "green" sperm was made in 1999 and the dogma has been challenged since then. I shall present the current status of the mouse AR with a fascinating imaging tool.

(COI: No)

S09-4

Regulation of fertilization competence of the egg-coating envelope by the interaction between *Xenopus* dicalcin and gp41

Miwa, Naofumi[1]; Ogawa, Motoyuki[2]; Hanaue, Mayu[1]; Takamatsu, Ken[1]
([1]*Dept Physiol, Sch Med, Toho Univ, Tokyo, Japan;* [2]*Dept Med Learn, Sch Med, KItasato Univ, Kanagawa, Japan*)

Mature oocytes of animals are surrounded by an extracellular egg-coating envelope (called vitelline envelope in frogs, VE) that plays important roles in the processes of fertilization and thereafter, up to implantation of fertilized egg. Evidence from lectin staining of oocytes has pointed out divergent structural patterns among the filamentous egg-coats of unfertilized eggs, but yet no study has shown precise structural basis on varied fertility. We previously found that *Xenopus* dicalcin, present in the VE, constitutively suppresses the efficiency of fertilization through binding to gp41, a glycoprotein of the egg envelope. By using synthetic peptides that correspond to amino-acid regions responsible for the interaction between two proteins, we clamped the VE either in fertilization-competent or -incompetent status, and investigated its nanoscale structure. Our electron microscopy analyses revealed a disorganized filamentous meshwork within the competent VE, but a well-organized meshwork was observed in the incompetent one. *In vivo* lectin-staining pattern of the VE also revealed remarkable differences between two VE statuses. These results demonstrated the structural basis on the fertilization competence of the egg-coats and observed transition of the fertilization competence by extrinsic treatment promotes the development of efficacious drugs for contraceptive strategy and the treatment of infertility in animals. Molecular insights of fertilization competence will also be discussed.

(COI: No)

S09-5

Equatorin-mediated sperm-egg interaction

Toshimori, Kiyotaka; Ito, Chizuru; Yamatoya, Kenji (*Grad.Sch.Med.Chiba Univ., Chiba, Japan*)

Mammalian fertilization is consisted of multistep processes mediated by sperm and egg factors. The molecular mechanism controlling gamete interaction requires mediation by gamete factors but remains unsolved. Now it is widely accepted that sperm Izumo1 and egg Cd9 are essential for gamete fusion. However, since no fusogenic domain is detected in Izumo1 and Cd9 and no direct interaction between them is reported, additional factors are expected. Recently Juno and Cd9 are reported as egg Izumo1 receptor and Juno partner, respectively. We first reported equatorin as a MN9 antigen against a monoclonal antibody MN9 in the mouse and human sperm. Equatorin is registered as a sperm protein (EQTN) encoded by Eqtn (EQTN). Inhibition assays with MN9 reduce the fertility without inhibition of sperm penetration into zona pellucida (ZP) but inhibit sperm-egg interaction and egg activation. Equatorin is type 1 membrane protein with single transmembrane domain and short cytoplasmic tail. High resolution fluorescence microscopy using transgenic B6-Tg(Eqtn-EGFP) male mice and immunogold electron microscopy with MN9 revealed that equatorin is Golgi-derived and integrated into the acrosomal membrane in spermatids and then relocated on the equatorial segment during the acrosome reaction. B6-Tg(Eqtn-EGFP) distribution pattern at the equatorial segment was uniform before penetration on the ZP, but much perturbed, reducing the staining intensity, after penetration through ZP. This change is presumed to correlate for sperm to gain the competency to fuse with oolemma. The function is currently underway using Eqtn-deficient male mice.

(COI: No)

Symposium 10

Forefront of exo- and endocytosis research

(March 21, 14:00～15:30, Room H)

S10-1

Visualizing exocytosis of single synaptic vesicles at the calyx-type presynaptic terminal

Midorikawa, Mitsuharu; Sakaba, Takeshi (*Grad Sch Brain Sci, Doshisha Univ, Kyoto, Japan*)

To support fast and reliable synaptic transmission at central nerve system, presynaptic terminals need to organize exocytosis precisely. However, the fate of each synaptic vesicle inside presynaptic terminals remains largely unknown. Applying TIRF microscopy to the presynaptic terminal of rat calyx of Held synapse at the brainstem, we visualized synaptic vesicles near the plasma membrane, and sites of calcium influx.The presynaptic terminal of a rat calyx of Held was acutely dissociated. Synaptic vesicles were labeled with FM dye. The presynaptic terminal was whole cell voltage-clamped, and was stimulated by depolarizing pulses. The sites of calcium influx were visualized using fluorescent calcium indicator, which was loaded into the terminal through a recording patch pipette.Upon stimulation, a fraction of FM-labeled vesicles underwent fusion, which could be identified as the release of FM-dye from the vesicles. We also found docking of synaptic vesicles and examined the distribution of these events against the time of stimulation. When calcium indicator was loaded into the cell, the sites of calcium entry could be defined as sites where the amplitude of fluorescence increase was large. We visualized vesicles and calcium entry sites from the same cell by applying two-color TIRF imaging, and examined the spatio-temporal relationship between those two sites.

(COI: No)

S10-2

2-photon FLIM analysis of SNARE assembly in neuron and endocrine cells

Takahashi, Noriko[1]; Sawada, Wakako[1]; Watanabe, Satoshi[1,2]; Noguchi, Jun[1]; Ohno, Mitsuyo[1]; Kasai, Haruo[1] ([1]*Dept Struct Physiol, Grad Sch med, Univ Tokyo, Tokyo, Japan;* [2]*Dept Bioengineering and Robotics, Grad Sch Engineering, Tohoku Univ, Sendai, Japan*)

We have investigated the SNARE assembly at the presynaptic terminals of neurons and plasma membranes of pancreatic beta cells, using two-photon fluorescence lifetime imaging (2p-FLIM) of Forster resonance energy transfer (FRET). We constructed the FRET probes of SNAREs, VAMP2 and syntaxin1 and SNAP25, by labeling with Turquoise (donor) or Venus (acceptor). In pancreatic beta cells, most SNAREs were unassembled in the plasma membranes, except for a small fraction (6%) of SNAP25 forming a binary complex with syntaxin. In contrast, a significant fraction (20%) of syntaxin formed the ternary trans-SNARE complexes in the boutons. Most trans-SNAREs were converted to cis-SNARE after exocytosis, and they were rapidly spread along axons by diffusion. The FRET ratio was correlated with the post-synaptic spine sizes and release probability. Thus, the SNARE configurations are diverse and regulated to allow the ultrafast exocytosis in the active zone, while slow exocytosis in the islet beta cells. There is huge diversity in resting SNARE complexes, and our FRET/2pFLIM enabled imaging of fusion readiness in intact live or chemically fixed secretory preparations.

(COI: No)

S10-3

Comprehensive functional analysis of Rab family small GTPases in dense-core vesicle exocytosis

Fukuda, Mitsunori (*Lab Membr Trafficking Mech, Grad Sch Life Sci, Tohoku Univ, Sendai, Japan*)

Rab-type small GTPases are key players in membrane trafficking, which underlies a variety of cellular events, including regualted secretion from secretory cells. Rabs function as switch molecules that cycle between two nucleotide-bound states, a GTP-bound active state and a GDP-bound inactive state, and the active Rabs drive various steps or types of membrane trafficking by recruiting their specific effector molecules. In mammals, more than 60 Rab isoforms have been reported, but because of their large numbers the precise functions of most mammalian Rabs remain largely unknown. To comprehensively analyze mammalian Rab isoforms, we have recently developed new tools, named "Rab panels", which include collections of Rab expression plasmids, siRNAs, and antibodies. By using these tools, we systematically screened for Rabs that are involved in dense-core vesicle exocytosis in neuroendocrine PC12 cells and succeeded in identifying three Rabs, Rab3A, Rab27A, and Rab33A, as dense-core vesicle-resident Rabs. We showed by TIRFM that two closely related isoforms, Rab3A and Rab27A, regulate the docking step of dense-core vesicles through interaction with their shared effectors, e.g., Slp4-a and rabphilin. By contrast, Rab33A is involved in both basal and regulated hormone secretion likely through interaction with an autophagic protein Atg16L1. We also applied these tools to other types of secreting cells and identified several cell-type specific secretory Rabs. Based on these results, we discuss the diversity and uniformity of Rab proteins in the regulation of various secretion events.

(COI: No)

S10-4

Study of Secretory Pathway Using Optogenetics

Nakata, Takao (*TMDU, Tokyo, Japan*)

Optogenetics is considered to be a study of neural circuit by regulating certain populationof neurons by channelrodopsin and light in vivo. We use optogenetics to study subcellular mechanism of cell signaling. Today we introduce our development of optogenetic tool and application to the study of secretory pathway in cell level.Ca is a champion of signaling molecules, in its diversity of the targets, number of researchers. In synaptic transmission, its speed is msec order, the vesicle size was small(50nm). Howeever, there are many other phenomenon slower. Endocrine cells such as insulin secreting cells, Ca was the 2nd messenger, and secretory vesicles are bigger. We made a tool which regulalte Ca concentration by light.Similar attempt was reported in USA but, it appeared to be too slow as a tool. We show the characterization of the switch and report on its application in cell biology.

(COI: No)

S10-5

Imaging of individual endocytosis of AMPA receptor around postsynaptic membrane

Hirano, Tomoo (*Dept Biophys, Grad Sch Sci, Kyoto Univ, Kyoto, Japan*)

AMPA-type glutamate receptors (AMPARs) dynamically change during synaptic plasticity. In the hippocampal long-term depression (LTD), the number of AMPARs decreases at the postsynaptic membrane. During LTD endocytosis of AMPAR takes place. However, when and where each subtype of AMPAR is endocytosed remains unclear. To address this question, we have developed an experimental method to visualize individual endocytosis of AMPAR with a high signal to noise ratio. We coated a glass coverslip with Neurexin, a cell adhesion molecule involved in synapse formation, and cultured hippocampal neurons on the coverslip. This procedure induced formation of postsynaptic-like membrane (PSLM) on the glass surface. Then, AMPAR whose extracellular domain is tagged with pH-sensitive fluorescent protein SEP is expressed in a neuron, and observed with total internal reflection microscopy. SEP is fluorescent at neutral pH, but not at low pH such as in the endosome. After endocytosis of SEP-AMPAR the fluorescent signal decreases as pH of intracellular vesicle gets lower, but it takes several seconds. We changed pH of extracellular solution quickly and repeatedly using U-tube. When pH of external solution is 6, the fluorescent signal from SEP-AMPAR on the cell-surface becomes undetectable, and only the fluorescent signal from SEP-AMPAR endocytosed during several seconds before the pH change to 6 was detected. We observed individual endocytosis of SEP-AMPAR both in the vicinity of PSLM and in the extra-synaptic membrane. Furthermore, a LTD-inducing chemical stimulation increased the frequency of individual endocytosis of SEP-AMPAR.

(COI: No)

Symposium 11

Expression, Structure and Function of Thermosensitive TRP channels

(March 21, 14:00～15:30, Room I)

S11-1

Thermosensitive TRP channel contributes to oral membrane protection

Kido, Mizuho A (*Dept.Molecular Cell Biology, Grad.Sch.Dent.Kyushu Univ., Fukuoka, Japan*)

The oral cavity provides an entrance to the alimentary tract, for which it serves as a protective barrier against environmental stimuli. The oral mucosa sense dynamic changes in the oral cavity; they are required to detect changes in the external environment and then the epithelia adapts to the environment structurally because of its location. However, the molecular basis of oral epithelial maintenance in response to the changes in the oral cavity is still not clear. We explored the expression of transient receptor potential (TRP) channels in oral epithelia and found the most abundant expression of TRPV3 and TRPV4 among thermosensitive TRP channels observed. Through calcium imaging and patch-clamp analyses, we confirmed that the oral epithelial cells express functional TRPV3 and TRPV4 with thermosensitive properties. Since we found more delayed wound healing after tooth extraction in TRPV3 gene deleted mice than in wild type mice, TRPV3 suggested a contribution to wound healing via EGFR signaling. Cell-cell adhesion is also important for epithelial integrity. We found impaired cell- cell contact in TRPV4 gene deleted mice, suggesting that TRPV4 contribute to the epithelial barrier in the oral membrane. Warm temperatures activated oral epithelia via TRPV3 and TRPV4, which suggested they play an important role in the epithelial barrier and its maintenance.

(COI: No)

S11-2

Temperature elevation in epileptogenic zone promotes epileptic events through TRPV4 activation

Shibasaki, Koji (*Dept. of Mol.Cell. Neurobiology, Gunma Univ. Grad. Sch.of Med., Maebashi, Japan*)

Physiological brain temperature is an important determinant for neuronal functions, and it is well established that changes in temperature have dynamic influences on brain neuronal excitabilities. We have clearly revealed that a thermo-sensor TRPV4 (activated above 34℃) is activated by physiological temperature in hippocampal neurons and thereby controls their excitability. Therefore, if local brain temperature could dynamically elevate depending on the neuronal activities, a thermo-sensor TRPV4 can enhance electrical excitability in neurons, and might lead to hyperexcitability. In this study, we focused on epilepsy, since it was caused by hyperexcitability of neurons. We generated a model of partial epilepsy by utilizing kindling stimuli in ventral hippocampus of wild type (WT) or TRPV4KO mice, and measured electroencephalogram (EEG). The frequencies of epileptic EEG in WT mice were significantly larger than those in TRPV4KO mice. These results strongly indicate that TRPV4 activation is involved in disease progression of epilepsy. We expected that the disease progression enhanced hyperexcitability, and lead to hyperthermia in the epileptogenic zones. To confirm it, we developed a new device to measure exact brain temperature only in restricted local area. From the recording results by the new device, we revealed that the brain temperatures in epileptogenic zones were dramatically elevated compared with normal regions. Furthermore, we demonstrated that the temperature elevation was critical for disease progression.

(COI: No)

S11-3

Roles of redox-sensitive TRPA1 in painful peripheral neuropathy induced by chemotherapy

Nakagawa, Takayuki[1,2]; Kaneko, Shuji[2] ([1]*Dept Clin Pharmacol & Ther, Kyoto Univ Hospital, Kyoto, Japan;* [2]*Dept Mol Pharmacol, Grad Sch Pharmaceu Sci, Kyoto Univ, Kyoto, Japan*)

Peripheral neuropathy is a common side effect of chemotherapeutics. Notably, oxaliplatin (L-OHP), a platinum-based agent, causes peculiar acute peripheral neuropathy, which appear in almost all patients during or within hours after infusion, and is triggered or exacerbated by cold, but the mechanisms are poorly understood. In this study, we examined the roles of redox-sensitive TRPA1 in L-OHP-induced acute peripheral neuropathy in mice. An i.p. administration of L-OHP or its metabolite oxalate induced cold hypersensitivity within 2 h, which was abolished by a TRPA1 antagonist or deficiency. TRPA1 agonist-evoked nocifensive behaviors were significantly enhanced in mice pretreated with L-OHP. Pretreatment of cultured mouse DRG neurons with L-OHP for 1-4 h increased the responsiveness of TRPA1, but not TRPM8 and TRPV1. In hTRPA1/HEK293 cells, high concentrations of L-OHP evoked a Ca^{2+} response and increased whole-cell currents, which were mediated through ROS production. On the other hand, pretreatment with relatively low concentrations of L-OHP for 2 h enhanced H2O2-evoked Ca^{2+} response and currents in hTRPA1/HEK293 cells. Furthermore, the L-OHP-enhanced responsiveness of TRPA1 was inhibited by co-expression of mutated proline hydroxylase (PHD)1-3 or disappeared in mutated TRPA1 of Pro394, a residue hydroxylated by PHDs. These results suggest L-OHP could sensitize TRPA1 function via dehydroxylation of Pro394 in TRPA1 N-terminal by inhibition of PHD activity, and the sensitized TRPA1 is activated by ROS production.

(COI: No)

S11-4

Modulatory mechanisms of TRP channels TRPA1/V1

Noguchi, Koichi (*Hyogo College of Medicine, Nishinomiya, Hyogo, Japan*)

It is well suggested that TRPA1 is an important component of the transduction machinery through which noxious irritants and endogenous proalgesic molecules depolarize nociceptors to elicit pain, in addition to the established effect of TRPV1 on noxious heat transduction. The regulatory mechanisms of TRPA1/V1 in persistent pain collect much attention, and we previously reported a short-term treatment of artemin, a GDNF family member, significantly suppressed the AITC-induced TRPA1 currents using a whole-cell patch clamp analysis, and suggested a rapid and inhibitory role of artemin in regulation of sensory neurons. Here, we examined the long-term effect of artemin on the gene expression. We found that artemin increases locally in skin over long periods of time after peripheral inflammation, and the synthesis of artemin increased at a site distal to the nerve injury. In vivo repeated artemin injections into the periphery increased the gene expression of TRPA1/V1 in DRG, and also induced mechanical and heat hyperalgesia. All data indicate the positive regulatory role of periphery-derived artemin on the TRPV1/A1 expression in DRG neurons in pathological conditions such as inflammatory and neuropathic pain. Next, we examined another inhibitory modulation of TRPA1/V1 by the resveratrol, which is widely contained in natural food and historical medicines. We found that resveratrol dose-dependently suppressed the AITC-induced currents in HEK cells that express TRPA1, as well as in rat DRG neurons. In conjunction with behavioral data, we could suggest that resveratrol may have an inhibitory effect on TRP channels.

(COI: No)

S11-5

A pain-enhancing mechanism through the interaction between TRPV1 and anoctamin

Tominaga, Makoto (*Div Cell Signaling, Okazaki Inst Integrative Bioscience, Okazaki, Japan*)

Capsaicin receptor TRPV1 is activated by various noxious stimuli, and converted the stimuli into electrical signals in primary sensory neurons. It is believed that cation influx through TRPV1 causes depolarization, leading to the activation of voltage-gated sodium channels, followed by action potential generation. We report that the apparent capsaicin-evoked firing is induced by two components: a cation influx-mediated depolarization due to TRPV1 activation and a subsequent anion efflux-mediated depolarization via activation of anoctamin 1 (ANO1), a calcium-activated chloride channel, due to the entry of calcium through TRPV1. This interaction between TRPV1 and ANO1 is based on the physical binding of the two proteins. Capsaicin activated chloride currents in an extracellular calcium-dependent manner in HEK293T cells expressing TRPV1 and ANO1, and capsaicin-evoked inward currents were significantly inhibited by a specific ANO1 antagonist, T16Ainh-A01 (A01) in mouse DRG neurons. In addition, capsaicin-evoked action potential generation was drastically inhibited by A01. Furthermore, pain-related behaviors in mice treated with capsaicin were significantly reduced by the concomitant administration of A01. These results indicate that the TRPV1-ANO1 interaction is a significant pain-enhancing mechanism in the peripheral nervous system. Therefore, the TRPV1-ANO1 interaction would be a promising target for the development of novel analgesic agents.

(COI: No)

Symposium 12

Frontier of the structural and functional investigation of the kidney

(March 21, 14:00～15:30, Room J)

S12-1

Ciliary subdomains and abnormality in the kidney of inv mutant mice

Yokoyama, Takahiko[1]; Tsuji, Takuma[1,2] ([1]*Grad. Sch. Med. KPUM, Kyoto, Japan;* [2]*Grad. Sch. Med. Nagoya univ. Nagoya, Japan*)

Primary cilia in the kidney are a hair like structure projecting from the surface of nearly all cells. Inside of the cilia is the axoneme that is consisting of 9 circumferentially arranged microtubule doublets. The cilia are structurally divided longitudinally into sub-compartments that include the rootlet, the basal body, the transitional zone, the ciliary shaft and the tip. Nephronophthisis is a most common genetic disease that causes the end-stage renal failure. Sixteen causative genes have been identified, and all examined products are localized in the primary cilia and/or basal body. The INV compartment is a proximal region of the ciliary shaft in which inv/nephrocystin2 is localized.In the presentation, we show that the doublet of the axoneme is surprisingly short in renal cilia and that the region is likely to corresponding to the Inv compartment. The length of the microtubule doublet region is not altered in the inv mutant. However, in the inv mutants, the ciliary rootlets are not well developed and singlet microtubules of the axoneme are turbulent.

(COI: No)

S12-2

Role of calcium sensing receptor (CaSR) in type-B intercalated cell of mouse kidney collecting duct during acid/base and Ca salts-loadings

Yasuoka, Yukiko[1]; Kawahara, Katsumasa[1]; Sato, Yuichi[2]; Nonoguchi, Hiroshi[3]
([1]*Dept of Physiology, Kitasato U. Sch. of Med, Sagamihara, Kanagawa, Japan;* [2]*Dept of Mol. Diagnostics, Kitasato U. Sch. of Allied Health Sci, Sagamihara, Kanagawa, Japan;* [3]*Internal Med., Kitasato U. Medical Center, Kitamoto, Japan*)

It is believed that hypercalciuria stimulates the urinary acid excretion in type-A intercalated cell (IC-A) and inhibits luminal water permeability in principal cell (PC) to prevent urolithiasis through activation of apical calcium-sensing receptors (CaSR) in collecting ducts (CD). However, we found that CaSR, genetically same as RaKCaR in TAL, only localized in the basolateral membrane of type-B intercalated cell (IC-B), not at PC and IC-A through the CD by using a high sensitive in situ hybridization technique and immunohistochemistry (Yasuoka et al. 2014). The levels of CaSR mRNA and protein expression in IC-B were increased and decreased, respectively, during alkali- and acid-loading. The CaSR of IC-B may contribute to alkali excretion. On the other hand, neutral high calcium ($CaCO_3$ + Ca phosphate) loading for 28 days decreased urine pH, and the expression levels of H^+-ATPase, AE1 mRNA in IC-A and Pendrin, CaSR mRNA in IC-B increased co-operatively. High Ca diet (neutral CaP/CaC salts) promotes co-operative activation of IC-A and IC-B for increasing urinary acid and alkali excretion. The basolateral CaSR of IC-B may maintain plasma acid-base balance in accordance with preventing urolithiasis during high Ca diet.

(COI: No)

S12-3

Regulation of podocyte structure and function: roles of Rho family proteins and their modulators

Nagase, Miki; Sakai, Tatsuo (*Grad.Sch.Med.Juntendo Univ., Tokyo, Japan*)

Rac1, a member of the Rho-family small GTPases, regulates diverse cellular functions, including organization of the actin cytoskeleton (formation of lamellipodia and membrane ruffles), cell adhesion and motility, generation of reactive oxygen species, and gene transcription. Recent studies implicate a role of Rac1 overactivation in podocyte injury and glomerulosclerosis. Rac1 activity is enhanced in podocytes by proteinuric stimuli such as angiotensin II, puromycin aminonucleoside, lipopolysaccharide, diabetic condition, and HIV infection, causing motile phenotype and foot process effacement. We reported that mice deficient in RhoGDI α, a negative regulator of Rac1, resulted in Rac1 overactivation in the kidney, and spontaneously developed Rac1-dependent podocyte injury and focal glomerulosclerosis. In these mice, Rac1 potentiated the activity of mineralocorticoid receptor (MR) in a ligand-independent manner, thereby accelerating podocyte injury. We subsequently demonstrated the crosstalk of Rac1 and MR pathways in several kidney injury models. Podocyte-specific RhoGDI α knockout mice exerted podocyte injury similar to systemic knockout mice. Again, MR antagonist perfectly ameliorated the injury. On the other hand, podocyte-specific Rac1 depletion lead to podocytopathy and glomerulosclerosis by a different mechanism, because MR signaling was suppressed and MR antagonist was ineffective in these mice. These findings suggest that proper level of Rac1 activity is essential for the morphological and functional integrity of podocytes.

(COI: No)

S12-4

The role of podocyte injury in progression to glomerulosclerosis

Asanuma, Katsuhiko (*Kyoto Univ. Grad. Sch. Med., Kyoto, Japan*)

Podocytes, are highly specialized glomerular visceral epithelial cells that cover the outer layer of the glomerular basement membrane (GBM). Based on their cytoarchitecture, podocytes may be divided into three structurally and functionally different segments: cell body, major processes, and foot processes (FPs) that attach to the underlying GBM. The FPs of neighboring podocytes regularly interdigitate, leaving between them filtration slits that are bridged by an extracellular structure, known as slit diaphragm (SD). Therefore, podocytes form the final barrier to protein loss, which explains why podocyte injury is typically associated with marked proteinuria. Chronic podocyte injury may cause detachment from the GBM, resulting in depletion. FP effacement represents the most characteristic change in cell shape of injured podocytes. FP effacement depends on the disruption of both the actin cytoskeletal network, and the SD in the podocytes. This symposium highlights some of our recent findings for translating podocyte biology into new therapies and examinations of podocyte injury.

(COI: No)

Symposium 13

Space Medicine I : Living with Gravity

(March 21, 15:30～17:00 Room B)

S13-1

Skeletal muscle plasticity in response to gravitational loading

Goto, Katsumasa (*Dept Physiol, Grad Sch Health Sci, Toyohashi SOZO Univ, Aichi, Japan*)

Human skeletal muscle system has evolved by adapting to the environment of a gravity. Therefore, the functional and structural properties of skeletal muscles, especially antigravitational soleus muscle, change in response to gravitational level. For example, overloading causes to increase in skeletal muscle mass, so-called muscle hypertrophy. On the other hand, skeletal muscle atrophy is induced by unloading as well as aging. It is well known that these changes in skeletal muscle mass are reversible. Although skeletal muscle exhibits a large plasticity in response to gravitational levels, the molecular mechanism(s) for loading-dependent adaptation of skeletal muscle is not fully elucidated. It has been generally accepted that muscle satellite cells, a skeletal muscle-specific stem cell, play a crucial role in skeletal muscle plasticity, especially the regeneration of injured skeletal muscle cells. Muscle satellite cells are activated by muscle injury, and consequently proliferate and fuse to injured muscle cells or form a new muscle fiber. However, unloading prevent the proliferation of muscle satellite cells in injured skeletal muscle. The observations suggest that muscle satellite cells have a sensitivity to gravitational level. This study was supported, in part, by Grants-in-Aid for Scientific Research from JSPS, Grants-in-Aid for Challenging Exploratory Research from JSPS, The Uehara Memorial Foundation, and The Naito Foundation.

(COI: No)

S13-2

Unique epigenetic properties in anti-gravitational muscle

Kawano, Fuminori (*MSPA, Grad Sch Med, Osaka Univ, Japan*)

Slow skeletal muscle, such as soleus, shows a tonic neural activity to maintain the posture against gravity, so-called anti-gravitational muscle. Acquisition of the characteristics of slow muscles is resulted from such muscular activity during the growing period. It is well known that loss of the gravitational load causes a shift of muscle properties toward faster phenotype and metabolism, as well as the sever atrophy. However, it is unknown what is an essential factor accounted for the ontogeny of slow muscle characters. Histone modification, known as one of the epigenetic regulations of gene transcription, plays a critical role for the transcriptional onset via the conformational change of chromatins. We have identified the major differences of histone modification between slow- and fast-twitch skeletal muscles by using ChIP-seq. Generally, it is known that transcriptionally active histone modification, H3K4me3 and H3 acetylation, maps near the transcription start site. In fast-twitch plantaris muscle of rat, this typical pattern was noted in the loci of activated genes. However, genome-wide analysis by ChIP-seq also revealed that no relationship was observed between the expression of specific genes and active histone marks in slow-twitch soleus muscle. We also found that the up-regulation of slow genes in plantaris muscle, which are related to enhanced muscular activity, were not associated with additional active modifications. These findings indicate that the anti-gravitational, slow-twitch, muscle has a unique set of histone modification, may be due to the muscular activity present under gravity.

(COI: No)

S13-3

Effects of hypergravity on coordinated left and right arm movements

Wada, Yoshiro (*Dept Otolaryngology, Nara Med, Univ, Kashihara, Japan*)

We can move left and right arms symmetrically even with no visual feedback under various conditions because the brain generates appropriate motor commands to each arm. To examine the contribution of sensory inputs and the central nervous system (CNS) to these coordinated arm movements, the horizontal symmetric reaching arm movements (finger-finger docking tasks) were conducted with/without a weight on one arm (0.5 kg and 1.0 kg) under one- and two-gravity conditions in six normal subjects. The main results were: 1) the arm with a weight went down compared to the control arm under one-gravity condition; 2) on the contrary, the arm with a weight went up compared to the control arm under two-gravity condition. Those observations cannot be explained by somatosensory inputs. We will discuss the contribution of otolith inputs and/or the CNS to those results based on data from additional experiments.

(COI: No)

S13-4

Influence of the gravitational acceleration on body balance and gaze stability during walking

Hirasaki, Eishi (*Primate Res. Inst. Kyoto Univ., Inuayama, Japan*)

We have been studying body, head and eye movements during walking gait to understand the strategies for controlling posture and body balance during locomotion. Our results revealed that the coordinated motions of the eyes and head play important roles in maintenance of gaze stability. For example, when the humans walk, the body moves up and down, according to the reciprocal motions of the legs. These movements, which disturb clear vision, are partly compensated by head pitch rotation. The facts that the compensatory head rotations are deteriorated in the patients with vestibular disfunctions and in the postflight astronauts suggest the idea that it is induced by the vestibular signals via the linear vestibulocollic reflex. As a consequence of the compensatory head rotation, lines representing the naso-occipital axis of the head intersect at approximately a common point during walking, which is referred to as the "Head fixation point". It is constantly located approximately 1m in front of the head, and provides a stable platform on which the angular vestibuloocular reflex does the rest of the work to maintain gaze. During curved walking, things are more complicated due to visual flow and tilt of the gravito-inertial axis (GIA). The former induces the eye nystagmus, but coordinated motions of head yaw and slow phase of eye movements intermittently stabilize gaze in yaw plane. In coronal plane, the head tilts in the same direction of tilts of GIA. The head tilt increases as walking speed increases, independently from body tilt, suggesting that the head tilt is induced by the tilt of GIA via otolith input.

(COI: No)

S13-5

Variation of orthostatic arterial pressure response related to vestibular function

Tanaka, Kunihiko[1]; Nakamura, Koji[2]; Abe, Chikara[3]; Morita, Hironobu[3] ([1]*Dept Radiol Tech, Gifu Univ Med Sci, Seki, Japan;* [2]*Dept Med Tech, Gifu Univ Med Sci, Seki, Japan;* [3]*Dept Physiol, Grad Sch Med, Gifu Univ, Gifu, Japan*)

The vestibular system contributes to determination of the body orientation with respect to gravity. We clarified that maintenance of mean arterial pressure (MAP) at the onset of head-up tilt (HUT) is controlled by the vestibular system. However, changes in MAP at the onset of HUT vary among each subject. Some subjects show increase in MAP (UP), compared with that during supine position, but the others show decrease (DOWN). In healthy subjects, balance between the left and right otolith function is involved in the difference. To investigate the vestibular function for autonomic control of circulatory system in detail, heart rate variability (HRV) was measured with applying galvanic vestibular stimulation (GVS), which stimulates vestibular nerves from both semicircular canals and otolith organs. Changes in high frequency component of HRV (HF), an index of parasympathetic nerve activity, was inversely correlated with changes in MAP at the onset of HUT. Thus, parasympathetic nerve activity might be relatively higher in DOWN subjects at the onset of HUT, compared with that in UP subjects. Furthermore, changes in HF were also correlated with the changes in MAP with applying sound pressure, which stimulates only otolith organs. Those observations suggest presence of vestibulo-parasympathetic reflex, and the reflex is involved in variability of changes in MAP at the onset of HUT.

(COI: No)

Symposium 14

Sensory and motor mechanisms regulating feeding behavior

(March 21, 15:30～17:00, Room D)

S14-1

The roles of oral-brain-gut interaction in detection, transmission and modulation of taste signals and regulation of food intake

Ninomiya, Yuzo[1,2]; Takai, Shingo[1]; Yoshida, Ryusuke[1]; Shigemura, Noriatsu[1] (*[1]Sect Oral Neurosci, Grad Sch Dental Sci, Kyushu Univ, Fukuoka, Japan; [2]Div Sens Physiol, Res Dev Center for Taste and Odor Sensing, Kyushu Univ*)

In the brain, leptin reduces food intake by acting on hypothalamic receptor, Ob-Rb and endocannabinoids increase food intake by acting on cannabinoid CB_1 receptors in hypothalamus, limbic forebrain and brainstem. These anorexigenic and orexigenic mediators also modulate peripheral sweet taste sensitivity. Leptin selectively inhibits behavioral, taste nerve and taste cell responses to sweet compounds whereas endocannabinoids enhance sweet taste responses. However, potential roles of endogenous leptin and endocannabinoids in sweet taste still remain unclear. We used pharmacological antagonists for Ob-Rb (LA) and CB_1 (AM251) and examined effects of their blocking activation of endogenous leptin and endocannabinoid signaling on taste responses in lean control, leptin receptor deficient *db/db*, and diet induced obese (DIO) mice. Our results suggest that circulating leptin, but not local endocannabinoids, may be a dominant modulator for sweet taste in lean mice; however, endocannabinoids may become more effective modulators of sweet taste under conditions of deficient leptin signaling. In the gut, enteroendocrine cells express sweet taste receptor. Mouse enteroendocrine cell line STC-1 also expressed sweet taste receptor and their responses to sweet compounds were affected by leptin and endocannabinoids similar to sweet taste cells. Thus, leptin and endocannabinoids may regulate food intake and energy homeostasis via the oral-brain-gut axis.

(COI: No)

S14-2

Oscillation and synchronization of neuronal activity in the insular cortex implicated in the feeding behavior

Kang, Youngnam; Toyoda, Hiroki; Saito, Mitsuru; Sato, Hajime; Kawano, Tsutomu (*Dept Neurosci & Oral Physiol, Osaka Univ Grad Sch Dent, Osaka, Japan*)

The taste sensation arising from the taste cells in the tongue that express G-protein-coupled receptors is processed in the gustatory region of the insular cortex, while the chemosensation arising from the enterocytes in the gastrointestinal tract that express similar G-protein-coupled receptors is likely to be processed in the gastrointestinal region of the insular cortex. We found an oscillatory synchronization between the neuronal populations in the gustatory and gastrointestinal regions of the insular cortex, which may be crucial in the regulation of feeding behavior.

(COI: No)

S14-3

Cerebral cortical projections to trigeminal premotoneurons controlling jaw-movements in rats

Yoshida, Atsushi; Sato, Fumihiko; Ohara, Haruka; Fujio, Takashi; Tsutsumi, Kanako; Kato, Takafumi (*Dept. of Oral Anatomy and Neurobiology, Grad. Sch. Dent. Osaka Univ., Osaka, Japan*)

In rats, the trigeminal premotoneurons (interneurons directly projecting to the trigeminal motor nucleus [Vmo] which contains jaw-closing [JC] and jaw-opening [JO] motoneurons) were widely distributed in the intertrigeminal region (Vint), trigeminal mesencephalic nucleus (Vmes), reticular formation medial to the JO component of the Vmo (rmJO), juxtatrigeminal region (Vjuxt), trigeminal oral subnucleus (Vo), and solitary tract nucleus (Sol). The Vint and Vmes mainly contained the JC premotoneurons while the rmJO mainly contained the JO premotoneurons. The Vjuxt, Vo and Sol contained both types of the premotoneurons. The Vint received projections mainly from the lateral part of agranular cortex (Agl) which possibly corresponds to the primary somatomotor cortex (M1) while the rmJO received projections mainly from the medial part of agranular cortex (Agm); the Agm possibly corresponds to the secondary somatomotor cortex (M2) and is also considered to be a part of the prefrontal cortex involved in the autonomic and limbic function. The Vjuxt and Vo received projections mainly from the primary somatosensory cortex (S1) and Agl. The Vmes received projections mainly from the lateral part (insular cortex) and medial part of the prefrontal cortex while the Sol from the insular cortex. These findings suggest that the jaw-movements are regulated through the three types of trigeminal premotoneuron areas by the somatic sensorimotor cortex and the prefrontal cortex.

(COI: No)

S14-4

Properties of neuronal circuitry composed of supratrigeminal premotor neurons and trigeminal motoneurons

Inoue, Tomio; Nakamura, Shiro; Nakayama, Kiyomi; Mochizuki, Ayako; Yoshida, Atsushi; Kiyomoto, Masafumi (*[1]Dept Oral Physiol, Sch Dent, Showa Univ, Tokyo, Japan; [2]Dept Oral Anat, Grad Sch Dent, Osaka Univ, Osaka, Japan*)

Feeding is one of the most important survival functions for mammals. To understand neural mechanisms underlying jaw motor function during feeding, we examined properties of neuronal circuitry composed of supratrigeminal (SupV) premotor neurons and trigeminal motoneurons in rat brainstem slice preparations. SupV premotor neurons targeting the trigeminal motor nucleus (MoV) were detected on the basis of antidromic responses to electrical stimulation of the MoV using Ca^{2+} imaging and whole-cell recordings. The premotor neurons were divided into 2 groups according to their discharge patterns of the steady-state responses to 1 s current pulses: those firing higher (HF neurons) or lower (LF neurons) than 33 Hz. Intracellular labeling revealed that the axons of all HF neurons entered the MoV from its dorsomedial aspect, whereas the axons of half of the LF neurons entered the MoV from its dorsolateral aspect. Furthermore, the dendrites of a half of HF neurons penetrated into the principal sensory trigeminal nucleus, whereas the dendrites of all LF neurons were confined within the SupV. Laser photolysis of caged glutamate in the SupV induced burst excitatory postsynaptic currents especially in jaw-closing motoneurons. These results suggest that the SupV premotor neurons targeting the MoV with different firing properties have different dendritic and axonal morphologies, and these SupV neuron classes may play distinctive roles in suckling and chewing.

(COI: No)

S14-5

Effects of pharyngeal electrical stimulation on masticatory performance

Inoue, Makoto; Takeishi, Ryosuke; Hayashi, Hirokazu; Magara, Jin; Tsujimura, Takanori; Watanabe, Masahiro (*Div Dysphagia Rehab, Niigata Univ Grad Sch Med Dent Scis, Niigata, Japan*)

Purpose: The aim of this study was to examine the effects of repeated pharyngeal electrical stimulation on swallowing performance in healthy humans.
Method(s): Ten minutes pharyngeal electrical stimulation (5Hz, 1ms pulse duration) was applied in 9 healthy adults once/day for 5 days. The effects of stimulation were evaluated both on voluntary and involuntary swallowing behavior. For the effects on voluntary swallowing, the repetitive saliva swallowing test (RSST) was used, in which subjects were instructed to swallow their own saliva as quickly as possible for 30 sec and the number of swallows was counted. Changes in involuntary swallow performance was measured with the swallowing response time (SRT), where water was injected repeatedly into the pharynx at 0.1 ml/sec and the initiation latency of first swallow was measured. RSST and SRT were recorded before stimulation (baseline) and every 10 minutes up to an hour after 10-minutes stimulation.
Result(s): While SRT was not affected by pharyngeal stimulation, the number of swallows in RSST significantly increased at 60 minutes. In addition, 5-day stimulation resulted in gradual increase of number of swallows in RSST.
Conclusion: Current results suggest that repeated pharyngeal stimulation can lead to neuroplastic changes in the cortical excitability responsible for swallowing initiation.

(COI: No)

Symposium 15

Recent progress in differentiation and regeneration of vessels

(March 21, 15:30〜17:00, Room E)

S15-1

Vascular morphogenesis between the brain and spinal cord in zebrafish

Kimura, Eiji (*Iwate Med. Univ., Iwate, Japan*)

Zebrafish (*Danio rerio*) is an excellent model organism to investigate developmental process of the initial vascular formation during early ontogeny because of its transparent body and exo-utero development, and it contributed to show how the cranial and truncal vasculatures were formed over the past decade. These vasculatures developed individually and conjugated at their border. However, the stepwise process bridging these systems are not uncovered enough. In this study, we demonstrated how the vascular systems of the brain and spiral cord, that is, how internal carotid arteries and vertebral arteries were integrated via basilar artery using time-lapse imaging of living transgenic zebrafish embryos, in which endothelial cells specifically expressed the EGFP. As a result, we succeeded to show the first connecting process that the primordial hindbrain channel and basilar artery extended caudally and bridged with dorsal longitudinal anastomose vessel via first intersegmental arteries. The primary connection was soon remodeled and finally internal carotid arteries were integrated with the vertebral arteries via basilar artery. The vascular cast with micro-resin revealed that the basal vasculature was conserved in adult fish. Furthermore, we confirmed the primary vascular connection was not influenced by flow dynamics, and this leads us to propose the vascular integration in this region is also controlled by genetic cues such as in the truncal region. Our morphological data of vascular formation between the brain and spinal cord will help us to understand its regulatory mechanisms.

(COI: No)

S15-2

Unveiling the cellular and molecular mechanism of vascular development by fluorescence-based bio-imaging in zebrafish

Fukuhara, Shigetomo; Mochizuki, Naoki (*Dept. Cell Biol., Natl. Cereb. Cardiovasc. Res. Inst., Osaka, Japan*)

Vascular networks develop through two distinct processes; vasculogenesis and angiogenesis. Vasculogenesis is defined as the formation of primitive vascular plexus, while angiogenesis refers to the subsequent growth and expansion of developed blood vessels. Formation of functional vasculature also requires lumen formation, arterial venous specification and pericyte coverage. However, the cellular and molecular mechanisms of vascular development in vivo remain largely unknown, because a method of addressing these questions has not been established. To overcome this problem, we have adopted fluorescence-based bio-imaging techniques using zebrafish as a model animal. To investigate the cellular and molecular mechanisms of vascular development, we have developed the transgenic zebrafish lines in which endothelial cells express various types of fluorescence-based biosensors. Those have enabled us to simultaneously visualize cellular structure, including cytoskeleton and cellular signaling, including activity of various signaling molecules and transcription factors. By performing live imaging of these transgenic lines, we successfully visualized cell-cycle progression of endothelial cells during vascular development, delineated the signaling pathways underlying the endothelial cell migration during angiogenesis and demonstrated a crucial role of beta-catenin-mediated transcription in the development of venous vessels. In this symposium, we will introduce how fluorescence-based bio-imaging technique can be exploited for vascular biology research.

(COI: No)

S15-3

Construction of biological elastic vessels by extracellular matrix nanofilm-based cell accumulation technique

Yokoyama, Utako[1]; Ishiwata, Ryo[1]; Matsusaki, Michiya[2]; Akashi, Mitsuru[2]; Ishikawa, Yoshihiro[1] ([1]*Cardiovascular Research Inst., Yokohama City Univ.;* [2]*Graduate School of Engineering, Osaka Univ.*)

Background: Construction of biological artificial vessel with high elasticity is not currently feasible within short period, and biological arterial grafts have not been available in clinical. We previously fabricated vascular smooth muscle cells (SMCs) into three-dimensional cellular multilayers (3DCMs) using a hierarchical cell manipulation technique, in which cells were coated with fibronectin-gelatin nanofilms to provide adhesive nano-scaffolds. Based on these results, we aimed to make modifications on 3DCMs to obtain biological arterial graft with high elasticity.
Methods and Results: Elastica stain and electron microscopic analysis demonstrated that 3DCMs, which consisted of seven layers of neonatal rat aortic SMCs cultured in 1% fetal bovine serum in DMEM, exhibited layered elastic fibers within 7 days of being in a static culture condition. Radioimmunoassay using [^{3}H]valine confirmed the greater amount of cross-linked elastic fibers in 3DCMs than in monolayered SMCs. However, the 3DCMs did not show measurable elasticity. We then preincubated SMCs at hyperconfluency for 5 days, followed by hierarchical cell manipulation for 7 days, and found that more differentiated SMCs provided 3DMCs with high elasticity, in which 3DCMs could be stretched more than two times in length.
Conclusion: The use of an extracellular matrix nanofilm-based cell accumulation technique provided biological elastic arterial graft within 10 days of static culture condition.

(COI: No)

S15-4

Realization of iPSC-organ bud transplantation therapy

Takebe, Takanori[1,2]; Taniguchi, Hideki[1] ([1]*Dept Reg Med, Grad Sch Med, Yokohama City Univ, Yokohama, Japan;* [2]*PRESTO, JST*)

A critical shortage of donor organs for treating end-stage organ failure highlights the urgent need for generating organs from human induced pluripotent stem cells (iPSCs). Despite many reports describing functional cell differentiation, no studies have succeeded in generating a three-dimensional vascularized organ such as liver. Towards this end, we recently established a proof-of-principle approach to grow a vascularized and functional human organ from iPSCs by transplanting in vitro-derived organ bud into immunodeficient animal (T. Takebe et al. Nature 499 (7459), 481-484, 2013). With this technology, a transplantable iPSC-derived liver bud (rudimentary liver) could be self-organized from mixed human progenitors under a specific 3-D culture platform by recapitulating early organogenetic cellular interactions. Here, I will summarize the concept and adaptability of organ bud transplantation therapy, and and share the state-of-art technology currently used by us in mass production, quality evaluation, and optimal transplantation strategies towards clinical translation. Given the unsatisfactory clinical outcomes of the cell-based therapies that are currently the main goal of stem cell therapy, this proof-of-principle could revolutionize the application of regenerative medicine in the treatment of end-stage organ failure. This technique could also elucidate aspects of human developmental biology and disease modelling, and could provide a drug-screening platform.

(COI: No)

S15-5

Generation of in vitro vascular disease models using disease-specific iPS cells

Osafune, Kenji (*CiRA.Kyoto Univ., Kyoto, Japan*)

Disease modeling research using patient-derived iPS cells has been carried out with various intractable disorders. Notably, it has already been demonstrated that the iPS disease model can provide a platform for studies aiming at both understanding pathological mechanisms and discovering new drug compounds. Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent, potentially lethal, monogenic disorder, characterized by the development of multiple renal cysts and various extrarenal manifestations. Cardiovascular complications are the main cause of death in ADPKD and intracranial aneurysms, causing subarachnoid hemorrhage, are among the most serious. The pathogenesis of vascular lesions as well as cyst formation remains largely unknown. We derived iPS cells from seven ADPKD patients, among whom four had intracranial aneurysms. These iPS cells differentiate into vascular endothelia and smooth muscle cells in vitro, which recapitulate the defective intracellular Ca2+ regulation, similar findings to those reported in vascular cells of mouse ADPKD models. Furthermore, by microarray analyses, we have identified several molecules whose expression levels are specifically altered in the iPS cell-derived vascular cells from ADPKD patients and in those from ADPKD patients with aneurysms. We are currently examining the diagnostic performance of the molecules using clinical samples. These results suggest that vascular cells differentiated from patient-derived iPS cells can be used for studying the pathogenic mechanisms of vascular diseases and for identifying possible biomarkers.

(COI: Properly Declared)

Symposium 16

Zinc signaling: An emerging regulatory system in physiology and pathogenesis

(March 21, 15:30～17:00, Room F)

S16-1

Essential role of zinc transporter-mediated zinc signaling in lymphocyte homeostasis and immunity

Fukada, Toshiyuki[1,2] (*[1]Pathology, Showa Univ Sch Dentistry, Tokyo, Japan; [2]RIKEN-IMS*)

Zinc is an essential trace element required for a variety of cellular functions and molecular events. Zinc homeostasis is controlled by zinc transporters, channels and metallothioneins, and their loss or gain of functions cause serious health problems. Recent advances of experimental approaches have unlabeled that zinc ion mediated by transporters/channels/metallothioneins acts as a signaling factor recognized as **zinc signal**, which participates in regulation of numbers of cellular phenomena, thereby in health and disease conditions. This symposium will aim to share the updated information about the significant roles of zinc signaling in cellular functions and diseases, and to discuss about the next directions and problems to be solved.
I will address that zinc transporter ZIP10-mediated zinc signaling is essential for B-cell homeostasis and related immune responses, and discuss that zinc signal selectively controls signal transduction pathways that may help us understanding the role of zinc signaling in physiology and pathogenesis.

Reference:
Proc Natl Acad Sci USA 2014; 111:11780-11785 Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development.
Proc Natl Acad Sci USA 2014; 111:11786-11791 Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength.
Zinc Signals in Cellular Functions and Disorders Fukada and Kambe (eds): Springer, 2014

(COI: No)

S16-2

Zinc deficiency and cutaneous immunity

Kawamura, Tatsuyoshi (*Dept Dermatol., Univ Yamanashi, Yamanashi, Japan*)

Zn deficiency can be inherited or acquired. It has many clinical manifestations, including bacterial and fungal infection of skin lesions due to impaired immune function. Despite their impaired immune function, Zn-deficient individuals develop skin inflammation (Acrodermatitis enteropathica) through an unknown mechanism. We have found that, despite diminished allergic contact dermatitis in mice fed a zinc-deficient (ZD) diet, irritant contact dermatitis (ICD) in these mice was more severe and prolonged than in zinc-adequate mice. Histological examination of ICD lesions in ZD mice revealed subcorneal vacuolization and epidermal pallor, histological features of AE. Nucleotides released from chemically-injured keratinocytes are known to serve as a causative mediator for ICD. We found that the severe ICD response in ZD mice was significantly attenuated by local injections of soluble NTPDase. In addition, the zinc-chelating reagent TPEN significantly increased ATP levels released from cultured keratinocytes treated with chemical irritants in vitro. Moreover, ZD mice exhibited significantly decreased levels of TGF-b1 in the epidermis and loss of epidermal Langerhans cells (LC), known to play a protective role against ATP-mediated inflammatory signals by hydrolyzing extracellular nucleotides. The clinical significance of these mouse data was highlighted by the observation that Zn-deficient individuals lacked epidermal LCs. Thus, our findings suggest that ATP released from injured keratinocytes, which accumulates because there are insufficient epidermal LCs to hydrolyze it, causes the skin inflammation observed in Zn-deficient individuals.

(COI: No)

S16-3

SOD1 as a molecular switch for initiating the homeostatic ER stress response under zinc deficiency

Ichijo, Hidenori; Homma, Kengo (*Lab. Cell Signaling, Grad. Sch. Pharm. Sci., Univ. of Tokyo*)

Zinc is an essential trace element, and impaired zinc homeostasis is implicated in the pathogenesis of various human diseases. However, the mechanisms cells use to respond to zinc deficiency are poorly understood. We previously reported that amyotrophic lateral sclerosis (ALS)-linked pathogenic mutants of SOD1 cause chronic endoplasmic reticulum (ER) stress through specific interactions with Derlin-1, which is a component of the ER-associated degradation machinery. Moreover, we recently demonstrated that this interaction is common to ALS-linked SOD1 mutants, and wild-type SOD1 (SOD1WT) comprises a masked Derlin-1 binding region (DBR). Here, we found that, under zinc-deficient conditions, SOD1WT adopts a mutant-like conformation that exposes the DBR and induces the homeostatic ER stress response, including the inhibition of protein synthesis and induction of a zinc transporter. We conclude that SOD1 has a function as a molecular switch that activates the ER stress response, which plays an important role in cellular homeostasis under zinc-deficient conditions.

(COI: No)

S16-4

Zinc starvation-induced autophagy in yeast

Kawamata, Tomoko; Horie, Tetsuro; Matsunami, Miou; Ohsumi, Yoshinori (*Frontier Research Center, TITECH*)

Transition metal ions such as iron, copper, zinc, and manganese are essential nutrients for every organism. Among them, zinc is abundant, essential transition metal to all forms of life. Most cellular zinc seems to be bound to intracellular ligands, such as protein. More than 5 % proteins are reported as zinc binding protein. Zinc serves as a catalytic and/or structural cofactor for many proteins. For this reason abnormal zinc homeostasis causes serious problems, including cell growth. When available zinc becomes scarce, cell must properly partitioning intracellular zinc to be prioritized. Autophagy is evolutionally conserved, cellular degradation/recycling process whereby cytoplasmic proteins and organelles are sequestered for degradation in the vacuole/lysosome. We found that autophagy has an important role to support growth under zinc starvation in yeast, which suggests that autophagy contributes to zinc ion homeostasis. Indeed, zinc depletion induces autophagy. In this symposium, we will discuss zinc economy and autophagy, and molecular mechanisms for induction of autophagy under zinc starvation in yeast.

(COI: No)

S16-5

A wide range of cellular functions of zinc transporters in the secretory pathway

Kambe, Taiho (*Grad.Sch.Biostudies.Kyoto Univ., Kyoto, Japan*)

Zinc is an essential trace element life, because it plays a pivotal role as a structural, catalytic and regulatory element in protein functions. Thus, zinc homeostasis is tightly controlled through the highly integrated processes of zinc uptake, sequestration and efflux across the cell membrane, in which zinc transporters are essential in these processes. Two solute carrier transporters, Zn transporter (ZnT) and Zrt, Irt-like protein (ZIP), primarily control zinc transports, and enable various zinc-dependent proteins functions and signalings to play physiologically important roles in numerous biological processes. A number of studies have shown that zinc mediated by ZnT and ZIP transporters has specific crucial roles in numerous cellular events. However, the molecular mechanisms have virtually remained unknown. Here, I would like to discuss this point using a model of the activation process of zinc-requiring enzymes by ZnT transporters localized to the secretory pathway. To clarify how zinc transporters mobilize zinc to a specific target protein and functions in a specific physiological role would move zinc signaling research to the next phase, and may lead to therapeutic progress.

(COI: No)

Symposium 17

Role of the auditory cortex in hearing

(March 21, 15:30～17:00 Room G)

S17-1

Anatomical study on neural circuits of the mouse insular auditory field

Takemoto, Makoto; Hasegawa, Kayoko; Song, Wenjie (*Dept Sens Cogn Physiol, Grad Sch Med Sci, Kumamoto Univ, Kumamoto, Japan*)

The auditory cortex is divided into several subfields based on their response properties. The role of each subfield is a most intriguing issue in the research on the auditory cortex, and neuronal connectivities of these subfields can help gain an insight into their characteristic functions. We have recently identified an insular auditory field (IAF) in mice. To reveal the neural circuits of the IAF, we performed retrograde and anterograde neuron tracing by using fluorophore-conjugated cholera toxin subunit B and adeno-associated virus vectors encoding fluorescent protein genes, after area identification by optical imaging. We show that the IAF receives input from a ventromedial portion of the ventral division of the medial geniculate body, a core auditory thalamic nucleus. Moreover, distinct subsets of IAF neurons send their axons to the motor cortex or dysgranular insular cortex that contains medulla-projecting neurons. These results suggest that the IAF activated by auditory input could modify motor and autonomic functions.

(COI: No)

S17-2

Re-definition of the primary auditory cortex by separating a newly identified region and their functional specialization in mice

Tsukano, Hiroaki; Shibuki, Katsuei (*Dept of Neurophysiol, Brain Res Inst, Niigata Univ, Niigata, Japan*)

The auditory cortex is composed of several regions in mice. However, the regional function is yet to be identified clearly because delineation of regions including the primary auditory cortex (AI) is still under debate. We investigated the mouse auditory cortex using flavoprotein fluorescence imaging. AI was divided into two areas, dorsoventrally, in response to high frequency tones. We performed immunostaining using SMI-32 and found the dorsal AI high frequency area had totally different cytoarchitectural patterns from those of the rest of the AI, i.e., the low frequency area and ventral high frequency area. This data suggests that the AI dorsal high frequency area is a distinct region to be separated from the AI. We named this new region the dorsomedial field (DM). The responses of the DM to ultrasonic courtship songs presented by males were significantly greater in females than in males. In contrast, there was no difference between sexes in responses to artificial pure tones. These data suggest that courtship songs by male mice might be processed in DM. On the other hand, AI is likely to process harmonic sounds. Harmonic sounds of simultaneously presented 20 kHz and 25 kHz, which produced missing f0 perception at 5 kHz, activated the 5 kHz area of AI, while inharmonic sounds of simultaneously presented 19 kHz and 26 kHz did not. Furthermore, in mice reared in the presence of 5+19+26 kHz, the 5 kHz area in AI responded to inharmonic sounds of 19+26 kHz. These results indicate importance of experience for producing f0 responses.

(COI: No)

S17-3

Sound coding in auditory cortex; studies from single unit activities in the primary auditory cortex (A1) of awake animals

Chimoto, Sohei (*Dept Physiol, Interdisciplinary Grad Sch of Med and Eng, Univ of Yamanashi, Chuo, Yamanashi, Japan*)

To reveal the mechanism of sound coding in auditory cortex, many studies have investigated neural responses to various sound stimuli by using electrophysiological techniques of single unit recordings in awake cats. A1 neurons showed diversity of the response time-courses from phasic to sustained patterns to pure tone stimuli. The sustained response cells have different spectral edge sensitivities. The edge-sensitive cells had tuning to the high-edge or low-edge frequencies of sound stimuli, while the edge-insensitive cells were driven by any stimuli with energy on the cell's frequency response field (FRF) or only very narrowband stimuli with energy confined to FRF. They have different sensitivities to the fundamental frequency (F0) of harmonic complex tones. The F0-sensitive cells discriminated between harmonics and noise, while the F0-insentive cells did not. All aspects of the sustained responses were consequences of the spectral filtering properties. The phasic response cells showed paired onset and offset responses. Their frequency filtering property was dynamic, changing between sound onsets and offsets. Each response was precise and salient for effectively encoding sound onsets and offsets. A1 neurons showed various response patterns during amplitude modulation sounds, frequency modulation tones, and natural sounds. The presence of phasic and sustained response cells explained the diversity of response time courses for the various sounds. The same cell responds to pure tone and other kinds of sound in the specific response pattern.

(COI: No)

S17-4

Steady-state neuronal activity pattern in response to long-lasting continuous tone in the auditory cortex of rat

Takahashi, Hirokazu (*Research Center for Advanced Science and Technology, The University of Tokyo, Japan*)

Acoustic stimuli elicit distinct onset responses in the auditory cortex, which were followed by obscure long-latency responses. The onset responses exhibit clear tonotopic map and learning induced plasticity, which have been fully investigated to date. On the other hand, post-onset, long-latency responses have been poorly characterized because of less reproducibility across trials. Our conscious experience that lasts beyond the time course of onset responses implies that the long-latency responses play an important role in perception. In the present study, we investigate whether and how post-onset, steady-state activities in response to long-lasting continuous tones encode sound information and exhibit learning-induced plasticity. A microelectrode array with a gird of 10 × 10 recording sites was inserted into the 4th layer of the auditory cortex of rats to record local field potentials (LFPs) within the steady-state activity. For all possible pairs of recording sites, band-specific phase locking values (PLVs) of measured LFPs were calculated. We demonstrate that the PLV pattern represented rich information about sound such as frequency, consonance and tonality of chords. Furthermore, appetitive and aversive classical conditioning modified these patterns differently. These results support our hypothesis that long-latency, steady-state responses play important roles in encoding auditory information.

(COI: No)

Symposium 18

Mechanism of host defence and homeostatic maintenance by phagocytes

(March 21, 15:30～17:00, Room H)

S18-1

Multistep regulation of ROS production by voltage-gated proton channel Hv1/VSOP in neutrophils

Okochi, Yoshifumi[1]; Aratani, Yasuaki[2]; Adissu, Hibret A[3]; Miyawaki, Nana[1]; Sasaki, Mari[1]; Suzuki, Kazuo[4]; Okamura, Yasushi[1] (*[1]Integrative Physiol, Grad Sch Med, Osaka Univ, Osaka, Japan; [2]Grad School of Nanobioscience, Yokohama City Univ, Yokohama, Japan; [3]Physiol & Experimental Med, Hospital for Sick Children, Toronto, Canada; [4]Asia International Institute of Infectious Disease Control, Teikyo University, Tokyo, Japan*)

Reactive oxygen species (ROS) is a strong weapon for pathogen killing in phagocytes, whereas ROS is known to damage host itself. The ROS in neutrophils is generated by NADPH oxidase and myeloperoxidase, and it production is strictly regulated by various factors and ways such as pH, membrane potential and granule exocytosis. We discovered the molecule of voltage-gated proton channel Hv1/VSOP and have been analyzing the function of Hv1/VSOP in cellular and animal levels using Hv1/VSOP knockout mouse. We and collaborators have reported that Hv1/VSOP helps O_2^- and H_2O_2 productions through the regulation of intracellular pH and membrane potential in neutrophils, where these factors are known to affect the activity of NADPH oxidase that produce O_2^-. Recently, we found that Hv1/VSOP regulates ROS production in another way: Hv1/VSOP negatively regulates HOCl production, which is made from H_2O_2 by myeloperoxidase, by inhibiting exocytosis of myeloperoxidase-containing granules (azurophilic granules). These results indicate that Hv1/VSOP controls each ROS production by multiple means in neutrophils. Hv1/VSOP may be necessary for balancing competing goals for pathogen killing and protection of host in ROS production.

(COI: No)

S18-2

Optogenetic analysis of spatiotemporal regulation of macropinocytosis and phagocytosis through Rac1 switching in macrophages

Araki, Nobukazu (*Sch.Med.Kagawa Univ., Miki, Japan*)

In phagocytes such as macrophages, macropinocytosis of fluid-phase and phagocytosis of particulate materials play pivotal roles in the immune system. Mechanisms of both macropinosome and IgG-mediated phagosome formation share many common features including actin cytoskeleton dependence and its regulatory components, as well as a large vacuole size (larger than 0.5 μm), although there are some differences in detail. Recently, we have investigated the molecular mechanisms underlying macropinosome and phagosome formation which are spatiotemporally coordinated by dynamic actin organization. Rac1, a member of the Rho family GTPases, acts as a molecular switch which regulates actin polymerization and remodeling. Optogenetics of photoactivatable Rac1 (mCherry-LOV-Rac1Q61L) enables us to manipulate Rac1 activity both in space and time using blue light irradiation under a microscope. Using this technique, we could induce cell surface ruffling and macropinocytic cup formation by photoactivation of Rac1 in RAW264 macrophages. Although the number of macropinocytic cups increased with prolonged time of Rac1 photoactivation, the cup closure into macropinosomes was restrained. When the blue light irradiation was turned off, ruffling immediately receded. Thereafter, macropinosomes were formed by closing cups. In the case of Fc γ R-mediated phagocytosis, activation of Rac1 facilitated pseudopod extension around IgG-opsonized particles. However, subsequent deactivation of Rac1 was also required for shaping phagocytic cups tightly grasping IgG-opsonized particles.

(COI: No)

S18-3

Targeting and assembling mechanisms of NADPH oxidases at phagosomal and apical membranes

Ueyama, Takehiko; Saito, Naoaki (*Biosignal Res. Ctr., Kobe Univ., Kobe, Japan*)

Among the seven known mammalian Nox family NADPH oxidases (Nox1-Nox5, Duox1, Duox2), several are known to serve essential roles based on the effects of disease-related mutations. Defects in the phagocytic oxidase (Nox2) have been known resulting in chronic granulomatous disease (CGD), which is characterized by susceptibility to infection. A Nox3-based oxidase is indispensable for development of otoconia. Mutations in Duox2 have been reported to cause congenital hypothyroidism due to deficiency of thyroid hormone biosynthesis. We have explored targeting and assembly of Nox2, Nox3, and Duoxes.In the phagocytic oxidase, cytosolic regulators (p47phox, p67phox, p40phox, Rac, PKC) translocate and associate with the membrane-spanning flavocytochrome b558, leading to superoxide production. The ternary complex (p47phox-p67phox-p40phox), Rac, and PKC translocate to phagosomes with independent mechanism. Although p67phox is not targeted to phagosomes by itself, p47phox functions as an adaptor for the ternary complex in early stages of phagocytosis, while p40phox functions in later stages. Duoxes are functional only in combination with Duox activators (DuoxAs), and work on the apical surfaces of epithelial cells. Besides, NADPH oxidases produce the primary product superoxide by directly transferring an electron to O2. Duoxes produce superoxide as an intermediate product, but the final product generated by Duoxes is H2O2.In this symposium, I will demonstrate targeting mechanisms of Nox2 to phagosomes and Duoxes to apical membranes, and also conversion mechanism of superoxide to H2O2 by Duoxes.

(COI: No)

S18-4

Splenic dendritic cells phagocytose donor T-cells and induce anti-donor MHC antibody forming cell response

Matsuno, Kenjiro; Ueta, Hisashi; Kitazawa, Yusuke; Sawanobori, Yasushi (*Dokkyo Med.Univ., Tochigi, Japan*)

Aim: Donor-specific blood transfusion (DST) is one of the tolerance-inducing protocols used in clinical transplantation, where a donor blood transfusion can readily induce alloantibody production in recipients. In this study, we examined a mechanism for this antibody response in rats and mice.

Result: DST-treated recipients produced donor class I MHC antigen (MHCI)-specific alloantibodies. Among the donor blood components, T-cells were the most efficient immunogens. One day after the DST treatment, donor T-cells migrated to the splenic periarterial lymphocyte sheath (PALS), where donor MHCI+ fragments were found within the resident DCs. Resident DCs formed clusters with BrdU+ cells, where the recipient CD4+ T-cell proliferative response begun. From day 3, antibody-forming cell response begun followed by the germinal center reaction at day 5. Inhibition of T-cell entry from the red pulp into the PALS strongly suppressed the DST-antibody response. In mice, two-photon intravital microscopy elucidated that the resident DCs readily bound to donor allogeneic T-cells and then ingested them.

Conclusion: Donor T-cells can induce alloresponse most efficiently, because the trafficking pattern of recirculating T-cells is designed to freely enter the PALS and cluster with the resident DCs, thus having a chance to provide alloantigen to DCs. Recirculating T-cells pulsed with antigens might be applicable as vaccine vectors, targeted to the resident DCs for the prophylactic antibody production. (Collaborators: Tomoya Katakai and Takamasa Ueno)

(COI: No)

Symposium 19

Physiological functions of membrane transporters that regulate signals for tooth morphogenesis and differentiation

(March 21, 15:30～17:00, Room I)

S19-1

Analysis of tooth development and bone remodeling using a3 isoform of V-H$^+$ATPase -GFP and -deficient mice

Harada, Hidemitsu[1]; Fujiwara, Naoki[1]; Otsu, Keishi[1]; Sahara, Yoshinori[2]; Horie, Sawa[2]; Nakanishi, Mayumi[3]; Matsumoto, Naomi[3]; Ohshima, Hayato[4]
(*[1]Dpt. Anat., Iwate Med. Univ., Iwate; [2]Dpt. Physiol., Iwate Med. Univ., Iwate; [3]Fac Pharm. Sci., Iwate Med. Univ., Iwate; [4]Niigata Univ. Grad. Sch. MDS, Niigata*)

Vacuolar proton-ATPase (V-H$^+$ATPase) is a multi-subunit enzyme that regulates proton transport and creates the acidic microenvironment. Recently, it has been reported that the strong expression of the a3 isoform is closely associated with the exocytosis of some cells. To examine the localization and function of a3 isoform in the bone and tooth development, we used a3 isoform-GFP and -deficient mice. The strong expression of GFP was detected specifically at osteoclasts, but not dental epithelial cells. The size of body and head of a3 isoform-deficient mice was small, and the tooth eruption was inhibited or delayed, and the root was often morphologically short and anomaly. Though the tooth germs grew normally until bell stage in the development, ameloblasts structurally was unaffected and the mineral content of enamel was similar to that of wild type mice. Interestingly, the bone mineral content of the mutant was lower than that of wild type unexpectedly. Furthermore, to examine the character of the dental epithelial cells in detail, we produced a dental epithelial cell line from the mutant mice and compared the cell line with wild type cell line about organelle acidification. The results showed that there is no difference between these cells. Taken together, it is considered that the decline of bone metabolism resulted in tooth anomaly.

(COI: No)

S19-2

Role of glucose metabolism in amelogenesis

Ida-Yonemochi , Hiroko (*Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata, Japan*)

In organogenesis, cells exhibit various behaviors, such as proliferation, changes in cell shape, matrix production and secretion. It is generally believed that many cells utilize glucose as the basis of energy, and the glucose metabolic pathway is a critical event in determining cell behavior in organogenesis. Blood glucose is transported into cells by glucose transporters (GLUTs), and GLUTs are expressed in tissue- and cell-specific functional manners depending on various glucose requirements. Recently, we demonstrated that the expression of GLUT1/2 in the dental cells is precisely and spatiotemporally controlled depending on cell differentiation. In an in vitro organ culture experiment with an inhibitor of GLUTs1/2, the bud-stage tooth germs showed the developmental arrest of the explants. And the inhibition of GLUTs1/2 in cap-to-bell-stage tooth germs reduced tooth size. These findings suggest that the glucose uptake mediated by GLUT1/2 plays a crucial role in the early tooth morphogenesis and tooth size determination. Next, we examined the glucose metabolism in amelogenesis. We found that the timing of glycogen synthesis, accumulation and degradation is also tightly associated with the process of ameloblast differentiation. In vitro organ culture experiment, the inhibition of glycogen synthesis/degradation disturbed ameloblast differentiation and enamel matrix formation, and the activation of Akt signaling by IGF-1 consequent glycogen accumulation led to an increase in enamel matrix formation. Thus, the glycogen shunt governed by IGF-1-Akt signaling is an essential system for ameloblast differentiation.

(COI: No)

S19-3

Regulation of BMP-2 expression by extracellular-calcium ions/-phosphate ions in dental pulp cells

Nemoto, Eiji (*Grad.Sch.Dent.Tohoku Univ., Sendai, Japan*)

Dental pulp cells, which have been shown to share phenotypical features with osteoblasts, are capable of differentiating into odontoblast-like cells and generating a dentin-like mineral structure. Elevated extracellular calcium (Ca^{2+}_o) and extracellular phosphate (Pi) are known to play key roles in promoting osteoblastic differentiation by altering gene expression and cellular function; however, the roles of Ca^{2+}_o and/or Pi signaling in odontogenesis remain unclear. We found that elevated Ca^{2+}_o as well as Pi increase the gene expression of bone morphogenetic protein (BMP)-2, a crucial regulator of mineralization, in human dental pulp cells. The Ca^{2+}_o-mediated *BMP-2* increase was markedly inhibited by pretreatment with an extracellular signal-regulated kinase (ERK) inhibitor, PD98059, and partially inhibited by the L-type Ca^{2+} channels inhibitor, nifedipine. However, pretreatment with nifedipine had no effect on ERK1/2 phosphorylation triggered by Ca^{2+}, suggesting that the Ca^{2+} influx from Ca^{2+} channels may operate independently of ERK signaling. On the other hand, Pi-mediated *BMP-2* expression requires activation of cAMP/protein kinase A, which is indispensable but not sufficient for the *BMP-2* increase. Moreover, the *BMP-2* increase requires activation of ERK1/2 pathway, which may operate independently of cAMP-dependent signaling. Importantly, it may be possible to use this knowledge as a means to deliver Ca^{2+}_o and Pi to local sites to regenerate mineralized tissues associated with the oral cavity.

(COI: No)

S19-4

Intercellular odontoblast-neuron signal communication via ATP

Sato, Masaki (*Dept Physiol, Tokyo Dent Col, Japan*)

Various stimuli to the dentin surface induce dentinal pain. There is, however, no clarity regarding the precise mechanisms of dentinal pain generation as well as the role of odontoblasts in the underlying sensory transduction pathway. In order to determine if odontoblasts act as sensory receptors in this pathway, we mechanically stimulated odontoblasts and investigated transient receptor potential (TRP) channel activation in these cells and the subsequent intercellular signaling between odontoblasts and neurons. Direct mechanical stimulation of single odontoblasts increased intracellular free calcium concentration ($[Ca^{2+}]_i$) via TRP channel activations. In a co-culture of odontoblasts and trigeminal ganglion (TG) neurons, direct mechanical stimulation of single odontoblasts increased $[Ca^{2+}]_i$ not only in the stimulated odontoblasts, but also in neighboring odontoblasts and TG neurons. The $[Ca^{2+}]_i$ increase in neighboring odontoblasts and TG neurons, but not that in stimulated odontoblasts, was inhibited by a pannexin-1 inhibitor. A $P2X_3$ receptor antagonist suppressed the $[Ca^{2+}]_i$ increase in neighboring TG neurons, but not in stimulated and neighboring odontoblasts. These results show that TRP channel activation in mechanically stimulated odontoblasts results in ATP release via pannexin-1 and the ATP transmits a signal to the $P2X_3$ receptors on TG neurons. These results also enhance understandings sensory of transduction sequence for dentinal pain, termed as "odontoblast hydrodynamic receptor theory".

(COI: No)

Symposium 20

A better understanding of liver metabolism by multifaceted approaches

(March 21, 15:30～17:00, Room J)

S20-1

Metabolite sensing and regulation of glucose metabolism in liver

Miki, Takashi[1]; Lee, Eunyoung[1]; Minokoshi, Yasuhiko[2] (*[1]Dept Med Physiol, Grad Sch Med, Chiba Univ, Chiba, Japan; [2]Dept Dev Physiol, NIPS, Okazaki, Japan*)

Liver plays a central role in the regulation of glucose metabolism. We found that the insulin receptor mutant mice (mIR) exhibit marked hyperglycemia under high fat diet (HFD). In mIR under HFD (mIR/HFD), lipolysis in white adipose tissues (WAT) and mRNA expression of glucose-6-phosphatase (G6pc) in liver were both increased on refeeding. Glycerol is the end product of lipolysis and insulin inhibits lipolysis by reducing phosphorylation of hormone sensitive lipase (HSL). As expected, phospho-HSL levels were significantly increased in WAT of mIR/HFD. In addition, transplantation of wild-type WAT into mIR/HFD markedly ameliorated the hyperglycemia, suggesting the pathophysiological relevance of unsuppressed lipolysis on development of hyperglycemia in mIR/HFD. Nevertheless, adipocyte specific insulin receptor knockout mice did not exhibit glucose intolerance. This prompted us to hypothesize that insulin dysfunction in liver is also involved in the pathogenesis. We found that glycerol administration induces mRNA expression of G6pc in to wild-type mice, suggesting that hepatocyte senses glycerol or its derivatives to regulate glucose metabolism. In addition, defective insulin action in liver is suggested to participate in the increased gluconeogenesis from glycerol. In this symposium, the mechanism of nutrient sensing in liver and its relevance in glucose homeostasis will be discussed.

(COI: No)

S20-2

Vitamin A-storing lipid droplets in hepatic stellate cells

Yoshikawa, Kiwamu[1]; Mezaki, Yoshihiro[1]; Hebiguchi, Taku[2]; Miura, Mitsutaka[1]; Imai, Katsuyuki[1]; Yamaguchi, Noriko[3] (*[1]Dep. Cell Biol. and Morp., Grad. Sch. of Med., Akita Univ.; [2]Dep. Ped. Sur., Grad. Sch. of Med., Akita Univ.; [3]Dep. Bas. Nurs., Grad. Sch. of Med., Akita Univ.*)

Hepatic stellate cells (HSCs) lie in the perisinusoidal space in the liver and store about 50-80% of total body vitamin A as retinyl esters in their cytoplasmic lipid droplets (LDs) in mammals. Therefore the vitamin A-storing LDs are considered specialized organelle for vitamin A storage. Under pathological conditions such as liver fibrosis, HSCs proliferate rapidly and produce large amounts of extracellular matrix components and lose their stored vitamin A (activation of HSCs). We investigated the involvement of perilipin 2 (ADRP) and perilipin 3 (TIP47) in the formation of vitamin A-storing LDs in cultured rat HSCs and observed very characteristic behaviors of the proteins. Perilipin 2 always localized on almost all vitamin A-storing LDs in any conditions examined; resting, growing (supplement of retinol and/or oleate), and diminishing conditions in both quiescent and activated HSCs. Perilipin 3 rarely localized on vitamin A-storing LDs in quiescent HSCs. However in activated HSCs it localized on newly induced vitamin A-storing LDs from small to very large ones as long as the constituents of LDs were supplied. In consequence, perilipin 2 and 3 co-localized on the same LDs for a long period irrespective of their size. Vitamin A-storing LDs positive for perilipin 3 were decreased after supplement of LD constituents was stopped. The manner of involvement of the two proteins for LD formation in HSCs will be discussed.

(COI: No)

S20-3

UBXD8 function in liver

Suzuki, Michitaka; Imai, Norihiro; Ohsaki, Yuki; Cheng, Jinglei; Fujimoto, Toyoshi (*Grad. Sch. Med. Nagoya Univ., Nagoya, Japan*)

Apolipoprotein B (ApoB) is the principal protein of VLDL, and lipidated cotranslationally by the microsomal tryglyceride transfer protein (MTP) activity in ER in hepatocyte. Previously we found that ApoB after lipidation accumulates in the ER fused to LDs (named ApoB-crescent structures) upon inhibition of proteasomes or autophagy and is partially ubiquitinated. The result suggested that ApoB-crescent structures function as a platform of lipidated ApoB degradation.

To elucidate the molecular mechanism of lipidated ApoB degradation, we searched for proteins that are engaged in the process and found UBXD8, p97 and Derlin-1. Knockdown of Derlin-1 increased ApoB in the ER lumen of ApoB-crescent structures. In contrast, knockdown of UBXD8 reduced p97 recruitment to LDs, and caused ApoB accumulation not only in the ER lumen but also on the LD surface facing the cytoplasm. Furthermore, UBXD8 bound ubiquitinated ApoB. The results inferred that UBXD8 together with p97 and Derlin-1 facilitates retrotranslocation and proteasomal degradation of lipidated ApoB in the vicinity of LDs.

To evaluate the physiological function of UBXD8 in liver in vivo, we generated hepatocyte-specific UBXD8 knockout (UBXD8-LKO) mice. After twenty-six weeks of high-fat diet feeding, VLDL secretion and the serum TG level were reduced in the UBXD8-LKO mice compared to the wild-type mice. Furthermore, the UBXD8-LKO mice showed steatosis both in the periportal area and the perivenular area, whereas the control mice showed steatosis only in the perivenular area. These data indicated that UBXD8 plays an important role in regulating ApoB secretion from hepatocytes.

(COI: No)

S20-4

Mechanism for biliary phospholipid efflux mediated by ABCB4 on canalicular membranes

Morita, Shin-ya (*Shiga University of Medical Science, Otsu City, Shiga, Japan*)

Bile salts have potent detergent properties and damage hepatocytes by affecting the integrity of cellular membranes, which are associated with hepatocellular necrosis. On the canalicular membranes of hepatocytes, ABCB4 plays an essential role in the secretion of phospholipids into bile. The biliary phospholipids are associated with bile salts and cholesterol in mixed micelles, thereby reducing the cytotoxicity of bile salts and preventing cholesterol crystallization. Mutations in the ABCB4 gene result in cholestasis, cholelithiasis, and cholangiocarcinoma. To elucidate the mechanism for ABCB4-mediated phospholipid efflux, we established HEK293 cells stably expressing ABCB4. Surprisingly, the phospholipid efflux from ABCB4-expressing cells was remarkably enhanced by taurocholate. ABCB4 mutants in ATP-binding domains did not mediate the phospholipid efflux. Mass spectrometry revealed that ABCB4-expressing cells preferentially secreted phosphatidylcholine (PC) rather than sphingomyelin (SM). In addition, by using enzyme-based fluorometric methods, we demonstrated that taurocholate stimulated the ABCB4-mediated efflux of PC, phosphatidylethanolamine (PE) and SM, while the efflux of PE and SM was much less than that of PC. ABCB4 was predominantly localized to nonraft membranes, and the ABCB4-mediated phospholipid efflux was completely abolished by BODIPY-verapamil, which hardly partitioned into raft membranes. In conclusion, ABCB4 localized in nonrafts, but not in rafts, mediates the efflux of phospholipids, preferentially PC, only in the presence of bile salts.

(COI: No)

Symposium 21

Space Medicine II: Complications of "Zero-Gravity" and their countermeasures

(March 21, 17:00～18:30, Room B)

S21-1

Medical challenges that need to be solved in super-long-duration stays in space facing Human Space Exploration

Furukawa, Satoshi; Ohshima, Hiroshi; Ogata, Katsuhiko; Miki, Takeo; Suzuki, Go; Abe, Takashi; Ohira, Takashi (*JAXA, Tsukuba, Japan*)

At the International Space Exploration Forum in January, 2014, there was a minister-level policy dialogue on international cooperation in space exploration. Long stay in space in human space exploration will cause physiological changes to human body. Below are examples. They are challenging and countermeasures are needed.1.MusculoskeletalOn International Space Station (ISS) muscle atrophy and bone loss induced by zero-G is almost controlled by good exercise devices. As for space exploration, limited room may require more compact ones. Plus, a countermeasure drug /food would be nice.2.NeurovestibularIt takes several months to get to Mars. On the way being exposed to zero-G, astronauts' vestibular system is adapted to zero-G. Arriving at Mars, astronauts experience "readaptation to gravity sickness". They need to work without the support of rescue team. Certain measures should be implemented.3.Immune SystemIn space, reduced number and proportion of lymphocytes and their cytokine production, decreased neutrophil and monocyte functions, decreased NK cell cytotoxicity were noted. JAXA is starting research on enterobacterial flora and immunity.4.RadiationAstronauts are subject to large amounts of space radiation due to lack of protection by the atmosphere or magnetic field around the Earth. Physical radiation shielding or modification of exposure by a drug/food would be great.5.VisualMore than 30% of ISS astronauts experience chronic visual acuity changes with papilledema and/or choroidal folds. The cause is not well known.

(COI: No)

S21-2

Our Space Biology Experiments in "Kibo (JEM)" of the International Space Station to Conquer Spaceflight-Associated Diseases

Nikawa, Takeshi[1]; Sokabe, Masahiro[2] (*[1]Dept Nutr Physiol, Inst Med Nutr, Tokushima Univ.; [2]Dept Biol Physics, Faculty Med, Nagoya Univ.*)

Skeletal muscles are vulnerable to rapid and marked atrophy under microgravity conditions. Unfortunately, despite the fact that almost of all astronauts are afflicted by debilitating atrophy, no treatment besides training exists to halt or reverse its progression, besides training. The muscle atrophy caused by microgravity is characterized by both decreased responsiveness to myogenic growth factors (e.g., IGF-1 and insulin) and increased proteolysis. Previously we showed that simulated microgravity conditions, such as tail-suspension and three dimensional (3D)-clinorotation, resulted in skeletal muscle atrophy through the induction and activation of the ubiquitin ligase Cbl-b. Upon induction, Cbl-b interacted with and degraded the IGF-1 signaling intermediate IRS-1. In turn, the loss of IRS-1 activated the FOXO3-dependent induction of atrogin-1/MAFbx, a dominant mediator of proteolysis in atrophic muscle. Furthermore, Cbl-b-deficient mice were resistant to tail-suspension-induced muscle atrophy and the loss of muscle function. Thus, the Cbl-b-dependent destruction of IRS-1 is a potent dual mediator of both increased protein degradation and reduced protein synthesis observed in microgravity conditions. In our space experiments, named "Myo Lab" and "Cell Mechanosensing", we aim to elucidate this hypothesis on "Microgravity-mediated Cbl-b expression" and Cbl-b-mediated skeletal muscle atrophy to develop a new therapeutic strategy (drug discovery in space) for muscle atrophy.

(COI: Properly Declared)

S21-3

Study and development of the countermeasure device on the disuse atrophy of the musculoskeletal system of the astronauts staying in the space for a long term -Participating in International Anouncement utilizing International Space Station-

Shiba, Naoto[1]; Matsuse, Hiroo[1]; Takano, Yoshio[2]; Omoto, Masayuki[1]; Hashida, Ryuuki[1]; Yamada, Shin[3]; Ohshima, Hiroshi[3] (*[1]Dept Orthop Surg, Kurume University School of Medicine; [2]Dept of Physical Therapy, Fukuoka Branch of Internatinal University of Health and Welfare; [3]Japan Aerospace eXploration Agency*)

The hybrid training system: HTS was developed as a countermeasure for the musculoskeletal atrophy common to astronauts and uses a contraction produced in a muscle by electrical stimulation of antagonist to resist the volitional contraction of the agonist. HTS, which utilizes electrically stimulated antagonist force as resistance to joint motion instead of gravity, would become a useful training method in weightlessness. In this experiment, HTS will be used for one of upper limbs of an astronaut (the non-dominant arm) for four weeks, and his/her muscular strength and bulk will be compared to those of his/her non-HTS arm (the dominant arm) to examine its orbital operation capability utility, as well as the preventive effect of HTS for musculoskeletal atrophy. The initial flight data together with the wealth of ground data obtained so far will be brought to the future planning of this project after the first flight.HTS will become a useful back-up for the large standard training devices in the ISS, or a useful training device in small space ships (CTV) for the exploration of the Moon and Mars.

(COI: No)

S21-4

Effects of artificial gravity by short arm centrifuge of 1.4 m with exercise as the countermeasures for spaceflight deconditioning

Iwase, Satoshi; Nishimura, Naoki (*Dept Physiol Aichi Med Univ*)

Crew members often suffer from space flight deconditioning including neurovestibular disorientation, cardiovascular deconditioning, myatrophy, and bone mineral loss. Several countermeasures have been introduced, but no single measure has been proved to be effective as the countermeasure. We have constructed the short arm centrifuge with ergometer with the radius of 1.4 m because the available space in international space station was reported to the inside of the cylinder with the diameter of 2.8 m. Subjects were required to lay down with supine position. Their legs were raised up to 70 cm high, and there, cycling pedals, which was fixed at the level of leg rotation, were stepped during centrifuge. G-level of 1.4 G with ergometric exercise of 60 W was loaded in the countermeasure group while control group were requested to lie down without exercise. Several measurements were applied to assess neurovestibular, cardiovascular, musculoskeletal, and bone metabolism, and bedrest studies were carried out comparing the countermeasure and control groups. As the results, centrifuge-induced artificial gravity with exercise has been provided significant difference from the control. We concluded artificial gravity is effective in mitigating spaceflight deconditioning in humans.

(COI: No)

S21-5

Long term stay in microgravity-induced suppression of vestibular function and its countermeasure

Morita, Hironobu; Abe, Chikara; Tanaka, Kunihiko (*Dept Physiol, Gifu Univ Grad Sch Med, Gifu Japan*)

Gravity, not only changes in amount but also changes in direction, is a major and the most common disturbance for our daily life. To cope with this, the vestibular system plays an important role in controlling body balance and arterial pressure upon gravitational stress. However, the vestibular system is known to be highly plastic, thus it is possible that the vestibular function might be altered, if subjects were in a different gravitational environment. This is true, since vestibular-mediated motor coordination and vestibulo-cardiovascular reflex were suppressed in rats raised in hypergravity for 2 weeks. Furthermore, vestibulo-cardiovascular reflex was also suppressed in astronauts who stayed in the International Space Station for 4-6 months. Thus, both hypergravity and microgravity elicit the suppression of the vestibular function, which might be due to use-dependent plasticity of the vestibular system, since phasic input to the vestibular system was reduced not only in microgravity but also hypergravity (less than 20% of 1 g environment). If this is the case, the suppressed vestibular function could be ameliorated by an artificial vestibular stimulation. Indeed, hypergravity-induced suppression of vestibular-mediated motor coordination and vestibulo-cardiovascular reflex were ameliorated by galvanic vestibular stimulation (GVS). Because posture imbalance and orthostatic intolerance are major complications of spaceflight, it is urgent to propose an effective countermeasure against the suppressed vestibular function, and GVS can be a candidate for this.

(COI: No)

Symposium 22

The effect of perinatal stress on brain function

(March 21, 17:00～18:30, Room C)

S22-1

Analyses of the effects of embryonic ischemia on brain development

Kubo, Ken-ichiro[1]; Deguchi, Kimiko[1]; Nagai, Taku[2]; Aramaki, Michihiko[1]; Yamada, Kiyofumi[2]; Inoe, Ken[3]; Nakajima, Kazunori[1] (*[1]Dept. Anatomy, Keio Univ. Sch. Med., Tokyo, Japan; [2]Dept. Neuropsychopharmacol. & Hospital Pharmacy, Nagoya Univ. Sch. Med., Nagoya, Japan; [3]Department of MR and BDR, NIN, NCNP, Tokyo, Japan*)

Environmental factors cause various neuropsychiatric diseases, such as schizophrenia, autism, and mood disorders, but their influence on brain development is largely unknown. To analyze the pathological mechanisms of environmental factors during brain development in detail, we produced ischemic brain damage in developing mouse embryos by occluding maternal uterine arteries. By combining *in utero* electroporation technique for tracking and visualization of neurons, we identified delayed neuronal migration and disruption of the final "inside-out" pattern of cortical neuronal alignment. We also performed behavioral analyses at postnatal stages to assess the neuropsychiatric functions of mice that had experienced ischemia during embryonic period. Importantly, cognitive impairments were associated with the mice that had experienced ischemia during development. Since cognitive deficits are known to underlie various neuropsychiatric diseases, ischemic insults in the developing cerebral hemispheres are thought to affect neurodevelopment, resulting in subsequent cognitive impairment that lead to increased susceptibility to neuropsychiatric diseases.

(COI: No)

S22-2

Effects of early life adverse experiences on the brain: Implications from maternal separation models in rodents

Nishi, Mayumi; Horii, Noriko; Sasagawa, Takayo (*Nara Med. Univ., Kashihara, Japan*)

During postnatal development, adverse early life experiences affect the formation of neuronal networks and exert long-lasting effects on neural function. Many studies have shown that daily repeated maternal separation (MS), an animal model of early life stress, can regulate the hypothalamic-pituitary-adrenal axis (HPA axis) and affect subsequent brain function and behavior during entire life including puberty and adulthood. However, the molecular basis of the long-lasting effects of early life stress on brain function has not been fully elucidated. In this symposium, we will present various cases of MS in rodents and illustrate the alterations in HPA axis activity by focusing on corticosterone (CORT). We then show a characterization of the brain regions affected by various patterns of MS, including repeated MS and single time MS at various stages before weaning, by investigating a neuronal activity marker, c-Fos. These CORT and c-Fos studies suggest that repeated early life stress may affect neuronal function in region- and temporal-specific manners, indicating a critical period for habituation to early life stress. Next, we introduce how early life stress can impact behavior, namely by inducing depression, anxiety or eating disorders, and alterations in gene expression in adult mice subjected to MS.This study has been supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

(COI: No)

S22-3

Maternal behavior of mice which suffered the early-life stress

Takatsuru, Yusuke (*Dept. Integrative Physiol., Gunma Univ. Grad. Sch. Med., Japan*)

Maturation of the brain function still continues during lactating in mammal including human. Thus, relationship between mother and neonate is important not only nutrition supply but also mental stability. The early-life stress during the lactation induces severe effect on brain function and it induces several disorders such as depression. This stress induces instability of synapse and disruption of glutamate homeostasis in brain not only in emotion-rerated area such as hippocampus and prefrontal cortex but also basic area such as somatosensory cortex (Takatsuru et al., 2009, Toya et al., 2014). It is also said that abused children tend to abuse their children when they become to be a mother. This sad phenomena possibly induced by dysfunction of brain which induced by early-life stress. However, underling mechanisms are not still fully understood. We recently showed that the maternal behavior is disrupted in maternal deprivation (MD) mice, one of the early-life stressed models. The success rate of mating decreased in MD mice and even if they became to be a mother, the rate of neglect was also increased. Sadly and interestingly, these changes were also detected in second generation mice. They also showed difficulty in mating and maternal behavior. These changes were detected without early-life stress application to 2nd generation. In conclusion, early-life stress affected the maternal behavior beyond the generation and this MD mouse is potentially good model for study on mechanisms underlying the effect of early-life stress on brain function of human patient case.

(COI: No)

S22-4

Effects of mental task and daily eating behavior on preference to fat and sweet taste in young females

Someya, Nami (*Chiba Prefectural University of Health Sciences, Chiba, Japan*)

It was reported that restrained eaters who intentionally restrict food intake to maintain or lose weight consumed more energy under stressful condition. The purpose of the present study was to evaluate an effect of acute mental task on preference to fat and sweet taste in high and low restrained eaters. Seventeen females (18-22 yrs) participated in the study. The participants were assigned to two groups based on the score of restrained eating scale of the Japanese version of the Dutch Eating Behavior Questionnaire, i.e., high restraint (HR, n=8) and low restraint (LR, n=9). In both groups, Stroop color-word test (CWT) was performed as a mental task for 10 min. Before and after the CWT, the preference to 2, 4, 8, 12, 16 and 20 % fat milk and 25, 50, 100, 150, 200 and 250 mmol/l sucrose solutions were assessed by visual analog scale. In the LR group, the preference rating for milk was peaked at 8 % fat before the CWT, while it was peaked at 12 % fat after the CWT. The preference rating for 12 % fat after the CWT tended to be greater than that before the CWT. On the other hand, there was no significant effect on fat preference in the HR group. In the HR group, the preference rating for sweet taste was peaked at 150 mmol/l sucrose before the CWT, while it was peaked at 200 mmol/l sucrose after the CWT. The preference rating for 200 mmol/l sucrose after the CWT tended to be greater than that before the CWT. There was no significant effect on sweet taste preference in the LR group. These results imply that preference to fat and sweet taste was affected by both mental task and daily eating behavior.

(COI: No)

Symposium 23

Possibility of Joint Lectures and Practicals on Central Nervous System Anatomy and Physiology

(March 21, 17:00～18:30, Room D)

S23-1

Lectures and Practical training of the Central Nervous System from an Anatomical View

Yoshimura, Ken (*Dept. of Anatomy, The Nippon Dental Univ. Sch. of Life Dent. at Niigata*)

We are providing lectures on the central nervous system (CNS) for students in dental faculty. The lecture of CNS from an anatomical view are as follows: (1) The Development of the CNS and the Cerebral Ventricle, (2) The Basic Structure of the Brain and Cerebral Association Fibers, (3) Typical Functional Localizations, (4) The Layers of the Cerebral Neocortex and Associating Pyramidal Cell like Betz, (5) Association areas of the Cerebral Cortex Including the Speech Center like Broca\'s and Wernicke\'s areas, (6) The Basal Ganglia, (7) The Diencephalon, (8) The Brainstem, (9) The Basic Structure of Spinal cord and (10) Several Typical Examples of Conducting Pathways. As well as the above-mentioned morphology-based lecture of the CNS, we also have a practical course. Students observe overviews of the excised whole brain that were covered with the dura mater, arachnoid and pia mater, and then carefully excised from its brainstem, then the brain was sliced. After the observations, students made correlating drawings.Most students seem to be interested in morphology in the CNS. However, they have difficulty understanding it because the CNS lacks morphological characteristics and structures which can be easily assume unlike other organs such as muscles.Therefore, we are providing lectures not only a knowledge-based style but also associating historical information and updates. I would like to introduce the overviews of our anatomical view in lectures.

(COI: No)

S23-2

Current education of practical training and lecture in the central nervous system at physiology side

Satoh, Yoshihide (*Dept Physiol, Sch Dent Niigata, Nippon Dent Univ, Niigata, Japan*)

Students of dental university must learn not only maxillofacial structure including teeth but also function of orofacial and tongue in basic dental science. For example, there are neural mechanisms of orofacial somatic sensation, gustation, mastication, jaw reflexes, swallowing, salivation, articulation and respiration. It is very important for dental students to understand neural pathways and reflex arcs. In the central nervous system, there are many sites that are related to them as follows; the main sensory trigeminal nucleus, the spinal trigeminal nucleus, especially the subnucleus caudalis, the supratrigeminal nucleus, the motor trigeminal nucleus, the facial nucleus, the hypoglossal nucleus, the nucleus of the solitary tract related to the swallowing and the gustation, the superior and inferior salivatory nuclei, the ventral posteromedial nucleus of thalamus, the lateral hypothalamic area as a feeding center, the primary motor cortex, the primary somatosensory cortex, the primary gustatory cortex and so on. However, understanding three-dimensionally of location of these sites is very difficult for dental students, because the structure of the central nervous system is very complicated. An anatomical knowledge is absolutely necessary for all lectures of physiology. On the other hand, physiology practical training about the central nervous system has not done in our university. In this symposium, I would like to talk about challenges of lecture of the central nervous system from the standpoint of physiology.

(COI: No)

S23-3

A possiblity of joint lectures and practicals for anatomy and physiology on the central nervous system

Kageyama, Ikuo (*The NDU, Niigata, Japan*)

A recent survey revealed that the time allotted for lectures in anatomy and physiology is drastically decreasing in medical and dental schools because students are required to study an increasing number of subjects. However, knowledge of anatomy and physiology remains fundamental and essential, especially for clinicians. No one would want a surgeon who lacks knowledge of anatomy and physiology. Student physicians and dentists should learn anatomy and physiology because it is critical for them and their colleagues in co-medical disciplines. The use of joint lectures and practicals might be an efficient method for such study. In this symposium, we will discuss the possibility of joint lectures and practicals on the anatomy and physiology of the central nervous system.

(COI: No)

S23-4

Current Trends in Teaching Neuroanatomy in Sri Lanka

Nanayakkara, Chinthani D. (*Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka*)

Human anatomy, which includes gross and neuroanatomy, represents a crucial component among the basic sciences providing relevant medical awareness to students in health sciences. The anatomic background is deemed a cornerstone of medical learning for centuries. Likewise, neuroanatomy is considered as the cornerstone in understanding the nervous system and its disorders. Neuroanatomy poses many challenges to students not only because of its numerous discrete structures, but also due to the difficulty in comprehension of topography and complicated spatial relations between them. Traditional undergraduate teaching in neuroanatomy comprise of a group lecture, a gross dissection as well as self study with 2 dimensional (2D) images in atlases. Over the years the amount of time dedicated to anatomy, and therefore to neuroanatomy, also has significantly decreased. Most institutions have developed curricula to overcome many of these constraints by integrating anatomy teaching with physiology and clinical material, and adopting innovative teaching methods using electronic tools providing three-dimensional information. Developing a module on the Nervous System in the new Basic Sciences Curriculum in our institution, and how neuroanatomy and neurophysiology teaching is conducted to increase the clinical relevance and self-directed learning will be discussed.

(COI: No)

S23-5

Current Status and Future Challenges in Teaching Neurophysiology in Sri Lanka

Pallegama, Ranjith W. (*Division of Physiology, Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya, Peradeniya 20400, Sri Lanka*)

The development, organization, structure and the general functions of the nervous system are taught in an integrated course. The teaching is done mainly through lectures, practical classes and tutorials; none of the sessions includes combined content in anatomy and physiology. The contents include, organization and function of the peripheral nerves, physiology of somatic sensations including pain, autonomic nervous function, reflexes and their supraspinal control, cortical, cerebellar and brain stem control of motor functions and a limited coverage on higher intellectual functions. The neurophysiology of mastication, swallowing, speech, and special senses are taught in two other separate courses with the relevant anatomy content. Clinical relevance of the content is shown throughout the course. The students practice on clinical examination of the nervous system in practical classes and they study around problems during tutorial classes.Increasing demand for resources and time allocation in curricula by expanding clinical sciences forces the basic medical sciences to shrink the contents. A constructively aligned outcome based education is promoted in Sri Lanka as a general policy in education. The challenges in teaching preclinical sciences in an integrated fashion aiming at the intended outcomes and the potential teaching-learning activities are discussed in this discourse. Qualitative feedback of students indicates their perceptions and is also useful in shaping the future directions in teaching neurophysiology integrated with anatomy.

(COI: No)

Symposium 24

A new vista of study on formation and function of lymphatic vessels

(March 21, 17:00～18:30, Room E)

S24-1

Morphogenetic mechanisms of the lymphatic endothelial cells in zebrafish and Medaka

Isogai, Sumio (*Dept.Anat. Sch.Med.Iwate Med. Uni., Morioka, Japan*)

Despite its importance, lymphangiogenesis had remained poorly understood because the lack of specific markers to identify the lymph vessels and of a small animal model to be genetically manipulated. We have worked to make the zebrafish and medaka new genetic models to unravel the function of candidate gene . We demonstrated that they possess a lymphatic vascular system that shares the morphological, molecular and functional characteristics of the lymphatic vessels in other vertebrates. The main function of the lymph vascular system is to return excess interstitial fluid back to the blood vascular system. In early developmental stages, multiphoton time-lapse imaging of transgenic-fish enabled us to visualize and trace the formation of the thoracic duct and the lymph sac at the jugular angle by following the migration of individual cells from their origin through their incorporation into lymphatic endothelium, which allowed us to definitively determine the ontogeny of this system. The conserved anatomical pattern allows this collecting system to function properly. Some cues and mechanisms guiding the lymph progenitor cells to the gross anatomical pattern were revealed. Using time-lapse imaging and various injection methods, we are investigating the collecting system formation in zebrafish and medaka, and are compiling the anatomical atlas of lymph vascular system from embryo through adult. Our studies provide the detailed morphogenetic characterization of the lymphatic endothelial cells in these organisms, as well as the definitive in vivo evidence for the mechanism underlying the formation of the vertebrate lymphatic system.

(COI: No)

S24-2

Engineering of three-dimensional tissues with blood and lymphatic vascular tubules fabricated by cell-accumulation technique

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Engineering of culture tissue with blood and lymphatic vascular networks, constructed by human cells, can be applied for biomaterials in medical treatment and/or for valuable tools in biomedical researches. We have established three-dimensional tissue-constructing technique named cell-accumulation method, which is based on coating of culture cells by extracellular matrix-nanofilms. By using this method, we have successfully fabricated the artificial tissues with tubular networks of human umbilical venous endothelial cells (HUVECs) and/or human dermal lymphatic endothelial cells (HDLECs) in multilayerd normal human dermal fibroblasts (NHDFs). In ultrastructural analysis, HUVECs and HDLEC formed luminal structures mimicking native blood and lymphatic capillaries, respectively, in connective tissue-like constructs by NHDFs. The artificial lymphatic capillaries showed irregular shapes, loose adhesive connections, and gap formations between endothelial cells, in comparison to artificial blood capillaries. By using our artificial tissues, we demonstrate 1) morphogenetic analysis of blood and lymphatic vascular networks, 2) transplantation of blood or lymphatic vascular tissues as biomedical grafts, and 3) establishment of human peritoneum model with vascular networks for researches of cancer metastasis.

(COI: No)

S24-3

Key roles of lymph flow in the lymphatic function

Kawai, Yoshiko[1]; Ohhashi, Toshio[2] ([1]*Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumogo, Japan;* [2]*Dept. of innovation of medical and health sciences research, Shinshu Univ. Sch. of Med., Matsumoto, Japan*)

To address physiological roles of shear stress produced by lymph flow in gene and protein expression in lymphatic endothelial cells with special reference to physiological function of collecting lymph vessels, we firstly investigated the effects of shear stress stimulation on ecNOS expression in cultured lymphatic endothelial cells (LEC). These results suggest that shear stress produces a significant release of ATP from LEC, which activates the purinergic P2X/2Y receptor, thereby facilitating ecNOS mRNA and protein expression through inositol 1, 4, 5-trisphosphate-mediated release of intracellular Ca^{2+} ions and the activation of Ca^{2+}-activated K^+ channels in LEC. We also investigated the effects of shear stress stimulation on release of ATP through activation of F_1/F_0 ATP synthase in the cultured cells, and the mechanisms of shear stress-mediated F_1/F_0 ATP synthase-dependent release of ATP in the cultured cells. Next, we investigated the effects of shear stress stimulation on ICAM-1 expression in cultured LEC. We studied whether shear stress-mediated adhesion molecule expression accelerates the attachment of carcinoma cells to human LEC. Finally, in in vivo experiments we evaluated whether exogenous ATP facilitates the expression of carcinoma cell-ligated adhesion molecules in rat lymph node. In conclusion, shear stress stimulation induces ICAM-1 expression on LEC by activating cell surface F_1/F_0 ATP synthase, which might contribute, in part, to the creation of a premetastatic environment within sentinel lymph node.

(COI: No)

S24-4

Roles of signal networks during the formation of lymphatic vessels

Watabe, Tetsuro[1]; Yoshimatsu, Yasuhiro[1]; Miyazono, Kohei[2] ([1]*Lab. Oncology, Sch. Life Sci., Tokyo Univ. Pharm. Life Sci., Japan;* [2]*Dept. Mol. Pathol., Grad. Sch. Med., Univ. Tokyo, Japan*)

Members of bone morphogenetic protein (BMP) family have been implicated in the formation of blood vessels. We have previously reported that BMP-9/ALK-1 signals enhance the proliferation of blood vascular endothelial cells (BECs). However, the roles of BMP-9/ALK-1 signals in lymphatic vessel formation remain largely unknown. Here we examined the effects of BMP-9/ALK-1 signals on lymphangiogenesis both in vitro and in vivo. BMP-9 significantly inhibited the proliferation of human dermal lymphatic endothelial cells (HDLECs) in vitro. Importantly, we found that BMP-9 decreased the expression of Prox1, a transcription factor critical for the differentiation and maintenance of lymphatic endothelial cells, concomitantly with decreased expression of lymphatic-specific genes such as VEGFR3 and with increased expression of blood vascular-specific genes such as VEGFR2. In order to study the in vivo functions of BMP-9, we used multiple types of human cancer xenograft models. We observed the decreased formation of lymphatic vessels in tumors derived from tumor cells lentivirally transduced with BMP-9 as compared with those from control tumor cells. Furthermore, we observed that BMP-9 inhibited inflammation-induced lymphangiogegensis in the corneas and diaphragms of immunocompetent mice. Taken together, these results suggest that BMP-9 inhibits lymphangiogenesis both in vitro and in vivo through down-regulation of Prox1 expression, which leads to reprogramming of LECs to obtain BEC phenotypes.

(COI: No)

S24-5

Individualized minimally invasive treatment based on sentinel node concept for early gastric cancer

Takeuchi, Hiroya; Kitagawa, Yuko (*Dept. Surg. Keio Univ. Sch. Med, Tokyo, Japan*)

Clinical application of sentinel node (SN) mapping for early gastric cancer had been controversial for years. However, single institutional results of SN mapping for these cancers are almost acceptable in terms of detection rate and accuracy to determine lymph node status. SN mapping may play a key role to obtain individual metastatic information and allows modification of the surgical procedures for early gastric cancer.The Japan Society of Sentinel Node Navigation Surgery conducted a prospective multicenter trial of SN mapping for early gastric cancer by a dual tracer method with radioactive colloid and blue dye. SN mapping had been performed for 397 patients with early gastric cancer at 12 comprehensive hospitals including our institution. As results, detection rate of hot and/or blue node was 98%. The sensitivity to detect metastasis based on SN status was 93%, and accuracy of metastatic status based on SN was 99%. Based on these results, minimized gastrectomy such as partial gastrectomy, proximal gastrectomy, segmental gastrectomy and pylorus-preserving gastrectomy with individualized selective and modified lymphadenectomy for early gastric cancer with negative SN has been performed in our institution. More recently the combination of endoscopic resection with SN biopsy also appears attractively.Sensitivity of intraoperative diagnosis of micrometastasis is crucial for SN mapping in gastric cancer. However, the clinical impact of micrometastasis or isolated tumor cells detected by histopathology and molecular analysis in SNs of patients with early gastric cancer remains controversial.

(COI: No)

Symposium 25

Auditory information processing in local ciruit of the inferior colliculus

(March 21, 17:00～18:30, Room G)

S25-1

Organization of local circuit in the inferior colliculus

Ito, Tetsufumi (*Faculty Med. Sci. Univ. Fukui, Fukui, Japan*)

The inferior colliculus (IC) receives ascending inputs from virtually all lower brainstem auditory nuclei in which auditory information is extracted in parallel and integrate the sound information to create de novo responses to features of sound. However, morphological evidence for the integration of parallel auditory streams remained unclear. Large GABAergic (LG) cells are tectothalamic inhibitory neurons and characterized by the dense excitatory axosomatic terminals. We hypothesized that dense excitatory axosomatic terminals on LG cells arise from both local and ascending neurons. Injection of Sindbis palGFP virus in auditory brainstem nuclei resulted Golgi-like labeling of neurons at a single axonal level. A single excitatory axon from the lower brainstem auditory nuclei and IC made 1-6 axosomatic contacts on an LG cell, suggesting convergence of inputs from many neurons. These inputs are unlikely to be from a single brainstem nucleus since lesions of single nuclei fail to eliminate most excitatory axosomatic terminals on the LG cells. A single local excitatory neuron made laminar axonal plexus and contacted on cell bodies of 10-30 LG cells, suggesting that activation of a local neuron elicits synchronized excitation of LG cells in the same laminae. Double injections of different viruses in IC and in a brainstem nucleus showed that LG cells received inputs from both. The LG cell bodies in different IC regions received inputs from different brainstem nuclei, suggesting that combination of ascending inputs may different between LG cells in different locations. Such neural circuitry may underlie the integration of auditory information on a single IC neuron.

(COI: No)

S25-2

A temporal integration mechanism enhances frequency selectivity of broadband inputs to inferior colliculus

Chen, Chen[1]; Read, Heather L[2,3]; Escabi, Monty A[1,2,3] ([1]*Electrical and Computer Engineering, UConn, USA;* [2]*Biomedical Engineering, UConn, USA;* [3]*Psychology, UConn, USA*)

Mammals can accurately resolve frequency components in sounds, a task that is essential for sound recognition. Despite its importance, there is little direct evidence for how frequency selectivity is preserved or newly created across auditory structures. Here we demonstrate that pre potentials with properties resembling broadly tuned brainstem inputs can be recorded concurrently with post synaptic action potentials in inferior colliculus (IC). These putative input neurons (PIN) are broadly tuned and exhibit temporally delayed and spectrally interleaved excitation and inhibition not present in the paired IC neurons (ICN). A radical sharpening of tuning is accomplished locally by temporal integration of the broad converging inputs. A neuron model replicates the finding and demonstrates that temporal integration degrades timing precision but enhances spectral selectivity through interference of spectrally in- and out-phase inputs. This contrasts current models that require local inhibition to enhance selectivity and supports an alternative computational strategy to quickly enhance frequency selectivity.

(COI: No)

S25-3

Deviance detection in the auditory brain

Ayala, Yaneri A[1]; Oliver, Douglas L[2]; Malmierca, Manuel S[1] ([1]*Institute of Neuroscience of Castilla y Leon, University of Salamanca. Spain;* [2]*University of Connecticut Health Center, USA*)

The ability to discriminate novel stimuli is important for survival and it requires neuronal mechanisms to extract relevant information, to code regularities and to detect changes occurring in the acoustic scene. Neurons that exhibit stimulus-specific adaptation (SSA) may be involved in these computational tasks. SSA is a form of neural habituation in which the neuronal response to a common sound is diminished but not generalized to other sounds that occur rarely. We studied the anatomical and physiological correlates of SSA exhibited by single neurons in the inferior colliculus of the anesthetized rat. Our results suggest that the inferior colliculus is the first auditory stage where SSA emerges, and that neurons with strong SSA responses are located in the non-lemniscal subdivisions. Moreover, our studies indicate that the inputs to SSA recording sites differ from those to the non-SSA recording sites. This suggests specific synaptic domains provide inputs to neurons sensitive to deviant sounds. Finally, we demonstrate in iontophoretic studies that inhibitory and cholinergic inputs modulated SSA differently. The blockade of inhibition increased the response to common and deviant sounds while the acetylcholine only increased the response to the common sound. These data describe features of the neuromodulation and connectivity of a distributed neural network for mapping the saliency of the auditory scene.

(COI: No)

S25-4

Excitatory and inhibitory synaptic interactions underlying binaural hearing in inferior colliculus neurons

Ono, Munenori; Oliver, Douglas L (*Dept Neurosci, University of Connecticut Health Center, USA*)

Localizing the sound source by binaural hearing is critical for animal to survive. The inferior colliculus (IC) is a pivotal auditory center in the midbrain, yet little is known about the synaptic mechanisms that underlie binaural hearing in the inferior colliculus (IC). Here, we study the synaptic currents that process binaural hearing in vivo by voltage clamp technique. Monaural stimulation in either ear produced EPSCs and IPSCs in most neurons. The temporal properties of monaural responses were well matched, suggesting connected functional zones with matched inputs. Further, we studied the responses to interaural level difference (ILD), an important sound localization cue, by using stimuli in which ILD varies around a constant average binaural level to approximate sounds on the horizontal plane. EPSCs and IPSCs were well correlated in the response to the stimuli with different ILDs. Summation of the monaural EPSCs predicted the binaural excitatory response but less well than the summation of monaural IPSCs. Binaural EPSCs often showed a nonlinearity that strengthened the response to specific ILDs. Extracellular spike and intracellular current recordings from the same neuron showed that the ILD tuning of the spikes was sharper than that of the EPSCs. Thus, in the IC, balanced excitatory and inhibitory inputs maybe a critical feature of synaptic coding in sound localization.

(COI: No)

S25-5

In vivo optical and electrical recordings from inferior colliculus neurons by micro-endoscope

Funabiki, Kazuo[1,2,3]; Yashiro, Hidetaka[2]; Nakahara, Ichiro[1,3]; Kobayashi, Kohta[2]; Riquimaroux, Hiroshi[2] ([1]*OBI, Systems Biology, Japan;* [2]*Doshisha Univ. Life Med. Sci., Japan;* [3]*Kyoto Univ. Biostudies, Japan*)

In vivo Ca2+ imaging is a powerful method for the functional assessment of neural circuits. Although multi-photon excitation and two-photon fluorescence microscopy are used widely, observation of circuits in deep brain regions remains challenging. Recently, observing these deep regions has become possible via an endoscope consisting of an optical fiber bundle or gradient-index lens. We have developed a micro-endoscope system that enables simultaneous optical recording of fluorescence and electrical recording of neural activity. Using this system, we recorded auditory responses by simultaneously detecting changes in the fluorescence intensity of a Ca^{2+} indicator dye, multiunit activities, and local field potentials in the mouse's inferior colliculus (IC). Optical and electrical recording methods supplemented each other by providing high-resolution spatial and temporal information, respectively. By systematically changing sound frequency and intensity, we determined the frequency tuning of the recording site. The best frequency shifted higher as the probe advanced more deeply, demonstrating that the system is capable of optically measuring the dorsoventral organization of IC (i.e., tonotopicity). Thus, our new micro-endoscope system will be useful in analyzing neural circuits, including those within the auditory system.(CIO:No)

(COI: No)

Symposium 26

Clinical needs and Clinical anatomic researches

(March 21, 17:00～18:30, Room H)

S26-1

The extension pattern of a deep anal fistula in comparison with three-dimensional structures of the anal sphincter muscles and ischiorectal fossa

Kagawa, Ryuzaburo (*Rakuwakai Otowa Hosp, Kyoto, Japan*)

The primary abscesses of most deep anal fistulas are present in the posterior intersphincteric space at level of the deep part of external anal sphincter, or in the adjacent damaged external anal sphincter. From this origin, the tract of the fistula extends according to the three-dimensional structure of the anal sphincter muscles and ischiorectal fossa.
The bilateral puborectal muscles, in their anterior and posterior parts, send muscle fibers to the upper areas of the deep parts of the external anal sphincter muscles and form a ring-shaped muscle in a deep part of the external anal sphincter muscle. In ischiorectal anal fistula, the primary abscess, formed at the level of the deep part of external anal sphincter, penetrates the sphincter muscle in the directions of 5 and 7 o'clock and forms abscesses in the ischiorectal fossa below the levator ani muscle, following the course of the confluent puborectal muscle fibers.
In the adipose tissue of the ischiorectal fossa, there is a septum surrounding the anal sphincter muscles from the anteroposterior and vertical sides. An abscess that has penetrated the deep part of the external anal sphincter muscle in its posterior part extends along this septum. This results in the characteristic morphology of anal fistulas of the ischiorectal fossa with flat tracts advancing anteroinferiorly in the ischiorectal fossa.
In this report, I show jack-knife position MRI that three-dimensional analysis is possible with the structure of all deep anal fistulas.

(COI: No)

S26-2

My fellow anatomists, ask not what the physicians can do for you, ask what you can do for the physicians

Ibukuro, Kenji (*Diag Rad, Mitsui Mem Hosp, Tokyo, Japan*)

When I was a medical student, one of the first things we learned was the cutaneous distribution of the nerve in the upper arm. When I became a resident in radiology, I was asked to puncture the vein for injection of the contrast media. However, I noticed that I did not know much about the relation between the cutaneous nerve and the vein of the upper arm, when the patient complained the pain. The MHLW allowed the nurses to puncture the vein and place the indwelling needle several years ago. So we radiologist asked them to do that, but I was asked to tell them how to do it safely. I tried to gather the anatomical information about it, but I found very few. I thought what I could do was to dissect the cadaver by myself and show the nurses the result. Although the venous puncture is a simple procedure, it is undeniable fact that some patients sued the health care provider for the contiguous pain, peripheral nerve paralysis, etc. So how do we avoid these troubles?When it is necessary to place the central venous catheter, we should puncture either the subclavian or internal jugular vein. Compared with the peripheral venous puncture mentioned above, the complications related to this procedure are sometimes fatal. We need to know at least the venous anatomy but the anatomical knowledge with surrounding structure is more important to prevent or less the complications. So how do we get the anatomical knowledge in detail?If the anatomist would like to remain in the faculty of "medicine", you should participate to solve these clinical problems and "present danger", otherwise, you should be transferred to anthropology.

(COI: No)

S26-3

Anatomy for the clinicians (especially for the surgeons) based on my experience as a surgeon

Watanabe, Koichi; Iwanaga, Joe; Tabira, Yoko; Saga, Tsuyoshi; Yamaki, Koh-ichi (*Kurume Univ. Sch.Med., Fukuoka, Japan*)

The presenter is in the anatomy department now, but used to be engaged in plastic surgery over fifteen years. In this presentation, I would like to report what clinicians require to the anatomy with the based on my personal experience and my colleagues'(both clinicians and anatomists) opinion. I believe anatomical knowledge is extremely important for surgeons of every levels from resident to operator. In the case of residents, over five years have passed after anatomical lectures in the school. I consider that two problems are involved in them. One is that they usually forget majority of anatomical knowledge which the learned. And another is that the knowledge of regional anatomy which is not taught fully in the school is suddenly required in the surgery. To teach accurate anatomical knowledge is considered to be extremely important for the surgical training. The full-trained surgeons usually have a wide variety of anatomical knowledge about the region they usually treat. However, they also become to meed new anatomical knowledge in the case they have to perform inexperienced or newly developed operations. Furthermore, they need anatomical knowledge when they develop new operations or improve previous operations. In our department, we often collaborate with other clinical departments about the anatomical research and post graduate education. I consider that anatomists cooperate with clinicians more than ever and provide the anatomical knowledge in accordance with the level of surgeons. It will be benefit for both patients and clinicians.

(COI: No)

Symposium 27

Relationship between cellular functions and membrane transporters/ion channels

(March 21, 17:00～18:30, Room I)

S27-1

Removal of uremic toxin amelioreates the down regulation of SLCO4C1 transporter through transcriptional pathway

Abe, Takaaki (*Tohoku University Graduate School of Biomedical Engineering and Tohoku University Graduate School of Medicine, Japan*)

The accumulated uremic toxins inhibit the expression of various renal transporters and this inhibition may further reduce renal function and subsequently cause the accumulation of uremic toxins. However, the precise mechanism of the nephrotoxicity of uremic toxins on renal transport has been poorly understood. Recently, we have report that indoxyl sulfate, one of the potent uremic toxins, directly suppresses the renal-specific organic anion transporter SLCO4C1 expression through a transcription factor GATA3. The promoter region of SLCO4C1 gene has several GATA motifs, and indoxyl sulfate up-regulated GATA3 mRNA and subsequently down-regulated SLCO4C1 mRNA. Overexpression of GATA3 significantly reduced SLCO4C1 expression, and silencing of GATA3 increased SLCO4C1 expression vice versa. Administration of indoxyl sulfate to rats reduced renal expression of slco4c1 and under this condition, plasma level of guanidinosuccinate, one of the preferable substrates of slco4c1, was significantly increased without changing plasma creatinine.
Furthermore, in 5/6 nephrectomized rats, treatment with oral adsorbent AST-120 significantly decreased plasma indoxyl sulfate level and conversely increased the expression of slco4c1, following the reduction of plasma level of guanidinosuccinate. These data suggest that the removal of indoxyl sulfate and blocking its signal pathway may help to restore the SLCO4C1-mediated renal excretion of uremic toxins in CKD.

(COI: No)

S27-2

Characterization of LAT1 as a central transporter of essential amino acids in activated human T cells

Hayashi, Keitaro; Jutabha, Promsuk; Anzai, Naohiko (*Dept Pharmacol and Toxicol, Dokkyo Med Univ Sch Med, Tochigi, Japan*)

Activation of T cells accompanies remarkable enhancement of metabolism. Sufficient and continuous supply of nutrient such as amino acids is thus considerable importance to support immune reaction in T cells. However, the molecular mechanism of efficient incorporation of amino acids into activated T cells has not been determined. We characterized L-type amino acid transporter 1 (LAT1) as an essential amino acids transporter in activated human T cells. The activation of primary human T cells by CD3/CD28 stimulation triggers the dramatic induction of LAT1 mediated by NF-kB and AP-1. JPH203, a specific inhibitor of LAT1 suppressed amino acid incorporation and induction of DNA-damage-inducible transcript 3 (DDIT3) to attenuate cytokine production via inhibition of NF-kB and NFAT activities in activated human T cells. These results indicate that LAT1 expression is induced by full activation of T cells and works as a central transporter for essential amino acids in activated T cells. Our result uncover the previously unknown mechanism by which T cells accelerate essential amino acid uptake upon activation and adapt to essential amino acid starvation.

(COI: No)

S27-3

Localization of ATP sensitive K+ channel subunits in different organs and their possible functions

Zhou, Ming[1]; Kawahara, Katsumasa[2]; Abe, Hiroshi[1] ([1]*Akita Univ.Grad.Sch.Med., Akita, Japan;* [2]*Kitasato Univ.Sch.Med., Sagamihara Japan*)

ATP sensitive K+ (KATP) channel originally discovered in cardiomyocytes. From then, several kinds of KATP channel subunits were found. KATP channel has specific characteristics of channel opening and closing controlled by changes of intracellular ATP in micromole concentrations. It was considered with important functions of insulin secretion, cell protection against cardiac ischemia or brain anoxia. In cellular localization, it was proved in pancreatic β-cells, in cardiomyocytes, and in neurons and glia. KATP channels are composed of pore-forming subunits, which allow the potassium ion passing through, and regulatory subunits controlling the channel activity. The pore-forming subunits are Kir6.1 and Kir6.2, belonging to inwardly rectifying K+ channel subfamily, well, the regulatory subunits are SURs (SUR1, SUR2A and SUR2B), belonging to ATP binding cassette superfamily. Recently, a prior study of our lab (Zhou et al., Neurosci Res 2012) showed KATP channel subunits SUR2A and SUR2B differently localized in neurons and glial cells. The SUR2A localized mainly in neurons and in some oligodendrocytes, well the SUR2B weakly in neurons but mainly in astrocytes and some oligodendrocytes. The results from our lab also revealed that KATP channel subunits localized in other organs such as heart, kidney, submandibular gland, testis, ovary and pituitary gland. The wide and differential distribution of KATP channel subunits in those target cells and tissues indicates their diversity of relationship between biomedical metabolism and related possible functions.

(COI: No)

S27-4

Water and electrolytes transport across kidney collecting ducts through vasopressin receptors

Kawahara, Katsumasa[1]; Yasuoka, Yukiko[1]; Nonoguchi, Hiroshi[2] ([1]*Dept Physiol, Kitasato Univ Sch Med, Sagamihara, Japan;* [2]*Kitasato University Medical Center, Kitamoto, Japan*)

Vasopressin V1a receptor (V1aR) is known to modulate luminal water permeability of kidney collecting duct (CD) principal cells (PC) by competing with signals of V2R-cAMP-AQP2 axis, however, localization and role of V1aR in the CD are still controversy. In wild-type (WT) and V1aR knockout (KO) mice, we examined localization and expression of V1aR mRNA along the kidney nephron under the conditions of control (normal) and chronic metabolic acidosis (CMA). In control animals, normalized levels of the V1aR mRNA expression were high in macula densa and CD, low in glomerulus (Glm), thick ascending limb of Henle loop (TAL), and distal convoluted tubule (DCT). Surprisingly, we found that in CD, V1aR mRNA expressed in type A and type B intercalated cells (IC-A and IC-B, respectively). Under NH4Cl load, the level of V1aR mRNA increased only at medullary TALis and outer medullary CDis (is: inner stripe). We also found that in KO mice, lower urine concentration ability and metabolic acidosis vs. WT mice. In conclusion, V1aR may play an important role for controlling the basal water permeability in PC and stimulating urinary acid excretion by IC-A.

(COI: No)

S27-5

Physiological Significance of Delayed Rectifier K+-Channels (Kv1.3) Expressed in T lymphocytes and Their Pathological Significance in Chronic Kidney Disease

Kazama, Itsuro (*Dept Physiol I, Grad Sch Med, Tohoku Univ, Sendai, Japan*)

T lymphocytes predominantly express delayed rectifier K+-channels (Kv1.3) in their plasma membranes. Patch-clamp studies revealed that the channels play crucial roles in facilitating calcium influx necessary to trigger the lymphocyte activation and proliferation. In addition to selective channel inhibitors that have been developed, we recently showed physiological evidence that the drugs, such as non-steroidal anti-inflammatory drugs, antibiotics, anti-hypertensives and anti-cholesterol drugs, effectively suppress the channel currents in lymphocytes, and thus exert immunosuppressive effects.Using experimental animal models, previous studies revealed the pathological relevance between the expression of ion channels and the progression of renal diseases. As an extension, we recently demonstrated that the overexpression of lymphocyte Kv1.3-channels contributed to the progression of chronic kidney disease (CKD) by promoting cellular proliferation and interstitial fibrosis. In our most recent study, benidipine, a potent dihydropyridine calcium channel blocker which also strongly and persistently inhibited the lymphocyte Kv1.3-channel currents, actually ameliorated the progression of renal fibrosis in rat models with advanced chronic renal failure. Together with our in vitro results, the studies indicated the therapeutic potency of Kv1.3-channel inhibitors in the treatment or the prevention of CKD.

(COI: No)

S27-6

Therapeutic implications of myofibroblast TRP channels for stenotic fibrosis in Crohns disease

Kurahara, Lin[1]; Hiraishi, Keizo[1]; Sumiyoshi, Miho[1]; Aoyagi, Kunihiko[2]; Inoue, Ryuji[1] ([1]*Dept Physiol, Sch Med, Fukuoka Univ, Fukuoka, Japan;* [2]*Dept Gastroenterol, Sch Med, Fukuoka Univ, Fukuoka, Japan*)

Intestinal fibrosis is a frequent complication of Crohns disease (CD) and often leads to detrimental stricture formation. Myofibroblasts play active roles in mediating fibrotic changes in various tissues. In this study, we investigated whether transient receptor potential (TRP) channels in myofibroblasts are involved in CD-associated intestinal fibrosis, for the purpose of exploring its possible therapeutic targets.A profibrotic factor TGF-β1 treatment transformed spindle-shaped InMyoFibs into filament-shaped cells with enhanced α-SMA, N-cadherin, TRPC4 and TRPC6 expression. Augmented Ca^{2+} influxes due to TRPC6 upregulation facilitate stress fiber formation and strengthen cell-cell interactions by negatively regulating the synthesis of anti-fibrotic factors IL-10 and IL-11 in TGF-β1-treated myofibroblasts. Similar changes were observed in stenotic areas of CD patients, suggesting the therapeutic significance of targeting TRPC6. Active ingredients of Daikenchuto (TU-100) a traditional oriental herbal medicine used for post-operative ileus and constipation, such as hydroxy α-sanshool and [6]-shogaol induced Ca^{2+} influxes due to TRPA1. 24 hour incubation with TU-100 accelerated the mRNA and protein expression of TRPA1 in InMyoFibs. TU-100 also ameliorated Type I Collagen, α-SMA, N-cadherin expression and the phosphorylation of Smad2 and p38-MAPK at the downstream of TGF-β1.These results suggest that TRPC6 and TRPA1 channels could be promising targets for anti-fibrotic therapies in the gut.

(COI: No)

S27-7

Expression, function and phenotype of CFTR mutants found in Japanese CF patients

Sohma, Yoshiro[1]; Yu, Yingchun[1]; Nakakuki, Miyuki[2]; Ishiguro, Hiroshi[2] ([1]*Dept Pharmacol, Sch Med, Keio Univ, Tokyo, Japan;* [2]*Dept Human Nutrition, Sch. Med. Nagoya Univ. Nagoya, Japan*)

Cystic Fibrosis Transmembrane conductance Regulator (CFTR) functions as an ATP-dependent anion channel after a PKA-dependent phosphorylation. CFTR is expressed primarily in epithelial cells and its dysfunction causes Cystic fibrosis (CF), a life-shortening hereditary disease mainly affecting white Caucasians through insufficient exocrine. On the other hand, we recently reported that CFTR was expressed in pancreatic β-cells and its dysfunction induced a diabetes mellitus [1], which suggested that a group of Japanese diabetes patients might be caused by acquired CFTR dysfunctions. We investigated expression and function of nine CFTR mutants found in Japanese CF patients. In whole cell (WC) clamp experiment, R347H-, T633P- and T1220I-CFTR showed a WC current comparable to WT-CFTR. L441P- and R1066C-CFTR showed a smaller but significant WC current than WT-CFTR, however, no or little currents on Y517H-, E267V, M152R- and T1086I-CFTR. In the western blotting, R347H- and T633P-CFTR showed the mature C band which intensity was higher than the premature B band. R1066C-CFTR showed the B band higher than the C band whereas E267V- and T1086I-CFTR showed minimal signals for both B and C bands. These results are generally consistent with their phenotype.

[1] Guo JH, Sohma Y, *Chan HC. Glucose-induced electrical a ctivities and insulin secretion in pancreatic islet B -cells are modulated by CFTR. Nat Commun. 15;5: 4420, 2014.

The authors have declared no conflicts of interest.

(COI: No)

Symposium 28

New research focuses on the structure and function of gastric parietal cells

(March 21, 17:00～18:30, Room J)

S28-1

New and multiple functions of parietal cells

Ueyama, Takashi (*Wakayama Med.Univ., Wakayama, Japan*)

Gastric parietal cells are well-known in exocrine function of secreting hydrochloric acid into the gastric juice, which is antagonized by proton pump inhibitor (PPI). Here, new functions of parietal cells and PPI are proposed.

First, gastric parietal cells serve an endocrine function, whereby estrogen is synthesized and secreted into the portal vein. Gastric estrogen is not a simple sex steroid specific to female but a steroid common to both sexes. As one possibility, gastric estrogen may act as a local regulator of the gastro-hepatic axis.

Second, gastric hydrochloric acid supports the homeostasis of bone. Gastrectomy (GX) was thought to result in osteomalacia due to deficiencies in Vitamin D and calcium. Using a GX rat model, GX induced high turnover of bone with hyperosteoidosis, prominent increase of mineralization and increased mRNA expression of both osteoclasts and osteoblasts-related genes. The increased 1, 25 $(OH)_2$ D_3 level and unchanged PTH and calcitonin levels suggested that conventional bone and calcium metabolic pathways were not involved or changed in compensation. Gene expression profiles through microarray analysis and data mining using Ingenuity Pathway Analysis indicated 9 genes were hubs connected with tissue development and immunological diseases. These results suggest that chronic systemic inflammation might underlie the GX-induced pathological changes in bone.

Third, lansoprazole, a potent PPI, has an alternative indication in the prevention and treatment of oxidative hepatic damage through the induction of both phase I and phase II drug-metabolizing systems, i.e. the AhR/Cyp1a1/Nrf2 pathway in hepatocytes.

(COI: No)

S28-2

Localization and function of ion-transporting proteins involved in gastric acid secretion

Sakai, Hideki; Fujii, Takuto; Shimizu, Takahiro (*Dept. Pharm. Physiol., Grad. Sch. Med. Pharm. Sci., Univ. Toyama, Toyama, Japan*)

Hydrochloric acid (HCl) secretion by gastric parietal cells is accompanied with dramatic morphological changes. In resting parietal cells, tubulovesicles are present in intracellular compartments underlying the apical membrane. Upon stimulation, the tubulovesicles translocate and connect with the apical membrane, resulting in massive acid secretion. Proton is actively secreted by H, K-ATPase, a gastric proton pump, present in both the tubulovesicular and apical membranes. However, it has not been established what molecules contribute to Cl^- transport for HCl secretion. Here, we focus on two Cl^--transporting molecules (ClC-5, a chloride-proton exchanger and KCC4, a potassium-chloride co-transporter). We found that both ClC-5 and KCC4 are expressed in the parietal cells which secrete actively HCl at the luminal region of gastric glands. ClC-5 was co-immunoprecipitated with H, K-ATPase in tubulovesicles (TV), whereas KCC4 was co-immunoprecipitated with H, K-ATPase in the stimulation-associated vesicles (SAV) derived from the apical membrane. ClC-5 and KCC4 were functionally coupled with H, K-ATPase in TV and SAV, respectively. KCC4 may function as a K^+-supplying molecule for H, K-ATPase and also as a Cl^--transporting molecule for HCl secretion. In the resting phase of the parietal cells, KCC4 and H, K-ATPase in the apical membrane of the parietal cell are the main machineries for the basal gastric acid secretion. In the stimulated phase, they also contribute to acid secretion together with ClC-5 and H, K-ATPase in tubulovesicles.

(COI: No)

S28-3

Visualization of the intracellular signaling that integrates the gastric secretion

Fukushi, Yasuko; Sakurai, Takashi; Terakawa, Susumu (*Medical Photonics Research Center, Hamamatsu Univ. Sch. Medicine, Hamamatsu, Japan*)

The gastric gland consists of many kinds of cells such as parietal cells, chief cells and ECL cells; each secretes HCl, mucus and enzymes, respectively. These cells might cooperate for smooth gastric secretion without leaving any damage to each other. The presence of the gap junctions is known in the gastric gland. These gap junctions, mainly formed by connexin32, might be important for the defense system and for the regulation of acid secretion. However, the actual signal transmission through these gaps has not physiologically been demonstrated in the gastric gland yet. Moreover, there is few report elucidating such an intercellular signaling related to the cooperative secretion. In this study, we demonstrate a fluorescence image showing a histamine-induced intracellular signal that propagates from cell to cell in a living gastric gland isolated from the guinea pig. They were stained with acridine orange (AO), and then stimulated with 100 μM histamine. The green fluorescence of AO in the parietal cells was transiently increased, and the same response propagated along the gland for a long distance over many cells. Moreover, local stimulation with histamine on a couple of cells in the presence of suramin induced the propagation also. However, the fluorescence response was suppressed by addition of H89, an inhibitor of the PKA. It was concluded that the propagation of fluorescent signal along the gland reflects cAMP-dependent intercellular signals and that these signals indicate coordinated activations of a combination of many secretory cells in the gastric gland.

(COI: No)

S28-4

E-cadherin on the parietal cell in Helicobacter pylori infected gastric mucosa

Murakami, Motonobu; Fukuzawa, Mayu (*Doshisha Women's College of Liberal Arts, Kyoto, Japan*)

Atrophic gastritis caused by Helicobacter pylori is characterized by loss of parietal cell, which plays an important role in the maintenance of the normal structure of gastric mucosa. The gastric glandular unit provides a model for studying the processes that occur during deregulation of Helicobacter pylori infected gastric mucosa such as increase of proliferation in the isthmus, dysregulation of differentiation, and induction of cell death. We investigated the effects of gastric Helicobacter pylori infection on E-cadherin on parietal cells, generation of parietal cell, and deregulation of gastric glandular unit in Mongolian gerbils after inoculation with Helicobacter pylori. The number of parietal cells, staining of E-cadherin, and Ki67 in the stomach were investigated by immunohistochemistry with monoclonal antibodys against H+/K+-ATPase, E-cadherin, and Ki67 respectively. After inoculation, E-cadherin and parietal cells are lost and marked increase in proliferative activity of the proliferation zone in the isthmus with no new generation of parietal cells and microcirculatory disturbance of the mucosa were obserbed. Adherens junction plays a crucial role in the integrity of epithelium and E-cadherin is a key molecule in the morphogenesis, regulation of the fate of cells in proliferation and differentiation. Our study strongly suggests that disruption of E-cadherin in the inflamed mucosa plays an important role in the induction of parietal cell loss and deregulation in the gastric isthmus, leading to the gastric mucosal dysorganization observed in atrophic gastritis.

(COI: No)

S28-5

Exfoliation of gastric pit-parietal cells into the gastric lumen associated with autophagic degradation: an in vitro study discovered in iGM model

Toyoshima-Aoyama, Fumiyo; Takahashi, Nobuyasu; Sawaguchi, Akira (*Univ. Miyazaki, Miyazaki, Japan.*)

Parietal cells are produced in the isthmus of the gastric gland and migrate either upward to the pit region (referred to as pit-parietal cell) or downward to the glandular base via the neck region in rodents. Pit-parietal cells are highly differentiated cells responsible for the gastric hydrochloric acid secretion. We have recently noted that a number of pit-parietal cells were exfoliated into the lumen of isolated rat gastric mucosa (iGM) model, and applied the cryotechniques to elucidate the fine structure and histochemical characteristics in the process of cell exfoliation.As results, quantitative analysis clarified a time-dependent increase in the number of cell exfoliation in the iGM under histamine-stimulation compared to acid-inhibition with H_2-antagonist or proton pump inhibitor. Immunohistochemical staining of LC3 and beclin-1 demonstrated positive reactions in the exfoliated pit-parietal cells, suggesting that the cell exfoliation was preceded by autophagic degradation. In addition, immuno-electron microscopy verified characteristics of the autophagic cell death in the exfoliated pit-parietal cells, forming a number of autophagic ultra-structures in the cytoplasm. Another striking finding is that the pit-parietal cell exfoliation was usually accompanied by extension of the cytoplasmic processes from adjacent surface mucous cells. This finding indicates a crucial sealing of the basal region between the exfoliating pit-parietal cell and the basement membrane, to prevent an epithelial deficiency and possible gastric erosion and ulcer.

(COI: No)

Symposium 29

The structural cell physiology of tight junction protein claudin

(March 22, 9:00～10:30, Room C)

S29-1

Role of Tight Junction Claudins in Biological Systems-More than a Simple Paracellular Barrier in Biological Flow-

Tsukita, Sachiko; Tamura, Atsushi (*Grad. Sch. of Front. Biosci. and Grad.Sch.of Med., Osaka Univ., Osaka, Japan*)

Epithelial cell sheets cover the outer and inner surfaces of every compartment in the body, at every level, from the cell to the body surface, and at all of these levels, they function as a permselective barrier. The paracellular barrier, which is established by the formation of tight junctions between epithelial cells, has received considerable attention lately, because of new information about the claudin-family proteins, tight-junctional membrane proteins with four membrane-spanning regions. Claudins play a critical role both in paracellular permselectivity and as a structural component of the paracellular barrier. In this respect, the knockout mouse analyses, in which single claudins or multiple claudins in different combinations are targeted, are beginning to elucidate the in vivo biological relevance of paracellular barriers/permeability in many aspects of biological systems' functioning. In this respect, in these years we revealed the role of ion-leaky claudins claudin-2 and/or 15, which selectively permeate ions such as Na+, in the nutrient absorption systems. In digestive systems, the inflammation system backboned the safety guard for which tight junctional claudins play an important role. Thus, we would like to discuss the tight junction- based construction of biological systems in general.

(COI: No)

S29-2

Crystal structure of a claudin, a main component of tight junctions

Tani, Kazutoshi[1]; Suzuki, Hiroshi[1]; Tamura, Atsushi[2]; Tsukita, Sachiko[2]; Fujiyoshi, Yoshinori[1,3] (*[1]Cellular and Structural Physiology Institute, Nagoya Univ., Nagoya, Japan; [2]Laboratory of Biological Science, Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka Univ., Osaka, Japan; [3]Department of Basic Medical Science, Graduate School of Pharmaceutical Science, Nagoya Univ., Nagoya, Japan*)

Tight junctions are cell-cell adhesion structures in epithelial cell sheets that form boundaries to regulate the paracellular permeation of solutes inside and outside of multicellular organisms, and are crucial for maintaining homeostasis. Claudins are the major constituents of the tight junction strands and function as cell adhesion molecules and paracellular barriers. Recently we reported the first crystal structure of a mammalian claudin, revealing a transmembrane four-helix bundle that supports an extracellular domain with a unique β-sheet architecture. It comprises two extracellular segments and is anchored in a crevice between transmembrane helices by the highly conserved W-LW motif. Combined with the results of our mutational experiment, a linear arrangement of claudin-15 monomers in the crystal suggests that inter-molecular interactions for polymerization form potential paracellular ion pathways with distinctive surface charges. Our proposed model explains the dual functions of TJs in forming barriers as well as paracellular channels in epithelial cell sheets. Our findings provide insight into the molecular basis of the structure and function of tight junctions.

(COI: No)

S29-3

Structure and diversity of gap junction channels studied by electron microscopy

Oshima, Atsunori (*CeSPI, Nagoya Univ, Nagoya, Japan*)

Invertebrate-specific gap junction proteins, termed innexins, form a large family of four-transmembrane proteins. These proteins oligomerize to constitute intercellular channels that allow for the passage of small signaling molecules associated with neural and muscular electrical activity in invertebrates. In contrast to the large number of structural and functional studies of vertebrate connexin gap junction channels, there are few structural works on recombinant innexin channels. Here we show an electron microscopic analysis of solubilized and 2D crystallized Caenorhabditis elegans innexin-6 (INX-6) gap junction channels. Negative-staining electron microscopy (EM) of purified INX-6 gap junction channels revealed tandem particles. Class averages from those images indicated a longitudinal height of 220 Å, a channel diameter of 110 Å in the absence of detergent micelles, and an extracellular gap space of 60 Å. Single particle cryo-EM of purified INX-6 channels revealed eight rotational peaks related to the innexin subunit organization. We recently obtained 2D crystals of INX-6 channels, and cryo-EM crystallographic analysis revealed a projection map showing eight clear and separate densities around the pore. Initial 3D reconstruction at 10 Å resolution revealed that a single INX-6 full gap junction channel comprises 16 subunits, a hexadecamer. We also found plug densities in the opposing hemichannels, reminiscent of the Cx26M34A structure we previously reported. These results suggest that the oligomeric number of INX-6 channels is distinct from that of vertebrate connexin channels, and provide insight into innexin channel function.

(COI: No)

S29-4

Correlation of claudin molecular properties with its channel or barrier functions

Fromm, Michael; Krug, Susanne; Milatz, Susanne; Rosenthal, Rita; Piontek, Jorg; Gunzel, Dorothee (*Univ.med.Berlin, Berlin, Germany*)

Claudins connect neighboring epi- and endothelial cells by forming polymers which tighten the tissue layer against paracellular passage of solutes and water. Besides this general function, some claudins provide specific permeation sites. By this they form channels which, unlike common membrane channels, lead through extracellularly located pores. Several channel-forming claudins have been identified so far, some of them being selective for cations (claudin-2, -10b, -15), others for anions (claudin-10a, -17), and one for both, ions and water (claudin-2). Interestingly, some of the barrier-forming claudins form the barrier in a charge-selective manner too (e.g. claudin-4, -8, -14). Since the crystal structure of claudin-15 has been resolved it is a major topic of several labs to elucidate the structural design of a complete claudin-based channel. It is unambiguous that this channel is formed by extracellular loops originating from two or more claudin protomers arranged in cis and/or trans position. Recent data from our lab are based on structure-function studies investigating detailed channel or barrier properties. The proteins analyzed include claudin-2, -3, -5, -10a, -10b, and -17. Results comprise (i) structural features of the barrier-forming claudins 3 and 5, (ii) oligomerization properties of claudin-3, -10a and -10b, (iii) description of the pore formed by claudin-2 being common for cations and water, and (iv) structural features of claudin-17 protomers capable of anion channel formation.

(COI: No)

S29-5

Molecular mechanisms of claudin function and regulation: Implications for physiology, pathobiology, and therapy

Turner, Jerrold (*Univ. of Chicago, Chicago, USA*)

Intercellular tight junctions form paracellular seals that are selectively permeable. To define the mechanisms of paracellular permeability, we developed a trans-tight junction patch clamp approach and showed that individual pores are actively gated similar to transmembrane ion channels. To define regulation of these pores, we explored the molecular basis by which casein kinase-2 (CK2) inhibition reduces paracellular cation flux. CK2 inhibition increased the mobile pool of claudin-2 at the tight junction by triggering assembly of tripartite complexes that also included ZO-1 and occludin, thereby preventing claudin-2 from forming paracellular pores. Dephosphorylation of the CK2 target serine 408 within the occludin cytoplasmic tail regulates assembly of these tripartite complexes. Consistent with this, CK2 inhibition reversed IL-13-induced, claudin-2-dependent increases in intestinal epithelial paracellular cation permeability, both in vitro and in vivo. To determine the potential relevance of increased claudin-2 expression in inflammatory bowel disease (IBD), experimental IBD was induced in claudin-2-deficient mice and CK2 inhibitor-treated mice; both were markedly protected from disease. In contrast, CK2 inhibitor treatment had no effect on claudin-2-deficient mice, indicating that the benefit provided by CK2 inhibition was claudin2-dependent. As a whole, these data provide novel insight into molecular mechanisms of claudin-2 pore function, regulation, and impact in disease and suggest that exploitation of these processes, e.g. by CK2 inhibition, may be beneficial in human disease.

(COI: No)

S29-6

Recent advances in claudin binder platforms and their contribution to drug development

Kondoh, Masuo; Yagi, Kiyohito (*Grad Sch Pharm Sci, Osaka University*)

Claudins (CLDNs), a tetra-transmembrane protein family with 27 members, are components of tight junction-seals. They prevent the free movement of solutes across epithelial cell sheets. CLDNs are frequently overexpressed in malignant tumors and are co-receptors for hepatitis C virus (HCV). Thus, they are promising targets for drug development. A popular strategy for drug development against membrane proteins is the preparation of binders to their extracellular region. However, recombinant CLDN proteins are difficult to prepare, which slowed the proof-of-concept for CLDN-targeted drug development. Several studies have provided insights into CLDNs as targets for drug development. C-terminal fragment of *Clostridium perfringens* enterotoxin (C-CPE) was identified as the first CLDN binder, proving that CLDNs can be targets for enhancing mucosal drug absorption, treating cancer, and mucosal vaccination. C-CPE binding to CLDN-3, -4, -6, and -9 has low CLDN-specificity; CLDN-specific binding is a requirement for clinical application. Therefore, anti-CLDN monoclonal antibodies have been developed as CLDN binders; some of these have anti-tumor activity. Anti-CLDN-1 antibodies prevented *in vitro* and *in vivo* HCV infection without apparent adverse effects. Moreover, the first three-dimensional structure of CLDN was determined this April, which will greatly facilitate future CLDN-targeted drug development. Here, we present an overview of CLDN-targeted drug development from the perspective of advances in platforms to create CLDN binders. The authors have no conflicts of interest.

(COI: No)

Symposium 30

Contents and view points necessary for the co-medical education of anatomy and physiology

(March 22, 9:00～10:30, Room D)

S30-1

What is the goal of physiology in education for allied health professionals?

Watanabe, Masaru (*Grad Sch Front Health Sci, Tokyo Met Univ, Tokyo, Japan*)

There are many problems in physiology education in training courses for allied health professionals. First, the students who enter the courses are unequal in knowledge level of basic sciences. However, in most cases, the students have to study physiology in the first school year of the courses. Second, basic medicine education occupies only limited hours in the courses, although knowledge of medical sciences what the students have to know is expanding. Third, in many cases, there are only one or two teaching staffs of physiology in the courses. Finally, in some cases, questions about physiology in a state examination for the license to allied health professionals are not prepared by physiologists. In this talk, the author will give some suggestions what is the goal of physiology in education for allied health professionals under such a limited conditions.

(COI: No)

S30-2

What kind of anatomy education is required for pharmacy students?

Suzaki, Etsuko (*Sch.Pharm.Shujitsu Univ., Okayama, Japan*)

In co-medical fields such as nursing, physical therapy, and clinical radiology, there has been an increasing request for practical training in the education of human anatomy. In Hiroshima University School of Medicine, for example, almost 2,000 co-medical students from 29 different schools experienced dissection practices last year. Among these students, only about 40 students were from the pharmacy department, and the interest to human anatomy can hardly be said to be high in the pharmaceutical field. Eight years have passed since pharmacy department introduced the six-year system of education, in which pharmacists who can play an active role as a member of the team medical care are intended to be trained. Thus, the pharmacy students will be required to learn basic medicine such as anatomy and physiology as well as other co-medical students learn. Needless to say, they learn the working mechanisms of medicines that have specific target organs or cells in the human body. Therefore, it is naturally important and essential for them to understand the structure, function and regulation mechanisms of the body. Through anatomical and histological practices, pharmacy students are able to acquire deeper understanding and more useful knowledge about the human body. Such experiences will help them to become the pharmacist who can cope equally as a member of the team medical care. In School of Pharmacy, Shujitsu University, anatomical and histological practices including human dissection have been tried. What practices are being introduced and what students have learned will be explained and discussed.

(COI: No)

S30-3

Teaching anatomy and physiology in nursing education

Nakatani, Toshio (*Div.Nurs.Fac.HealthSci.Kanazawa Univ., Kanazawa, Japan*)

I here describe how I teach anatomy and physiology to nursing students at the Department of Nursing, School of Health Sciences, Kanazawa University. Anatomy and physiology course names are Basic Anatomy, Basic Physiology and Human Physiology. The textbook for them is Anatomy and Physiology, one of a series of systematic nursing lecture courses, Igakushoin. They are taught to 1st year nursing students. Basic Anatomy and Basic Physiology are each taught via one 90-minute class per week, with 15 lectures and 1 examination in the first semester, giving two credits. Human Physiology is taught via 7 lectures and 1 examination in the second semester, giving one credit. Regarding macroscopic anatomical practice, students who want to examine a dissected corpse go to the anatomical dissection room two times, in the morning on a Saturday in June, where they can observe the inner organs, muscles, blood vessels and nerves. Osteological practice is performed in one of the 15 lectures, during which the students observe the bones. I do not teach microscopic anatomical practice. I lecture according to the above textbook. I also distribute copies of papers and other books in combination with the textbook, as well as a collection of questions for self-teaching. I use Powerpoint with a computer touch panel, Windows 8 or a document presentation device in my lectures because I write various things while I am lecturing. I check attendance using a roll card, on which the students can also evaluate my lecture. The examination questions are true-false problems or multiple-choice questions.

(COI: No)

S30-4

Anatomy education for undergraduate health professionals needs improvement, particularly anatomy practice

Kawamata, Seiichi (*Inst.Biomed.HealthSci., Hiroshima Univ., Hiroshima, Japan*)

Anatomy education for undergraduate health professionals should principally deal with all parts of the body at macro- and microscopic levels and an appropriately long time should be allocated to classes and practice. The most important fields of anatomy differ among the great variety of courses for health professionals. Nursing students are mainly interested in the thoracic and abdominal organs, whereas physical therapy (PT) and occupational therapy (OT) students have to understand the musculoskeletal system. Thus, anatomy education should be customized for students depending on their specialty.Practice is very important. All students on health care professional courses should observe dissected human cadavers at least once in order to obtain a better understanding of human structures and to correct prejudices and avoid possible misunderstandings. Nursing, PT and OT students should observe the brain. Exposure to dissected cadavers provides a good opportunity for students to learn about individual variability, to think about life and death, and to nurture their professionalism. Microscopic observation is very useful to understand functions of cells and organs. Furthermore, PT and OT students should dissect the musculoskeletal system by themselves. However, anatomy practice differs considerably in terms of method, duration and quality in Japan, even within the same course. A considerable proportion of undergraduate health professionals have no chance to observe or dissect cadaveric materials. It is thus important to improve anatomy education, especially practice, for undergraduate health professionals.

(COI: No)

Symposium 31

Imaging studies of memory processes with various animal models

(March 22, 9:00～10:30, Room F)

S31-1

Learning-induced Changes of Neural and Behavioral Responses to Chemosensory Stimuli in *C. elegans*

Iino, Yuichi[1]; Kunitomo, Hirofumi[1]; Ohno, Hayao[1]; Sato, Hirofumi[1]; Satoh, Yohsuke[1]; Iwata, Ryo[1,2] ([1]*Dept Biol Sci, Grad Sch Sci, Tokyo Univ, Tokyo, Japan;* [2]*Present Addr, RIKEN Ctr Dev Biol, Kobe, Japan*)

The nematode *C. elegans* is an excellent model organism for elucidating the neural basis of behavioral plasticity because of its simple nervous system and genetic manipulability. It senses various chemicals, processes the sensory information and shows behavioral output called chemotaxis. The direction of chemotaxis changes depending on previous experience. For example, after cultivation at a high concentration of salt, it migrates to high concentration of salt, while it will avoid high concentration of salt after cultivation at a low concentration of salt. These observations suggested that worms form a memory of salt concentration and recognize the difference between current concentration and previous salt concentration to determine the direction of movement. Neural activity imaging suggested that transmission of information from the sensory neuron to the first-layer interneurons is the major site of neural plasticity. Mutants of the phospholipase C/diacylglycerol/protein kinase C pathway migrated to abnormally higher or lower salt concentrations, suggesting that the activity of this pathway, which acts in the sensory neuron, biases the movement towards higher salt concentrations. Observations using a FRET reporter indicated that diacylglycerol level in the sensory neuron changes by the change in sensory input, suggesting that the diacylglycerol pathway is involved in either formation or read-out of the concentration memory.

(COI: No)

S31-2

Hippocampal neural circuit dynamics imaged during spatial behavior in mice

Sato, Masaaki[1,2] ([1]*PRESTO, Japan Science and Technology Agency, Kawaguchi, Japan;* [2]*RIKEN Brain Science Institute, Wako, Japan*)

In 1940s, the Canadian psychologist Donald Hebb proposed the theory of "cell assembly", in which he postulated neurons acting together are arranged into groups to form the brain basis of mental representation. Our research aims to elucidate the principle of how such functional circuits emerge, operate and change during repeated experience and learning. To directly visualize the dynamics of neuronal circuit activity in awake behaving animals, we have developed a set of new technologies, such as transgenic mice that express new fluorescent calcium indicator proteins in the brain, a virtual reality (VR) set-up for head-fixed mice and automated image analysis software. Mice head-fixed above an air-supported spherical treadmill were allowed to run freely in a computer-generated VR environment rendered on a wide LCD monitor that provided visual feedback in response to running. The mice trained in a virtual linear track task learned to exhibit spontaneous alteration of running and standing still, which allowed us to study behavioral-state dependent changes of hippocampal neuronal ensemble dynamics with two-photon calcium imaging. To test whether mice can discriminate a particular place in VR, we have recently established a new hippocampus-dependent virtual spatial recognition task. Our ongoing imaging experiments in mice performing this task will provide important insights into hippocampal neural network dynamics underlying memory-guided spatial behavior.

(COI: No)

S31-3

Visualization of Neural Representations of Memory

Matsuo, Naoki[1,2] ([1]*Dept Mol Behav Neurosci, Grad Sch Med, Osaka Univ;* [2]*PRESTO, JST, Saitama, Japan*)

Memories are presumably stored in a specific small subset group of neurons sparsely distributed in the brain in response to various sensory experiences. One of the major difficulties in studying the mechanism of cognitive functions including learning and memory is the identification of the "functional" neuronal populations among the hundred billions of neurons in mammalian brain. We have developed a transgenic system in mice that allows us a genetic manipulation in those neurons activated by a given behavioral stimulus during a limited time window. The mice express tetracycline-regulated transactivator (tTA) under the control of promoter of the c-fos gene, one of the immediate-early genes whose expression is rapidly and transiently induced in response to neuronal activities. The transgenic system provides a distinctive tool for visualizing the dynamism of neuronal ensembles representing a given information or memory.

(COI: No)

S31-4

Chemical tools to control cellular chemistry

Furuta, Toshiaki (*Dept Biomol Sci, Faculty of Sci, Toho Univ, Chiba, Japan*)

Caged compounds are designed synthetic molecules so that their original biological activities are temporally masked by covalently attached photo-caging groups. After being applied into live cells or tissue samples, appropriately designed caged compounds can manipulate various cellular processes such as neuronal signaling, intracellular signal transduction and gene expression upon photo-irradiation. The purpose of our study is to develop new photo-responsive chemical tools for controlling and monitoring cellular processes with high spatial and temporal resolution. We designed and synthesized brominated hydroxycoumarin (Bhc) chromophores as photo-responsible protecting groups which can be activated under one and two-photon excitation conditions with improved photochemical efficiency. The protecting groups have been applied to making caged compounds of low molecular weight organic compounds including neurotransmitters and second messengers. Thus, we demonstrated photo-manipulation of sperm motility using Bhc-caged cyclic nucleotides. Intracellular signaling in immature T cells can be controlled with subcellular spatial resolution using Bhc-caged DAG and flashes of focused UV light. Modular approaches to preparing caged compounds of DNAs and RNAs enabled photo-mediated gene expression in mammalian cells. One barrier to using conventional caged compounds in in vivo applications is the lack of cell type specificity because the compounds are not genetically encoded. To overcome the problems, we have developed new modular Bhc-caged compounds which can be photo-activated with cell type specificity.

(COI: No)

S31-5

Morphological analysis of memory circuits in rat, rabbit, and marmoset brains

Honda, Yoshiko (*Dept.Anat.Sch.Med.Tokyo Women's Med.Univ., Tokyo, Japan*)

The development of various techniques for visualizing memory circuits has recently enabled us to connect morphological information to functional information more easily. Research using various genetically modified animals is also increasing, and it is now essential to gain a sufficient understanding of the morphological information, i.e., the normal pattern of neuronal connections in each animal species, in advance of functional analyses of the various model animals. Here, we introduce some of the morphological features of memory circuits in rat, rabbit, and marmoset brains that have been clarified to date. The most essential portion of a memory circuit can be predicted to be generally preserved over rodents to primates; to elucidate such fundamental connections, we investigated neuronal connections between the hippocampus and parahippocampal cortices (i.e., the presubiculum, parasubiculum, and entorhinal cortex), in each species. Standard tracers, such as HRP, CTB, and BDA, were injected into the hippocampal body or several parahippocampal cortices, enabling input-and-output connections between each area on the cell mass level to be investigated. In addition, the palGFP-expressing Sindbis virus vector was used to analyze axonal arborization and termination of single neurons, particularly in the rat.

(COI: No)

Symposium 32

Chrono-network ~Molecular Physiology/ Anatomy Cross-talking with Biological Time

(March 22, 9:00～10:30, Room G)

S32-1

The chrononetwork and hypoxia signaling pathways

Ikeda, Masaaki[1,2]; Kumagai, Megumi[1,2]; Nakajima, Yoshihiro[3]; Ueno, Munehisa[4]; Okabe, Takashi[4] (*[1]Dept Physiol, Sch Med, Saitama Medical Univ, Moroyama, Japan; [2]Mol Clock Project, Res Center for Genomic Med, Saitama Medical Univ, Hidaka, Japan; [3]Biofunctional Regulation Res Group, Health Res Inst, AIST, Takamatsu, Japan; [4]Dept Uro-Oncol, Saitama Medical Univ, Hidaka, Japan*)

Hypoxia sensing is an important homeostatic mechanism in mammals. Hypoxia-inducible factors (HIFs), which belong to the basic helix-loop-helix-Per-Arnt-Sim transcription factor family, function as sensors for hypoxia and are involved not only in the transactivation of hypoxia-induced genes but also in pathologic processes such as carcinogenesis and the progression and metastasis of many cancers. Recently, we demonstrated that HIF-1α activated transcription of the *Period2* (*Per2*) gene via an HIF response element and enhanced the amplitude of the *Per2* promoter rhythm in cells. HIFs and PER2 play important roles in cancer progression. We examined PER2 expression by Western blotting in renal cancer cell lines; however, almost no expression was detected. The protein level of HIF-1α was also negligible in these cells. Real-time monitoring of *Per2* promoter activity using a destabilized luciferase reporter fused with the *Per2* promoter revealed that the oscillation of *Per2* promoter activity was highly diminished in all of the renal cancer cell lines examined. Thus, the expression levels of PER2 and HIF-1α are determining factors in the circadian oscillation of clock genes in cancer cells.

(COI: No)

S32-2

Molecular signals connecting Chrono-network of the adaptation systems

Tamaru, Teruya (*Dept. of Physiol., Toho Univ. Sch. of Med.*)

Among various adaptation systems against environments/ stresses/ aging, circadian (clock) system has daily-periodicity and synchronizing properties to external stimuli. Circadian system is driven by cell-autonomous molecular clocks (core circadian oscillator) consisted with clock genes/ proteins; Bmal1, Clock, Cry, Per. Dysfunctional clocks become risk factor for various diseases, likely via down-regulated adaptation (protection, repair, etc.). Thus, manipulating (chrono-) network of the adaptation systems via circadian signal would become potential medical strategy. So far, we found; 1) protein modification-interaction oscillator controlling core circadian oscillator (Science 2005, Nature 2007, Nat Struct Mol Biol 2009), 2) cell injury stresses -evoked clock synchronization and activation of adaptation pathways (PLoS ONE 2011, 2013). Here, as molecular signals connecting chrono-network of the adaptation system, we will discuss about BMAL1, CRY, CK2, HSF1, etc., as the players in the chrono-networking among the core circadian oscillator, protein modification-interaction oscillator and adaptation systems

(COI: No)

S32-3

Direct interaction between tumor suppressors and the circadian rhythm

Miki, Takao (*Dept Mol Biol, Grad Sch Med, Kyoto Univ, Kyoto, Japan*)

Accumulating epidemiological evidence suggests that cancer and the circadian clock have close interplay, although direct molecular evidence in mammalian systems remains scarce. The circadian clock is the daily oscillation of biological processes and believed to have close interplay with many fundamental cellular pathways including metabolism. Previous studies have shown that mice lacking Period 2 (Per2), a critical component of circadian clock, was cancer prone, suggesting that clock genes could be involved in tumor suppression and hence, PER2 and other tumor suppressors may function in an integrated network. We screened several tumor suppressors for their possible clock functions and identified two major tumor suppressors, p53 and PML. We also found that p53 and PML exert their clock functions by regulating Per2. These studies highlight the direct connection between tumor suppression and the circadian clock. Since the circadian clock is known to regulate cellular metabolism, some of the metabolic changes found in cancer cells may also be explained by loss of tumor suppressors involved in circadian clock regulation.

(COI: No)

S32-4

A chemical biology approach to dissect chrononetwork

Hirota, Tsuyoshi (*ITbM, Nagoya Univ, Nagoya, Japan*)

The circadian clock is an intrinsic time-keeping mechanism that coordinates the daily rhythms of numerous physiological processes, such as sleep/wake behavior, hormone secretion, and metabolism. Circadian rhythms are generated in a cell-autonomous manner through transcriptional regulatory networks of the clock genes. To develop a new approach for the circadian clock research, we applied chemical biology that uses chemicals to investigate biological machinery. We have established a cell-based high-throughput circadian assay and conducted phenotype-based chemical screens. From hundreds of thousands of small molecules, we identified two compounds named longdaysin and LH846 that potently lengthen the period of the circadian clock through inhibition of casein kinase I family proteins. More recently, we discovered a new class of period lengthening compound named KL001 that specifically interacts with the core clock protein CRY to inhibit its degradation. We demonstrated that KL001 inhibits glucagon-dependent induction of gluconeogenesis in mouse primary hepatocytes, based on a regulatory role of CRY in the pathway. KL001 is the first compound specifically targeting CRY and may provide an opportunity to enable clock-based control of gluconeogenesis. Furthermore, quantitative manipulation using compounds in combination with mathematical modeling enabled systems level understanding of the clock oscillation. These studies indicate effectiveness of chemical biology approaches for a better understanding of chrononetwork.

(COI: No)

S32-5

Cytosolic calcium rhythms in circadian pacemaker neurons: Current issues and future perspective

Ikeda, Masayuki (*Grad Schl Sci Eng, Univ Toyama, Toyama, Japan*)

Since presence of tetrodotoxin-resistant circadian Ca^{2+} rhythms (CCR) in suprachiasmatic nucleus (SCN) neurons was reported (Ikeda et al, *Neuron* 2003), numerous of newer findings progress the understanding of ionic rhythms in circadian pacemakers. I will overview these and discuss about future perspective in this filed. Cytosolic free Ca^{2+} is a ubiquitous signaling messenger, with plant cells showing strong CCR. However, corresponding CCR have not been reported in animal cells, other than in mature SCN neurons. For example, endocrine circadian oscillators in pupae of *Drosophila melanogaster* failed to display dynamic CCR (Morioka et al, *Nat Commun* 2012). In addition, we analyzed a SCN progenitor cell line that stably expresses YC3.6 (SCN2.2YC), but failed to observe CCR in these cells (Takeuchi et al, *Sci Rep* 2014). Because SCN2.2YC displays Per1-luciferase oscillations and expresses voltage-sensitive Ca^{2+} channels, other unveiled component(s) that are present in mature SCN neurons may be essential for the generation of CCR. It should be emphasized that machineries specific for pacemaker neurons may be present as we demonstrated that differential involvement of the C-terminal motif of clock gene Bmal1 between SCN neurons and fibroblasts, and that nuclear translocation of BMAL1 is more strictly regulated in SCN neurons (Ikeda and Ikeda, *J Neurosci* 2014). Candidate molecules and/or machineries linking molecular oscillations to ionic rhythms will be argued by comparing rhythms in lateral neurons, which are central pacemaker neurons in *Drosophila melanogaster*.

(COI: No)

Symposium 33

Frontiers in morphological and functional studies of neocortical circuits

(March 22, 9:00～10:30, Room H)

S33-1

Compartmental organization of synaptic inputs to parvalbumin-expressing inhibitory neurons in mouse neocortex

Hioki, Hiroyuki (*Morphol. Brain Sci., Grad. Sch. Med., Kyoto Univ., Kyoto, Japan*)

Neocortical GABAergic neurons are divided into at least three distinct subgroups by chemical markers: 1) parvalbumin (PV); 2) somatostatin (SOM); 3) other markers such as vasoactive intestinal polypeptide (VIP). PV neurons are a major component of neocortical GABAergic neurons, and considered to play a key role in higher-order brain functions and psychiatric disorders.

We have recently succeeded in visualizing dendrites and cell bodies of PV neurons completely by generating transgenic mice. Using the mice, we first analyzed excitatory and inhibitory inputs to PV neurons in the primary somatosensory cortex. Corticocortical glutamatergic inputs were more frequently found on the distal dendrites than on the soma, whereas thalamocortical inputs did not differ between the proximal and distal portions. GABAergic terminals were more densely distributed on the cell bodies than on the dendrites.

We further investigated which types of neocortical GABAergic neurons preferred the cell bodies of PV neurons to the dendrites. We revealed that the dendritic compartment principally received GABAergic inputs from PV neurons, while the somatic compartment received inputs from VIP neurons. This compartmental organization of synaptic inputs suggests that PV neurons communicate with each other mainly via the dendrites, and that their activity is effectively controlled by the somatic inputs of VIP neurons. In addition, this further suggests that PV neurons located in the superficial and deep cortical layers are simultaneously inhibited by vertically running VIP axons.

(COI: No)

S33-2

Dynamic behavior of inhibitory synapse on pyramidal cell

Kubota, Yoshiyuki[1,2,3] ([1]*National Institute for Physiological Sciences;* [2]*SOKENDAI, Okazaki, Japan;* [3]*JST-CREST, Tokyo, Japan*)

While the adult brain has long been considered hard-wired, recent in vivo imaging studies using excitatory or inhibitory synaptic markers have revealed a capacity for remodeling of neuronal connections. Here, we simultaneously monitor in vivo inhibitory synapse and dendritic spine dynamics across the dendritic arbor of pyramidal neurons in the adult mouse cortex using large-volume, high-resolution dual-color two-photon microscopy. We chronically monitored postsynaptic markers onto Layer 2/3 pyramidal neurons in the mouse primary visual cortex in vivo. We found that inhibitory synapses on dendritic spines are exceptionally dynamic as compared to other synaptic populations, due to the fact that a large proportion of them disappear and recur again in the same location on a timescale of days. In contrast to these inhibitory synapses on dually innervated spines, excitatory synapses on the same spines, as well as the spines themselves were extremely stable. Electron microscopic observation revealed that when the postsynaptic element of recurrent synapses observed in vivo disappeared, a presynaptic inhibitory axon could be found adjacent to the site of the recently removed inhibitory postsynaptic structure. Inhibitory synapses are often found on dendritic spines which receive direct thalamic input. Thus, the function of inhibitory synaptic remodeling at this locale may serve as a mechanism for gating feedforward excitation, rather than, as proposed for excitatory synapses, as a mechanism to remodel circuitry and exchange partners.

(COI: No)

S33-3

Macroscopic functional organization of natural visual representation in the human cortex

Nishimoto, Shinji[1,2] ([1]*Center for Information and Neural Networks, National Institute of Information and Communications Technology, Osaka, Japan;* [2]*Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan*)

One of the long-term goals of systems neuroscience is to reveal the functional and anatomical principles underlying our natural perception and behavior. Recently, we developed a quantitative framework to understand the natural visual representation in the human visual cortex via modeling movie-evoked brain activity measured using fMRI (functional magnetic resonance imaging). The framework aimed to reveal feature spaces of visual representation within cortical areas of interest as well as elucidate how the representation was distributed across the cortex. Using this framework we recovered the representations of spatiotemporal and semantic information in the human visual cortex as well as their macroscopic functional structure. The framework is general and can also be used to decode visual experiences and assess quantitative differences in cortical representation between different cognitive states or individuals. Our framework is a powerful tool to facilitate the quantitative understanding of functional structures in the human cortex under natural conditions.

(COI: No)

S33-4

Visual object recognition based on short-term memory in mice

Shibuki, Katsuei (*Dept Neurophysiol, Brain Res Inst, Niigata Univ, Niigata, Japan*)

Mice are important experimental animals, since many new techniques are available in the experiments using mice. We found that mice have visual short-term memory of objects. In a memory-dependent task, a cue object was presented at center of the display in front of mice, and two choice objects including the original cue object were presented with a delay interval of 20 s. Mice could select the original cue object based on short-term memory. After this visual short-term memory sessions were finished, we confirmed that mice could similarly differentiate a pair of alphabets, which they had never seen before, based on short-term memory. These results indicate that mice can recognize and memorize visual objects as complex as alphabets. Higher visual areas responsible for object recognition are unknown in mice. To identify the responsible areas, we used an association memory paradigm. After mice were exposed a combination of an object and a sound, they could choose the object based on the associated sound cue only. We further investigated cortical responses to the sound cue using flavoprotein fluorescence imaging. The cortical responses to the sound cue were observed in the auditory cortex and higher visual areas located dorsally to the auditory cortex, suggesting that the activated higher visual areas may play an important role in objects recognition. As expected, we found object-specific neuronal activity in these areas using two-photon microscopy. Interestingly, the short-term memory, associative memory, and the memory based-cortical activities were not found in mice with reduced diversity of clustered protocadherin α.

(COI: No)

Symposium 34

Crosstalk between nervous and immune systems

(March 22, 9:00～10:30, Room I)

S34-1

Local Neural Activation Enhances Inflammation via Gateway Reflex

Murakami, Masaaki (*Inst. Genetic Medicine and Grad. Sch. Med., Hokkaido Univ., Sapporo, Japan*)

The CNS is an immune-privileged environment that can be compromised by an accumulation of immune cells, particularly pathogenic T cells. Using a transfer system from the multiple sclerosis model, EAE, we show autoreactive Th17 cells accumulate in the CNS via dorsal blood vessels in the 5th lumbar-cord (L5 cord). These vessels excessively express various chemokines including CCL20, which attracts the autoreactive Th17 cells, in a manner dependent on regional nerve activation mediated by anti-gravity responses on the soleus muscle, which enhances the NFkB signal in the L5 vessels. This activation increased vessel blood flow in the L5 cord via the activation of L5 sympathetic neurons. On the other hand, the inhibition of norepinephrine signaling in vivo suppressed CCL20 expression, pathogenic T cell accumulation in the L5 cord, and EAE-development. Consistent with these observations, norepinephrine enhanced a synergistic NFkB signal after IL-17A and IL-6 stimulation. We term these neuroinflammatory relationship as Gateway Reflex, which describes that neural activation can be transformed into an inflammatory-signal in the dorsal vessels of the L5 potentially leading to autoimmune diseases like EAE. Thus, the Gateway Reflex, may offer new therapeutic targets for various diseases and disorders particularly in the CNS.

(COI: No)

S34-2

RGMa modulates T cell responses and is involved in Th17 cell-induced neurodegeneration in autoimmune encephalomyelitis

Yamashita, Toshihide (*Grad.Sch.Med.Osaka Univ., Osaka, Japan*)

Multiple sclerosis (MS) is an autoimmune disease caused by myelin-specific T cells that induce an immune response against the brain and spinal cord. We demonstrated that repulsive guidance molecule-a (RGMa) is a promising new target for the treatment of MS. RGM was originally identified as a membrane-bound protein with repulsive and growth cone collapse-inducing activities in the chick retinotectal system. Expression analysis revealed that RGMa is expressed in bone marrow-derived dendritic cells (BMDCs) and that CD4+ T cells express receptor for RGMa. Treatment with neutralizing antibodies to RGMa prevented mouse experimental autoimmune encephalomyelitis (EAE) and reduced invasion by inflammatory cells. In humans, RGMa-specific antibody could modulate T cell proliferative responses and cytokine expression in peripheral blood mononuclear cells isolated from patients with relapsing-remitting MS. These results show that RGMa-specific antibody suppresses T cell response to antigens. Furthermore, we recently demonstrated that RGMa is associated with neurodegeneration in EAE. RGMa was highly expressed in interleukin-17-producing CD4+ T cells (Th17 cells). We induced EAE by adoptive transfer of myelin oligodendrocyte glycoprotein (MOG)-specific Th17 cells. Inhibition of RGMa improved EAE scores and reduced neuronal degeneration without altering immune or glial responses. Th17 cells induced cultured cortical neuron death through RGMa-neogenin and Akt dephosphorylation. Our results demonstrate that RGMa is involved in Th17 cell-mediated neurodegeneration.

(COI: No)

S34-3

Mechanisms of brain inflammation after stroke

Yoshimura, Akihiko; Ito, Minako; Shichita, Takashi (*Dept Microbiol & Immunol., Keio Univ. School of Med., Tokyo, Japan*)

It has been well established that the interleukin-23 (IL-23)-IL-17 axis plays essential role in experimental autoimmune encephalomyelitis (EAE) which is a well-established Th17 cell-mediated brain and spinal cord inflammation. Stroke or brain ischemia is one of the major causes of death and disability worldwide. Post-ischemic inflammation is an essential step in the progression of brain ischemia-reperfusion injury. In a mouse stroke model, we have reported that IL-23 and IL-17 play essential roles in infarct volume growth in the brain ischemia model. IL-23 is produced from infiltrating macrophages, which induces IL-17 from T cells. IL-17 is mainly produced from $\gamma\delta$ T cells and promotes delayed (day 3-4) ischemic brain damage. Furthermore, we demonstrate that peroxiredoxin (Prx) family proteins released extracellularly from necrotic brain cells induce expression of inflammatory cytokines including interleukin-23 in macrophages through activation of Toll-like receptor 2 (TLR2) and TLR4, even though intracellular Prxs have been shown to be neuroprotective. Extracellular Prxs are cleared by macrophages, which is important for the resolution of brain inflammation. We have clarified a scheme of brain inflammation after stroke.

(COI: No)

S34-4

Translational Research in Neuroimmunological Disorders

Yamamura, Takashi (*Dept Immunol, Nat Inst Neurosc, NCNP, Kodaira*)

Pathogenesis of neurological disorders was traditionally investigated by means of clinical, pathological, and electrophysiological techniques. More recently, genetics and radiology have contributed greatly to better diagnosis and classifications of rare diseases. However, immunological approaches in the past decade have been most successful in the development of drugs. In fact, interferon-β, anti-Vα4 integrin antibodies, and fingolimod have been developed for multiple sclerosis (MS), an autoimmune disease, accompanying inflammatory demyelinating lesions in the central nervous system (CNS). Of note is that these emerging therapies gained seeds from the academic research, indicating the importance of translational research. In this symposium, I will talk about two ongoing translational studies that we designed. The first one, application of anti-IL-6 receptor antibody therapy for neuromyelitis optica (NMO), was initiated following the discovery that IL-6 dependent plasmablasts producing autoantibodies are increased in the peripheral blood of patients with NMO (PNAS 2011). Another study, development of NKT cell ligand therapy for MS, aims at obtaining proof of concept to ask if the efficacy of glycolipid OCH is efficacious not only in mouse MS model (Nature 2001) but also in human. Unexpected results from these studies will be highlighted with regard to the scientific merit and novelty.

(COI: No)

S34-5

Gut immunity and neuroinflammation

Miyake, Sachiko (*Juntendo Univ. Sch Med., Tokyo Japan*)

Multiple sclerosis (MS) is a chronic autoimmune disease targeting the central nervous system (CNS). Recent increase in the number of MS patients in Japan is probably attributed to the environmental changes rather than genetic changes. The intestine has lately received much attention as a potential location for the regulation of immune cells. We and other groups previously showed that alterations of gut environment could lead to the amelioration of experimental autoimmune encephalomyelitis (EAE), a rodent model for MS. We investigated the characteristics of myelin reactive T cells in the gut and the molecular mechanism of the way they could influence on CNS autoimmunity using myelin oligodendrocyte glycoprotein (MOG) reactive T-cell receptor transgenic (2D2) mice. Adoptively transferred 2D2-CD4+ cells among intraepithelial lymphocytes (IELs) ameliorated EAE. The transferred IEL were found to migrate into the CNS and up-regulated several immune regulatory molecules. 2D2-CD4+ IELs exhibited the suppressive activities on T cell proliferation. These findings suggested that gut is an important place to regulate the function of autoreactive T cells and neuroinflammation.

(COI: No)

Symposium 35

Neuronal mechanisms of respiratory control in the medulla and spinal cord: integrative view of the anatomy and function

(March 22, 9:00～10:30, Room J)

S35-1

Recent progress in understanding of a respiratory rhythm generation center, pFRG

Ikeda, Keiko[1]; Onimaru, Hiroshi[2] (*[1]Biology, Hyogo Col. Med., Hyogo, Japan; [2]Dept Physiol, Showa Univ Sch Med, Tokyo, Japan*)

The pivotal role of the respiratory center in homeostasis is to control ventilation to maintain optimum pCO_2/pH and pO_2 in extracellular fluids. Such information is perceived by peripheral and central chemoreceptors. As regarding the central chemoreception, the understanding of the cytoarchitecture of respiratory control center in the brainstem has been progressed greatly in the last two decades. One of the milestones was the discovery of *PHOX2B* mutations in patients of congenital hypoventilation syndrome which shows abrogation or a great reduction of the sensitivity to hypercapnia. Another was the identification of unique expression pattern of the paired-type homeobox gene *Phox2b*. Others and we have reported the existence of a small population of Phox2b-expressing neurons in the parafacial respiratory group (pFRG)/the retrotrapezoid nucleus (RTN) in the brainstem. The preservation of CO_2 sensitivity even after blockade of Na^+ channels and Ca^{2+} channels in the Phox2b-positive pre-inspiratory (Pre-I) neurons in pFRG/RTN indicated that the Pre-I neurons indeed possess CO_2 sensor molecule(s) whose transcriptional expression may be directly regulated by Phox2b. To uncover the sensor molecules and to facilitate understanding of respiratory neural network, we have recently generated a bacterial artificial chromosome transgenic rat line harboring a fluorescent protein under the control of a mouse *Phox2b* promoter/emhancer. Here we show a new insight into cytoarchitecture together with electrophysiological function of the Phox2b-positive neurons using this transgenic rat.

(COI: No)

S35-2

Anatomy of the respiratory rhythmogenic kernel: pre-Bötzinger complex of the medulla

Okada, Yasumasa[1]; Yokota, Shigefumi[2] (*[1]Clin Res Ctr, Murayama Med Ctr, Tokyo, Japan; [2] Dept Anat Morphol Neurosci, Shimane Univ Sch Med, Izumo, Japan*)

The preBotzinger complex (preBotC) of the ventrolateral medulla is the kernel for respiratory rhythm generation. We analyzed anatomical connection of preBotC neurons to and from other respiratory related medullary regions in rats. In retrograde and anterograde tracing, Fluoro-gold (FG) and biotinylated dextran amines (BDA) were microinjected into the unilateral preBotC, respectively. Putative rhythmogenic neurons were identified in the preBotC by immunostaining of neurokinin-1 receptor (NK1R) and somatostatin (SST). A large number of FG-labeled neurons were distributed in the contralateral ventrolateral medulla throughout its rostrocaudal extent. One-third of FG-labeled neurons were immunoreactive for NK1R in the preBotC. In anterograde tracing, we found that BDA-labeled boutons were in contiguity with dendrites and somata of neurons that were double-labeled with NK1R and SST in the contralateral preBotC. When the preBotC region was electron microscopically examined, we found BDA-labeled axon terminals making synaptic contacts with somatic or dendritic profiles of NK1R-immunoreactive neurons in the contralateral preBotC, and most of the synapses observed were of an asymmetrical type. We elucidated the anatomical pathways (1) from the preBotC in one side to the contralateral preBotC, and (2) from the preBotC directly to the bilateral hypoglossal premotor and motor areas as well as to the nuclei tractus solitarius. These connectivities would be the anatomical basis for bilaterally synchronized respiratory rhythmogenis and robust control of breathing.

(COI: No)

S35-3

Physiology of the pre-Bötzinger Complex

Koshiya, Naohiro (*NIH-NINDS, Bethesda MD, USA*)

The ventrolateral medulla contains respiratory neurons. While many of them (the ventral respiratory group) project to spinal motor nuclei, a gap was found at a mesorostrocaudal level, where respiratory neurons were mostly propriobulbar. Discovered there was a bilaterally distributed population pacemaker for breathing rhythm, named pre-Botzinger complex (pBC). Within the heterogeneous reticular formation, some pBC inspiratory (pacemaker) neurons possess intrinsic rhythm generation mechanisms. They are capable to generate periodic bursts, even in isolated conditions, with balanced conductances of a persistent Na+ (gNaP) and K+ dominated leak (non-voltage-gated), arguably with other conductances (e.g., Ca2+). The pBC inspiratory pacemaker population is functionally connected together with mutual glutamatergic synapses, on each side of the brainstem and bilaterally by decussating axons, with which they synchronize their activities. Also within this positive recurrent recruitment system, other non-pacemaker (synaptic amplifier) inspiratory neurons conceivably contribute to the population-level generation of the central inspiratory drive. These pBC neurons express variety of synaptic and ligand receptors, with which the rhythmogenic kernel is functionally embedded in larger systems. Via such supersets' afferents, the pBC monitors environmental information, e.g., from the arterial and central chemoreceptors, as feedback; furthermore the pBC cells have chemosensitivity by themselves for a fundamental homeostasis. We will review the pBC's inspiratory motor rhythm generation mechanisms in a multiscale perspective from cellular biophysics to synaptic, microcircuit, population, and network levels.

(COI: No)

S35-4

Structure and function of respiratory neuronal circuits of the high cervical spinal cord

Oku, Yoshitaka[1]; Hayakawa, Tetsu[2] (*[1]Dept Physiol, Hyogo Col Med, Nishinomiya, Japan; [2]Dept Anat, Hyogo Col Med, Nishinomiya, Japan*)

Animals spinalized at the C1 level can generate respiratory rhythm (Aoki et al. 1980). However, since administration of curare abolishes phrenic activity, the respiratory rhythmicity is thought to be supported by feedback inputs from cutaneous and chest wall proprioceptors. In the current concept, eupnea, defined by the breathing pattern comprising inspiration, post-inspiration, and expiration, requires the integrity of the pontine-medullary respiratory network. Essential structures for eupneic breathing have been postulated to extend from the pons to the pre-Botzinger complex. Structures caudal to the obex are thought to be unnecessary for eupneic breathing. Optical imaging using voltage-sensitive dyes led to discoveries of novel respiratory regions: the parafacial respiratory group (Onimaru and Homma, 2003) and the high cervical respiratory group (HCRG) in the spinal cord (Oku et al., 2008). This latter discovery motivated a re-examination of the role of spinal cord in respiratory rhythmogenesis. Jones et al. (2012) recorded phrenic (PNA) and hypoglossal (HNA) nerve activity in the perfused brainstem preparation of rat. Transverse transections at the pyramidal decussation not only abolished PNA immediately, but also progressively deprived HNA amplitude and rhythm. Transverse transections at the first cervical spinal segment level did not abolish HNA rhythmicity. The result contradicts the current concept of the genesis of eupnea, and indicates the importance of structures at the medullo-spinal junction.

(COI: No)

S35-5

Respiratory activity in the thoracic spinal cord

Iizuka, Makito (*Dept Physiol, Showa Univ Sch Med, Tokyo, Japan*)

The inspiratory and expiratory motor outputs are larger in the intercostal muscles positioned at more rostral and caudal segments, respectively. Such rostro-caudal gradient is kept in the *in vitro* preparation from neonatal rat. It is well documented that there are many propriospinal respiratory neurons in the thoracic spinal cord and anatomical studies showed no evidences that the inspiratory bulbospinal neurons have systematic patterns of connections to different segments. Therefore, such gradients in part could be generated by the excitatory respiratory propriospinal neurons with similar distribution. To examine this hypothesis, the respiratory-related neuronal activities were optically recorded from thoracic segments in the brainstem-spinal cord preparations from neonatal rats stained with voltage-sensitive dye. Respiratory-related signals were detected from ventral surface of all spinal segments examined (T1-T13). The blockade of the synaptic transmission in the thoracic spinal cord by the low Ca^{2+} superfusate blocked all respiratory signals, suggesting that these signals should come from spinal neurons. Areas of the optical signals evoked by the antidromic activation of the motoneurons were restricted in the lateral areas where the respiratory signals were observed. Therefore, the medial areas would come from the spinal interneurons. In both areas, more rostral thoracic segments showed larger inspiratory-related signals. These results support our hypothesis.

(COI: No)

S35-6

Integrative view of respiratory control mechanisms in the pons, medulla and spinal cord

Onimaru, Hiroshi[1]; Koizumi, Hidehiko[2] (*[1]Dept Physiol, Showa Univ Sch Med, Tokyo, Japan; [2]USA*)

Central respiratory neuronal activity is primarily produced in the respiratory rhythm generator of the ventral medulla. The respiratory rhythm and motor patterns are regulated by various information from many regions in the lower brainstem including the pons while they are transmitted to motor neurons (e.g. in the spinal cord) via pre-motor neurons. The normal respiratory motor pattern consists of basically three or four phases; pre-inspiratory, inspiratory, post-inspiratory and late-expiratory. A number of different types of preparations from mainly mice or rats have been used for analyses of respiratory rhythm and pattern generation; medullary slice (newborn or juvenile), en bloc brainstem-spinal cord preparation (newborn), decerebrate and arterially perfused preparation (newborn or juvenile) and in vivo preparation (any ages). These experimental variations that show the different motor output pattern of respiratory activity caused some controversy in the field. Recent studies demonstrated the significant role of the pons in formation of the respiratory burst pattern. On the basis of our knowledge from recent studies, we provide an integrative view of respiratory control mechanisms involving the pons, medulla and spinal cord.

(COI: No)

Symposium 36

Frontier of functional and morphological research in epithelial tissues of digestive organs

(March 22, 16:00～17:30, Room C)

S36-1

Refined arrangement of monocarboxylate transporters (SMCT and MCT) in the intestine

Iwanaga, Toshihiko (*Grad. Sch. Med. Hokkaido Univ., Sapporo, Japan*)

Plant-derived dietary fibers and undigested carbohydrates are fermented by bacterial microflora in the large intestine, resulting in the productions of acetate, propionates, and butyrate, collectively called short-chain fatty acids (SCFAs). SCFAs are further classified into monocarboxylates together with lactate and ketone bodies. In order to absorb SCFAs as nutrients, the epithelium of the large intestine expresses a selective transporter, sodium-coupled monocarboxylate transporter (SMCT1). SMCT1 is localized only at the brush border of the large intestine. The gut epithelial cells also possess a proton-coupled transporter for SCFAs, MCT (monocarboxylate transporter)-1, on the basolateral membrane to transport SCFAs through the epithelium to internal milieu. Another type of SMCT2 with 59% identity with SMCT1 mediates the transport of monocarboxylates with low affinity as compared with SMCT1. Since its distribution was restricted to the jejunum and ileum, function of SMCT2 may be to absorb lactate from fermented milk and yoghurt and acetic acid rich in vinegar. The arrangement of high affinity SMCT1 and low affinity SMCT2 is biologically significant, as in the case of the kidney, where differential distribution of SMCT2 in the proximal urinary tubules and of SMCT1 in the distal part makes possible the effective re-absorption of lactate. MCT1 in the small intestine was expressed intensely in the basolateral membrane of crypt cells including dividing cells. Expression patterns of MCT1 in the skin, bone marrow as well as the small intestine suggest that monocarboxylates are favorite energy sources in self-renewing tissues.

(COI: No)

S36-2

The functional diversity of TJ-barrier in the digestive tract regulated by TJ-claudins in mice

Tamura, Atsushi; Tsukita, Sachiko (*Grad. Sch. of Front. Biosci. and Grad.Sch.of Med., Osaka Univ.*)

The properties of the tight junction (TJ) in digestive tracts are so different between organs/tissues. The permselective properties of TJ are thought to be mainly determined by the expression pattern of TJ component membranous protein claudins. What does different pattern of claudin based TJ permeability plays roles in multicellular organisms? This question has not been resolved well in many cases. In the liver, claudin-1, -2, -3 are dominantly expressed. Among them, claudin-1, -3 are barrier forming types of claudins and homogeneously expressed throughout the hepatic lobule while claudin-2 is channel forming type of claudin and predominantly expressed only around perivenous zone. Recently, we analyzed and found the bile flow of claudin-2 deficient mice were decreased by about a half compared to that of wild type mice because of the decreased expression of channel forming type of claudin-2 between sinusoidal flow and bile canaliculi flow. The bile canaliculi terminate in blind ends in the perivenous region and the bile flow starts from the perivenous region toward the periportal region. On the other hand, the sinusoidal blood streams start from the periportal region toward the perivenous region on the contrary to the bile flow. This counter current flow system seems adequatly maintained by the uneven distribution of cation/water permeable clauin-2. Thus, claudin based TJ function in the biological and pathophysiological system is discussed especially in claudin-2 deficient mice.

(COI: No)

S36-3

Molecular mechanism of morphofunctional regulation via PPARα autocrine modulation of Ca^{2+}-regulated exocytosis in mucous cells of gastric antrum

Tanaka, Saori[1]; Nakahari, Takashi[2] (*[1]Laboratory of Pharmacotherapy, Osaka University of Pharmaceutical Sciences, Takatsuki, Japan; [2]Department of Molecular Cell Physiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*)

In antral mucous cells, the Ca^{2+}-regulated exocytosis activated by ACh consists of an initial transient increase (initial phase) followed by a second slower decline (late phase). The ACh-stimulated exocytosis is modulated by PPAR α. However, we did not know how PPAR α modulates ACh-stimulated exocytosis. We studied the PPAR α actions in ACh-stimulated exocytosis of antral mucous cells. GW7647 (PPAR α agonist) enhanced the ACh-stimulated initial phase which was abolished by GW6471 (PPAR α antagonist). However, GW6471 produced a delayed, but transient increase (delayed increase) in the late phase. The inhibition of the initial phase and the delayed increase in the late phase were similarly induced by a PKG inhibitor or a NOS1 inhibitor. Moreover, in antral mucosae, ACh or GW7647 increased NO production and cGMP contents. On the other hand, Wortmannin (an inhibitor of PI3K) and Akt 2/2 kinase inhibitor (an inhibitor of Akt) also abolished the enhancement of initial phase and produced the delayed increase in the late phase during PPAR α activation in ACh-stimulated exocytosis. These observations suggest that AA/PPAR α autocrine mechanism stimulates NOS1 mediated via PI3K/Akt pathway leading to NO production and cGMP accumulation, which enhances the Ca^{2+}-regulated exocytosis in antral mucous cells.

(COI: No)

S36-4

Physiological effects of short-chain fatty acids on the intestinal epithelia - Difference between species and intestinal segments

Karaki, Shinichiro; Kuwahara, Atsukazu (*Lab Physiol, Sch Food Nutr Sci, Univ Shizuoka, Shizuoka, Japan*)

Short-chain fatty acids (SCFAs), 2-6 carbon monocarboxylic acids, are the predominant fermented products in the large intestine. They not only are absorbed as nutrients, but also stimulate intestinal mucosa inducing a variety of physiological responses including transepithelial ion transport. In the rat and guinea pig, it has been reported that SCFAs induce an anion secretion measured by the Ussing chamber technique. However, there had been no report that SCFAs induced a secretion in the human intestine until we reported the SCFA-induced anion secretion in the human terminal ileum at the Annual Meeting of the Physiological Society of Japan last year. We have found that SCFAs do not induce ion transport in any segments of the human colon, but induce anion secretion in the human terminal ileum. In the rat and guinea pig colon, it has been reported that propionate evokes anion secretion, but acetate does not. However in the human terminal ileum, the luminal addition of acetate also concentration-dependently evoked an anion secretion the same as propionate. Moreover, the luminal acetate-induced response was attenuated dependent on the concentration of propionate pretreated, and vice versa. These results suggest that the reflux of SCFAs from cecum to terminal ileum passing through the ileocecal valve may induce a fluid secretion in the human terminal ileum. These differences in the effects of SCFAS on intestinal epithelia between species and intestinal segments might be due to the food habit and/or the style of feces.

(COI: No)

S36-5

The molecular mechanism of intracellular Cl^- function in gastric cancer invasion and metastasis by regulating expression of cell adhesion molecules

Miyazaki, Hiroaki[1,2]; Marunaka, Yoshinori[1,2] (*[1]Dept Mol Cell Physiol, Grad Sch Med Sci, Kyoto Pref Univ Med, Kyoto, Japan; [2]Japan Inst Food Edu Health, Heian Jogakuin (St. Agnes) Univ, Kyoto, Japan*)

As tumors progress to increased malignancy, cells develop the ability to invade into surrounding normal tissues (the de-adhesion process) and through tissue boundaries to form new growths (the adhesion process) at sites distinct from the primary tumor; i.e., . It is generally accepted that the alterations of cell-cell and cell-matrix adhesion in metastatic tumor cells caused by changing their microenvironments may play critical roles in the metastatic process. Our recent studies show that the intracellular Cl^- plays important roles in fundamental cellular functions that would be involved in the cancer process. If there are differences between the cytosolic Cl^- concentrations ($[Cl^-]_c$) of primary and metastatic tumor cells due to ionic environments surrounding primary and metastatic tumor cells, and the activity of Cl^- transporters and/or Cl^- channels of primary and metastatic tumor cells, the change in $[Cl^-]_c$ of primary and metastatic tumor cells would be a candidate causing the de-adhesion and adhesion. Therefore, we investigated the effect of $[Cl^-]_c$ on the cell-matrix adhesion and the expression of cell adhesion molecules in several gastrointestinal tumor cell lines. Our study indicates that cytosolic Cl^- is a key factor regulating expressions of cell adhesion molecules, CD44 and EpCAM involved in tumor invasion, strongly suggesting that changes of $[Cl^-]_c$ would play important roles in invasions and metastasis of gastrointestinal tumor cells.

(COI: No)

S36-6

Molecular mechanism for morphological and functional regulation by scaffold proteins in the bile duct epithelium

Hatano, Ryo; Asano, Shinji (*Dept Mol Physiol, Col Pharm Sci, Ritsumeikan Univ, Shiga, Japan*)

Secretin dependent biliary secretion of ions and water by transporters and/or channels is essential for the regulation of biliary flow. Cystic fibrosis transmembrane conductance regulator (CFTR) plays a key role in the chloride secretion into the bile. In CF patients, totally 5 to 10% of patients develop the progressive biliary fibrosis. ERM (ezrin-radixin-moesin) proteins are identified as cross-linkers between the plasma membrane proteins and actin cytoskeleton. Ezrin interacts with Na^+/H^+ exchanger regulatory factor-1 (NHERF1) via its N-terminal binding domain and with actin cytoskeleton via its C-terminal actin-binding domain. CFTR is associated with NHERF1 via its c-terminal PDZ binding motif. In liver, Ezrin is exclusively expressed in the cholangiocytes and colocalizes with CFTR and NHERF1 at apical membrane of cholangiocyte. In the present study, we have found that ezrin knockdown ($Vil2^{kd/kd}$) mice develop severe hepatic injury characterized by extensive bile duct proliferation, periductular fibrosis, and intrahepatic bile acid accumulation. In these mice, apical membrane localizations of CFTR and NHERF1 were disturbed in the bile ducts. Reduced surface expression of these proteins was accompanied by reduced CFTR-mediated Cl^- efflux activity. Our data suggest that ezrin plays essential roles in the regulation of the bile duct morphology and functions.

(COI: No)

Symposium 37

Developmental insights into cellular communications during organogenesis

(March 22, 16:00～17:30, Room E)

S37-1

Linx: a transmembrane protein directing the establishment of neural circuits in the central and peripheral nervous systems during development

Mandai, Kenji (*Grad.Sch.Med.Kobe Univ., Kobe, Japan*)

The establishment of neural circuits relies on neuronal responses to guidance cues that are expressed spatially and temporally in a right place. To address molecular mechanisms underlying axonal growth, guidance, and target field innervation of developing neurons, we performed genome-wide screens and identified a novel gene coding a LIG family transmembrane protein, Linx. Linx forms complexes with receptor tyrosine kinases, TrkA and Ret, to control axonal extension, branching, and guidance of somatosensory and spinal motor neurons. In the brain, Linx is robustly expressed on corticofugal axons, but not on thalamocortical axons. The mice with a null mutation of Linx exhibit a complete absence of the internal capsule, although layer V cortical neurons and thalamic neurons are intact. Moreover, regional inactivation of Linx either in the prethalamus and lateral ganglionic eminence or in the neocortex leads to a failure of internal capsule formation. Furthermore, Linx binds to thalamocortical projections and promotes their outgrowth. Thus, Linx guides the extension of thalamocortical axons in the ventral forebrain and it mediates reciprocal interactions between thalamocortical and corticofugal axons at the pallial-subpallial boundary and guidance and extension of all ascending and descending projections of the mammalian neocortex. These observations indicate that Linx directs the establishment of neural circuits in both the central and peripheral nervous systems during development.

(COI: No)

S37-2

Crosstalk between hair follicle stem cells and their niche

Fujiwara, Hironobu (*RIKEN CDB, Kobe, Japan*)

It is well established that the special microenvironment for stem cells, the stem cell niche, sends signals to stem cells to regulate their behaviour. However, our recent studies indicate that stem cells themselves also act as a niche for neighbouring cells in the epidermal stem cell system. The hair follicle bulge in the epidermis associates with the arrector pili muscle that is responsible for the formation of goosebumps (Torihada in Japanese). We have recently demonstrated that mouse hair follicle bulge stem cells specialize the basement membrane in their niche, by depositing basement membrane protein nephronectin, thereby creating a special microenvironment for the development and anchorage of arrector pili muscles. We propose that bulge stem cells function as tendon cells in providing a physical connection for the muscles. The immobility of bulge stem cell compartment assures that muscle attachment is stable regardless of the stage of hair generation cycle. We expanded our study to different dermal cell populations and found that the epidermal stem cells also instruct morphogenesis and regeneration of skin dermal adipocytes. Periodical activation of epidermal Wnt/beta-catenin signalling during hair follicle morphogenesis and regeneration induces and synchronises the differentiation of adipocytes via secretion of adipogenic factors. Our findings indicate that the epidermal stem cells not only contribute to epidermal homeostasis and regeneration, but also act as unique environments for dermal cell populations to achieve coordinated skin morphogenesis and regeneration.

(COI: No)

S37-3

Evolution of the turtle shell: insights from developmental, paleontological and genomic perspectives

Kuratani, Shigeru (*Evolutionary Morphology, RIKEN CDB, Japan*)

The turtle shell consists of the dorsal half, or the carapace, which is formed of expanded ribs and the thoracic vertebral column, and the ventral dermal moiety, the plastron. The carapace represents an evolutionary novelty, since it shows an unusual topography. During the turtle development, growth of the turtle ribs is arrested in the axial part of the body only allowed to grow laterally towards the carapacial ridge (CR), a turtle-specific embryonic ridge, folding the body wall medially to encapsulate the scapula. The embryonic pattern of the turtle before this folding resembles the recently discovered fossil species, Odontochelys. The CR supports fan-shaped patterning of the ribs by specific expression of some regulatory genes apparently downstream of Wnt signaling. Analysis of draft genomes of Pelodiscus sinensis and Chelonia mydas confirmed a close relationship of turtles to the bird/crocodilian lineage, which split about 250 mya. Embryonic transcriptome analysis revealed an hourglass-like divergence between turtle and chicken embryogenesis, with maximal conservation around the vertebrate phylotypic period. Survey of the P. sinensis genome also allowed us to identify Wnt5a as the only Wnt ligand in the CR, apparently supporting the possible co-option of limb developmental program in the acquisition of the shell. However, our recent RNA^seq analyses sugegst that CR was more likely to have been obtained by modifying the proximal part of the lateral body wall.

(COI: No)

S37-4

On the origin of parasympathetic ganglia

Brunet, Jean-François (*IBENS*)

Neural crest cells migrate extensively and give rise to most of the peripheral nervous system. The formation of sympathetic, enteric, and dorsal root ganglia has been extensively documented. Much less information is available concerning the way in which parasympathetic ganglia form at numerous locations close to their target organ. I will present how parasympathetic precursors, in the form of Schwann Cell Precursors that coexpress the pan-autonomic transcriptional determinant Phox2b, invade the preganglionic branches of cranial nerves, accumulate at their tip and differentiate into the constituent neurons of their targets, the parasympathetic ganglia — a parsimonious solution to the wiring of autonomic pathways.

(COI: No)

Symposium 38

Anatomical and physiological approaches reveal the mechanism of memory retrieval in the Parabrachial Nucleus

(March 22, 16:00～17:30, Room F)

S38-1

Respiratory circuit in Parabrachial nucleus complex might involve in Panic disorder

Arata, Akiko (*Dept Physiol, Hyogo College of Med., Hyogo, Japan*)

The parabrachial nucleus complex (PB) of the pons is known as a respiratory modulating center and autonomic relay nucleus. The PB projected to paraventricular nucleus and amygdala controlling emotion and stress. The distribution of orexinergic axon fibers existed in the PB of neonatal rat. Orexin plays an essential role of establishing sleep-wakefulness cycle besides orexin also contributes to emotional stress and other state-dependent related regulation of ventilation, and the defense response. On the other hand, the PB is considered as an inspiratory termination and chemoreception. We reported previously the PB plays an active inspiratory-expiratory phase switching in neonatal rat. However, the effects of orexin on the relationship between respiration and chemoreception in neonatal stage of the pons had not been investigated. The firing rate of I-E neuron was increased by superfusion of orexin. Under the hypercapnia, orexin induced higher respiratory rate, and long duration of inspiratory activity appeared frequently in the C4 after 10 minutes from orexin application. Orexin increased respiratory rate by facilitating I-E neuron activity in the PB using inspiratory termination as an active phase-switching, and hypercapnia induced more facilitate and repetitive activity in the long duration of inspiratory phase, that kept for a half hour. These results suggested that PB might be involved in hyperventilation syndrome and panic disorder by controlling respiration using inspiratory termination as an active phase-switch under stress condition.

(COI: No)

S38-2

Pathway from the parabrachial nucleus to the phrenic nucleus is activated by hypercapnia

Yokota, Shigefumi[1]; Kaur, Satvinder[2]; Vanderhorst, Veronique G[2]; Saper, Clifford B[2]; Oka, Tatsuro[1]; Yasui, Yukihiko[1]; Chamberlin, Nancy L[2] (*[1]Dept. of Anat. & Morphol. Neurosci., Shimane Univ. Sch. of Med., Izumo, Japan; [2] Neurol., BIDMC & Harvard Med. Sch., Boston, USA*)

Elevated CO2 (hypercapnia) facilitates breathing by increasing the depth and frequency of ventilation. Recently, it is suggested that the parabrachial nucleus (PB) is a key mediator of respiratory facilitation in hypercapnia. Our previous studies demonstrated inspiratory facilitation after stimulation of the lateral PB and Kolliker-Fuse nucleus (KF) and the existence of glutamatergic pathways directly and indirectly via the ventrolateral medulla (VLM) from the KF to the phrenic nucleus (PhN). In this symposium, we first show the distribution of PB neurons that are activated by hypercapnia using Fos-immunohistochemistry. After 2 hours exposure to normoxic hypercapnia (10% CO2), a greater number of Fos-immunoreactive neurons were observed in the rostral KF as well as in the lateral crescent (cr), external lateral, and central lateral PB subnuclei compared to the control. Most of these neurons in the PB were positive for VGLUT2 mRNA but not for GAD67 mRNA. Using retrograde tracing combined with Fos-labeling, we secondly show that numerous hypercapnia-activated neurons in both the KF and the cr subnucleus or solely in the KF were labeled following cholera toxin b subunit injected into the VLM and into the PhN, respectively. These findings suggested that glutamatergic PB neurons activated by hypercapnia contribute to diaphragmatic contraction, thereby increasing ventilation through their direct and indirect pathways to the PhN.

(COI: No)

S38-3

Optogenetic demonstration of direct inputs from the lateral parabrachial nucleus to the nociceptive amygdala

Sugimura, Yae K[1, 2]; Takahashi, Yukari[1]; Watabe, Ayako M[1]; Kato, Fusao[1] (*[1]Dept Neurosci, Jikei Univ Sch Med, Tokyo Japan; [2]Research Fellow of Japan Society for the Promotion of Science*)

A large majority of neurons in the superficial layer of the dorsal horn project to the lateral parabrachial nucleus (LPB). The LPB neurons then project to the capsular part of the central amygdala (CeC), a key structure underlying nociception-induced emotional responses. It is demonstrated that LPB-CeC synaptic transmission is enhanced in various pain models by using electrical stimulation of the fibers arising from the LPB in brain slices. However this approach has limitations in examining direct monosynaptic connections devoid of contamination of synaptic inputs from locally stimulated neurons and fibers arising from other structures. To overcome these limitations, we transfected AAV vector for channelrodopsin (ChR2) expression to the LPB in rats and prepared brain slices containing amygdala with ChR2-expressing fibers 5-7 weeks after transfection. We found that blue light stimulation resulted in EPSCs with very small latency fluctuation and potent polysynaptic feed-forward inhibition in CeC neurons regardless of firing pattern type. Intraplanter formalin injection made 24 hours before the slice preparation resulted in a significantly larger EPSC amplitude than those with saline injection only in the CeC neurons showing late-firing pattern. These results suggest that direct inputs from the LPB are enhanced only in a specific type of CeC neurons in inflammatory pain model.

(COI: No)

S38-4

Genetic tracing reveals the architectural solution in the parabrachial nucleus that processes taste information and gates emotional memory

Sugita, Makoto; Yamamoto, Kuniyo; Hirono, Chikara; Shiba, Yoshiki (*Department of Physiology and Oral Physiology, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan*)

Bitter and sweet taste stimuli elicit contrastive behavioral and emotional responses. Therefore, the gustatory system provides a simple model to investigate neuronal mechanisms underlying behavioral and emotional responses, and learning. We used a genetic approach to visualize the neuronal circuitries of bitter and sweet taste processing by expressing the fluorescently labeled transneuronal tracer, tWGA-DsRed, in either bitter- or sweet-responsive taste receptor cells in mice. The spatial distribution of neurons labeled by tWGA-DsRed that originated from taste receptor cells suggests that gustatory neurons dispersed in the solitary tract nuclei, the parabrachial nuclei, the thalamic gustatory area, and the gustatory cortex may be organized with sweet inputs located rostral and with bitter inputs located caudal, except for bitter inputs into the external-lateral and external-medial subdivisions of the parabrachial nuclei, and the complex inputs in the amygdala. The tracer-labeled neurons in the parabrachial nuclei were further characterized by electrophysiological and immunohistochemical analyses, and mapping the induction of the immediate early gene by taste stimuli and aversion-eliciting stimuli from the gut. Our data suggest that the different types of taste-relaying neurons are clustered in distinct locations, showing the architectural solution in the parabrachial nucleus that processes taste information and gates emotional memory.

(COI: No)

S38-5

Parabrachial nucleus is a center of emotional expressions

Ohmura, Yoshiyuki; Kuniyoshi, Yasuo (*Dept. Mechano-Infomatics, Grad Sch Info and Tech., Univ. of Tokyo, Tokyo, Japan*)

The parabrachial nucleus (PB) has a great variety of connections. The PB receives inputs from Lamina I of spinal and trigeminal dorsal horns, nucleus of solitary tract, vestibular nucleus, area postrima, superior colliculus, inferior colliculus, periaqueductal gray(PAG), hypothalamus, amygdala, the bed nucleus of the stria terminalis(BNST), insular cortex, cerebellum. And PB projects to the nucleus raphe magnus, the reticular formation, the motoneurons controlling the diaphragm, the jaw closers, perioral musculature, orbicularis oculi, the tongue protruders, the pharynx, larynx and esophagus. All of these projections are reminiscent of the relations with emotional expressions. In a hierachical view of central nervous system, hypothalamus is a higher center of homeostatic control and PAG is a more complex modulator of social responses. However, because the projections of PB are generally reciprocal, we can make a hypothesis that PB will be a higher center of emotional expressions, PAG will be a reflex circuit for reproduction and hypothalamus will be a reflex circuit for endocrine secretion. Although the PAG has stronger connections with superior colliculus and inferior colliculus, the projections are limited to the regions related to reproduction (nociception, micturition and vocalization). The PB seems to have broader projections. CGRP inhibition neurons from PB potentiate anxiety-like behaviors and appetite suppressions. The PB will have a switch control between innate motions and voluntary motions, and relate to memory retrieval by activation of cerebral cortex.

(COI: No)

Symposium 39

Frontier researches on the suprachiasmatic nucleus, the center of the mammalian circadian timing system

(March 22, 16:00～17:30, Room G)

S39-1

Mechanisms of circadian rhythm generation in the suprachiasmatic nucleus of Cry1/2 deficient mice

Ono, Daisuke[1]; Honma, Ken-ichi[2]; Honma, Sato[2] ([1]*Photonic Bioimaging Section, Hokkaido Univ, Grad Sch of Med, Sapporo, Japan;* [2]*Department of Chronomedicine, Hokkaido Univ, Grad Sch of Med, Sapporo, Japan*)

In mammals, the circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Circadian rhythms are generated by transcription and translation autoregulatory feedback loop involving several clock genes, such as *Pers*, *Crys*, *Bmal1*, and *Clock*, in which *Cry1* and *Cry2* play essential roles. However we recently reported circadian rhythms in the SCN slice of *Cry1/Cry2* double deficient (*Cry1*$^{-/-}$/*Cry2*$^{-/-}$) mice in neonatal period. The rhythms disappeared in adults due to desynchronyzation of cellular rhythms. In addition, exposure *Cry1*$^{-/-}$/*Cry2*$^{-/-}$ mice to constant light during neonatal period restored circadian behavioral rhythms in adult hood. It is still unclear how the rhythm is generated without *Cry1/Cry2*. Interlocking with the above mentioned feedback loop, *Dec1* and *Dec2* consists another feedback loop and suppress transcriptions of *Pers* and *Crys* through binding upstream E-box enhancers. *Dec1/Dec2* could be involved in the circadian rhythm generation in an absence of *Cry1/Cry2*. To examine the compensatory role of *Dec1/Dec2* in the *Cry1*$^{-/-}$/*Cry2*$^{-/-}$SCN, we made mice lacking these 4 genes by crossing *Cry1*$^{-/-}$/*Cry2*$^{-/-}$ mice and *Dec1*$^{-/-}$/*Dec2*$^{-/-}$. The results showed that *Dec1/Dec2* could not compensate *Cry1/Cry2*. However, they involved in determining oscillation speed. We will discuss the roles of molecular feedback loops involving *Dec1/Dec2*.

(COI: No)

S39-2

Molecular and neuronal mechanisms underlying jet lag

Yamaguchi, Yoshiaki; Okamura, Hitoshi (*Grad.Sch.Pharm.Kyoto Univ., Kyoto, Japan*)

Circadian clock is an essential biological property that coordinates behavioral, physiological, and metabolic systems. Generally, we are not aware of our biological clock process since it is completely synchronized with external light-dark (LD) cycles. However, travelling rapidly across multiple time zones makes us aware of desynchrony between internal clock and the environmental time, resulting in sleep disorder and gastrointestinal distress. Moreover, recent works have reported that repeated jet-lag exposure and rotating shift work increase the risk of cancer and metabolic insufficiency. Although jet lag is considered as a chronobiological problem, the molecular and neuronal mechanisms are poorly understood. Here, we show that mice genetically deficient in vasopressin V1a and V1b receptors (V1aV1bDKO) are resistant to jet lag. Circadian rhythms of locomotor activity, clock gene expressions, and body temperature rapidly re-entrained to phase-shifted LD cycles in V1aV1bDKO mice. Nevertheless, the behavior of V1aV1bDKO mice was still coupled to the internal clock, which oscillated normally under standard LD and DD conditions. Real-time imaging of hundreds of cellular rhythms in the suprachiasmatic nucleus (SCN, the master clock) suggested that V1a/V1b-mediated cell-cell communication confers on the SCN an intrinsic resistance to external rhythm perturbation. Pharmacological inhibition of V1a and V1b in the SCN of wild-type mice accelerated the speed of recovery from jet lag, which promises vasopressin signaling as a pharmaceutical target for jet lag and shift work-related diseases.

(COI: No)

S39-3

Towards Organisms-level Systems Biology

Ueda, Hiroki R (*Graduate School of Medicine, The University of Tokyo*)

The logic of biological networks is difficult to elucidate without (1) comprehensive identification of network structure, (2) prediction and validation based on quantitative measurement and perturbation of network behavior, and (3) design and implementation of artificial networks of identified structure and observed dynamics.Mammalian circadian clock system is such a complex and dynamic system consisting of complicatedly integrated regulatory loops and displaying the various dynamic behaviors including i) endogenous oscillation with about 24-hour period, ii) entrainment to the external environmental changes (temperature and light cycle), and iii) temperature compensation over the wide range of temperature. In this symposium, I will discuss the current and past studies on a mammalian circadian clock as an example of molecule-to-cell-level systems biology, and introduce two design principles, which may underlie biological timings. I would also like to discuss about the challenges and opportunities towards the organism-level systems biology.

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(COI: No)

S39-4

Cellular Circadian Oscillators in Vasopressin Neurons of the Suprachiasmatic Nucleus Play a Critical Role in Coupling between Morning and Evening Behavioral Rhythms in Mice

Mieda, Michihiro (*Dept Molecular Neuroscience and Integrative Physiology, Fac Med, Kanazawa Univ, Kanazawa, Japan*)

The suprachiasmatic nucleus (SCN) is the primary circadian pacemaker in mammals and entrains to the environmental light/dark cycle. It is composed of multiple types of neurons, and neuronal network properties are integral to normal function of the SCN. However, mechanisms underlying the SCN neuronal network have remained elusive. As a first step to understand the principle of the SCN network, we generated mice in which *Bmal1*, an essential clock component, is deleted specifically in the neurons producing arginine vasopressin (AVP), one of the primary neuronal types in the SCN (*Avp-Bmal1*$^{-/-}$ mice). *Avp-Bmal1*$^{-/-}$ mice showed marked lengthening in the free-running period and activity time of behavior rhythms. When exposed to an abrupt 8 hr advance of the light/dark cycle, *Avp-Bmal1*$^{-/-}$ mice reentrained faster than control mice did. In *Avp-Bmal1*$^{-/-}$ mice, the circadian expression of genes involved in intercellular communications, including *Avp*, *Prokineticin 2*, and *Rgs16*, was drastically reduced in the dorsal SCN, where AVP neurons predominate. In slices, dorsal SCN cells showed attenuated PER2::LUC oscillation with highly variable and lengthened periods. Thus, *Bmal1*-dependent oscillators of AVP neurons may modulate the coupling of the SCN network, eventually coupling morning and evening behavioral rhythms, by regulating expression of multiple factors important for the network property of these neurons.

(COI: No)

S39-5

Structures that deliver the circadian rhythm from the suprachiasamtic nucleus to neighboring brain regions

Masumoto, Kohei; Nagano, Mamoru; Koinuma, Satoshi; Sujino, Mitsugu; Shigeyoshi, Yasufumi (*Dept. of Anat. and Neurobiol. Kinki Univ. Sch. Med., Osaka, Japan*)

The suprachiasmatic nucleus (SCN) is the center of circadian clock. It has been known that the brain region neighbouring the SCN indicates circadian rhythm synchronized with that in the SCN, what underlying mechanism transfer the circadian rhythm to neighboring brain regions has been obscure. The paraventricular nucleus (PVN) and subparaventricular zone (SPZ) are located in the dorsal to the SCN, and have been known to relay the circadian rhythm phase of the SCN to the other brain regions. In order to delineate what biological structure transmits circadian rhythm to the PVN/SPZ from the SCN, we observed the coherence between the circadian rhythms in the SCN and PVN/SPZ by monitoring bioluminescence emitted from tissue slice from neonatal Per2::luc knock-in mice. In slices containing SCN and PVN/SPZ, the two regions showed antiphasic circadian rhythm, in contrast, slices containing PVN/SPZ but not SCN showed a circadian rhythm which was damped after a few days. However, when the slice showing damped oscillation was co-cultured with the SCN slice, PVN/SPZ restored a stable circadian rhythm antiphasic to that in the SCN about one week after the treatment. The findings suggest that the structure maintaining the coherence between the circadian oscillations in the SCN and PVN/SPZ was reconstructed.

(COI: No)

Symposium 40

Variety in neural circuit construction and underlying principles

(March 22, 16:00～17:30, Room H)

S40-1

Molecular and cellular basis for establishment and remodeling of dendritic fields

Emoto, Kazuo (*Grad.Sch.Sci., Univ.of Tokyo, Japan*)

The refinement of neural circuits involves dendrite pruning, a process to remove inappropriate projections that are formed during neural development. In Drosophila sensory neurons, compartmentalized calcium (Ca2+) transients in dendritic branches act as temporal and spatial cues to trigger pruning, yet how neurons define the dendritic branches with Ca2+ transients remains elusive. Here we report that local endocytosis in proximal dendrites induces the compartmentalization of the Ca2+ transients. Live imaging of single dendrites revealed a massive increase of endocytic activity in proximal dendrites that spatially and temporally correlates with dendrite thinning, an early step in pruning tightly coupled with compartmentalized Ca2+ transients. We identified two GTPases, Rab5 and dynamin, as critical regulators of the local endocytosis in proximal dendrites; blocking the activity of these GTPases prevented dendrite thinning and impaired the occurrence of compartmentalized Ca2+ transients. These data indicate that local endocytosis drives dendrite thinning in the proximal dendrites to promote compartmentalized Ca2+ transients.

(COI: No)

S40-2

Energy homeostasis in growing dendrites of cerebellar Purkinje cells

Kengaku, Mineko[1,2]; Fukumitsu, Kansai[1,2]; Fujishima, Kazuto[1]; Hatsukano, Tetsu[1,2] ([1]*WPI-iCeMS, Kyoto Univ., Japan;* [2]*Grad.Sch.Biostudies, Kyoto Univ., Japan*)

The highly branched dendrites of vertebrate CNS neurons possess huge volumes and surface areas, necessitating robust and specific mechanisms for maintaining proper homeostatic control of their intracellular environment. We have studied molecular and cellular mechanisms underlying energy homeostasis in growing dendrites of cerebellar Purkinje cells. We found that local ATP synthesis by dendritic mitochondria, at least when it is supported by the ATP buffering activity of creatine kinases, is required to maintain the ATP levels that dendrites need for their continuous outgrowth, both in vitro and in vivo. Additionally, our results suggest that actin turnover and organization, as regulated by the ATP-dependent phosphorylation cycle of ADF/cofilin, plays an important role in feedback control of ATP consumption during dendritic outgrowth.

(COI: No)

S40-3

Molecular composition and functional mechanism of AMPA receptor complexes

Nakagawa, Terunaga (*Vanderbilt University Medical Center, Nashville, TN, USA*)

AMPA-type ionotropic glutamate receptors (AMPARs) mediate the majority of excitatory synaptic transmission and their dysfunction involves a variety of neurological and psychiatric disorders. Understanding the molecular mechanism of AMPAR function is thus critical for continued development of new therapeutic agents that targets AMPARs and to understand the molecular basis of synaptic plasticity and neural circuit function. The majority of the AMPARs in the brain form complex with auxiliary factors, including TARPs, CNIHs, synDIG1, CKAMP44, and GSG1L. Auxiliary subunits do not constitute the channel pore but physically interact with pore forming alpha subunits of AMPAR, GluA1-4. Because they regulate AMPAR trafficking and gating, loss and gain of function of AMPAR auxiliary subunits impact synaptic function in animal models. Different auxiliary subunits of AMPA-Rs modulate receptor function in specific ways. Combinatorial effects of four GluA subunits binding to various auxiliary subunits amplify the functional diversity of AMPA-Rs. The significance and magnitude of molecular diversity, however, remain elusive. Our goal is to extract principles of auxiliary subunit function by studying their diverse molecular mechanism using cryo-EM and their function using rodent genetic models. Recent progress in our laboratory will be presented.

(COI: No)

S40-4

Molecular and cellular mechanisms underlying learning

Takahashi, Takuya (*Dept Physiol, Sch Med, Yokohama city Univ, Kanagawa, Japan*)

Learning induces plastic changes in synapses. However, the regulatory molecules that orchestrate learning-induced synaptic changes are largely unknown. Although it is well established that cholinergic inputs from the medial septum modulate learning and memory, evidence for the cholinergic regulation of learning-induced synaptic plasticity is lacking. We present that the activation of muscarinic acetylcholine (ACh) receptors (mAChRs) mediates the contextual fear-learning-driven strengthening of hippocampal excitatory pyramidal synapses, through the synaptic incorporation of AMPA-type glutamate receptors (AMPARs). Contextual fear learning also enhances the strength of inhibitory synapses on hippocampal pyramidal neurons, in a manner mediated by the activation of, not mAChRs, but nicotinic AChRs (nAChRs). Interestingly, we observed a significant cross-correlation between the learning-induced increases in excitatory and inhibitory synaptic strength at individual pyramidal neurons. Understanding the mechanisms underlying cholinergic regulation of learning-induced hippocampal synaptic plasticity may help the development of new therapies for cognitive disorders such as Alzheimer's disease.

(COI: No)

Symposium 41

Stem cell therapy for neuronal disorders

(March 22, 16:00～17:30, Room I)

S41-1

A subpopulation of fibroblasts, Muse cells, ameliorate rat stroke model

Morita, Takahiro (*Department of Neurosurgery, Grad.Sch.Med.Tohoku Univ., Sendai, Miyagi, Japan*)

Multilineage-differentiating stress-enduring (Muse) cells, a distinct subpopulation of fibroblasts corresponding to ~1% of the total, exhibit pluripotency in vitro and replace lost cells and repair tissues in vivo. We investigated whether human fibroblast-derived Muse cells facilitate functional recovery in ischemic stroke in rats. Human dermal fibroblasts, separated into stage-specific embryonic antigen-3(+) Muse cells and antigen-3(-) non-Muse cells, were injected into rat brains 2 days after infarction. Histologic and behavioral tests were examined for up to day 87 after transplantation. The Muse-transplanted group exhibited significantly improved neurologic outcomes at the chronic stage of stroke, after day 70, compared with the non-Muse-transplanted group, without a change in the infarct size. Muse cells survived in the host brain and differentiated spontaneously into β-tubulin(+)and GFAP(+) cells by day 87 after transplantation, whereas almost all of the non-Muse cells disappeared from the host brain. Retrograde labeling further revealed that the integrated Muse cells extended their neurites into the brainstem. No tumorigenicity was observed after transplantation. Muse cells possess high potential to spontaneously differentiate into neural lineage cells in the host brain and facilitate functional recovery from stroke. Our results demonstrated that general fibroblasts are strong cell candidates for treating stroke if the Muse cell component is fully utilized by purification or enrichment.

(COI: No)

S41-2

Functional recovery after rat spinal cord injury by tissue regenerating factors derived from mesenchymal stem cells

Yamamoto, Akihito (*Grad.Sch.Med.Nagoya Univ., Nagoya, Japan*)

Human adult dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) are self-renewing mesenchymal stem cells residing within the perivascular niche of the dental pulp. They are thought to originate from the cranial neural crest, expresses early markers for both mesenchyme and neuroectodermal stem cells and are able to differentiate into the functional neurons and oligodendorocytes under appropriate conditions. Studies have reported that engrafting these pulp stem cells promote functional recovery from various types of acute and chronic CNS insults. Here we show that the intrathecal administration of conditioned serum-free medium (CM) from SHED into the severe adult rat spinal cord injury (SCI) led to a marked recovery of hindlimb locomotor function. SHED-CM treatment inhibited SCI-induced apoptosis, preserved neural fibres and myelin sheaths, and promoted the growth of the descending 5-HT+ axons. Importantly, we show here that these neuroregenerative activities were supported by a marked immunoregulatory function of SHED-CM, by which the pro-inflammatory M1 microglia/macrophages were directly converted to the anti-inflammatory/tissue repairing one. We have identified a novel set of M2-inducer in SHED-CM, which is necessary and sufficient for SHED-CM-mediated M2 induction and functional recovery after SCI. Thus, our data suggest that the anti-inflammatory/tissue-regenerative condition generated by the dental pulp stem cells play a central roles in functional recovery from SCI. I declare that there are no competing interests.

(COI: No)

S41-3

Mesenchymal stem cell therapy of spinocerebellar ataxia type 1 model mice

Nakamura, Kazuhiro; Hirai, Hirokazu (*Dept Neurophysiol, Grad Sch Med, Gunma Univ, Maebashi, Japan*)

Spinocerebellar ataxia (SCA) is a devastating progressive neurodegenerative disorder. Currently, no effective treatments have been developed. However, some studies have shown that an intracerebellar injection of mesenchymal stem cells (MSCs) was partially effective in some mouse models of cerebellar ataxia. MSCs likely exert their therapeutic efficacy by secreting innate factors to induce neuronal growth, synaptic connection and reduce apoptosis. We tested if the factors released from MSCs bring the therapeutic effects for SCA type 1 (SCA1) transgenic mice by intrathecally injecting MSCs conditioned medium into 5 weeks old mice, which was followed by weekly intravenous injections. The conditioned medium successfully reversed the disturbed motor coordination observed using rotarod test. Likewise, the conditioned medium also corrected delayed nerve conduction in the spinal motor neurons of SCA1-knockin mice. Collectively, the unknown factors released from MSCs work to correct functional disturbances seen in SCA1 model mice. We also introduce potential mechanisms by which the unknown factors exert the therapeutic effects on SCA1 mice.There are no potential conflicts of interest in the content of this presentation.

(COI: No)

Symposium 42

Birthplace, birthtime and molecular mechanisms of oligodendrogenesis

(March 22, 16:00～17:30, Room J)

S42-1

Molecular mechanisms underlying the production of cortical oligodendrocytes from neural stem cells

Hitoshi, Seiji (*Dept Integrative Physiol, Shiga Univ Med Sci, Otsu, Japan*)

Oligodendrocyte precursor cells (OPCs) appear in the late embryonic brain, mature to become oligodendrocytes (OLs) and form myelin in the postnatal brain. Recently, it has been proposed that early-born OPCs derived from the ventral forebrain are eradicated postnatally and that late-born OLs predominate in the cortex of the adult mouse brain. However, intrinsic and extrinsic factors that specify the ability of self-renewing multipotent neural stem cells in the embryonic brain to generate cortical OL-lineage cells remain largely unknown. Using an inducible Cre-loxP system to permanently label Nestin- and Olig2-lineage cells and using an in utero electroporation technique, we determined when and where cortical OL-lineage cells differentiate from neural stem cells in the developing mouse brain. We show that neural precursor cells in the dorsal VZ/SVZ are inhibited by Wnt signaling from contributing to cortical OLs in the adult brain. By contrast, neural precursor cells present in the dorsoventral boundary VZ/SVZ produce a significant amount of OLs in the adult cortex. Our results suggest that neural stem cells at this boundary are uniquely specialized to produce myelin-forming OLs in the cortex.

(COI: No)

S42-2

Origin of optic nerve oligodendrocyte in the developing mouse

Ono, Katsuhiko[1]; Ikenaka, Kazuhiko[2] (*[1]Kyoto Pref Univ Med, Kyoto, Japan; [2]Natl Inst Physiol Sci, Okazaki, Japan*)

Oligodendrocytes (OLs) are myelinating cells in the central nervous system (CNS). Oligodendrocyte precursor cells (OPCs), which express Olig2 and PDGFRalpha, are reported to originate in the restricted region of each subdivision in the CNS at early developing stage. In the present study, we examined whether optic nerve OL originate in the basal forebrain in the fetal mouse. In the early stages of the ventral forebrain, Olig2+ or PDGFRalpha+ OPCs were distributed around the third ventricle and first OPCs in the optic nerve appeared at E15.5. High titer retrovirus vector carrying lacZ gene was injected into the lateral and third ventricles of fetal mouse at e12.5, E14.5 or E15.5 at which optic ventricle was disconnected from the third ventricle, and LacZ+ cell distribution was examined in the adult optic nerve. LacZ+ cells with OL-like profiles were observed in 4 optic nerves out of 106 nerves. Once LacZ+ cells were observed in the optic nerve, several hundred cells were distributed in the single nerve. We next used Olig2-CreER/Rosa26-GEFP-reporter double heterozygous mice with tamoxifen treatment at E12.5 or E15.5 and adult optic nerves were examined. In 7 out of 15 animals, EGFP+ cells were observed and some of their processes were PLP+ and some of somata were CC1+. These results clearly demonstrated that optic nerve OPCs are derived from ventral basal forebrain and that they enter the optic nerve at round E15.5, and that they differentiate into mature OL throughout the optic nerve.

(COI: No)

S42-3

Microglia enhance oligodendrogenesis in the early postnatal subventricular zone

Sato, Kaoru (*Lab. Neuropharmacol., Div. Pharmaol., NIHS, Tokyo, Japan*)

Microglia have long been considered as resident immune cells, which are activated in response to pathological events. However, the physiological importance of microglia in the normal CNS has been clarified these days. We recently found a new physiological role of microglia in brain development. We found large numbers of activated microglia in the forebrain subventricular zone (SVZ) of the rat from P1 to P10. Pharmacological suppression of the activation, which produces a decrease in levels of a number of proinflammatory cytokines, i.e., IL-1beta, IL-6, TNF-alpha, and IFN-gamma, significantly inhibited oligodendrogenesis together with neurogenesis in the SVZ. In vitro neurosphere-assays reproduced the enhancement of oligodendrogenesis and neurogenesis by activated microglia and showed that the cytokines revealed the effects complementarily. These results suggest that activated microglia accumulate in the early postnatal SVZ and that they enhance oligodendrogenesis and neurogenesis via released cytokines.

(COI: No)

S42-4

Pathophysiology of oligodendrocyte in multiple sclerosis

Nakahara, Jin (*Dept. of Neurol., Keio Univ., Tokyo, Japan*)

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) affecting nearly 2.5 million people worldwide. It has long been assumed that MS is a T-cell-mediated autoimmune disease targeting CNS myelin, however recent neuropathological and radiological studies suggested that neurodegeneration (i.e. brain atrophy) also occurs in MS, independently of inflammatory demyelination. Although myelin is an exceptional structure that spontaneously regenerates within the CNS, remyelination failure may jeopardize recovery after demyelinating insults in a subset of MS patients. Such failure is attributable in part to the degeneration of preserved oligodendrocytes in MS lesions, together suggesting inflammatory demyelination, brain atrophy and "oligodendrogliopathy" are key pathologies in the disease. Understanding the pathophysiology of oligodendrocytes in MS is essential to overcome the disease, especially for the future remyelination strategy. In this presentation, the pathophysiology of oligodendrocytes in MS will be reviewed and possible remyelination strategies will be discussed.

(COI: Properly Declared)

Symposium 43

Generation of Physiological Functions During Ontogenesis : Looking for the Frontier of "Functiogenesis"

(March 22, 17:30～19:00, Room E)

S43-1

Functiogenesis of the embryonic CNS revealed by multiple-site optical recording with a voltage-sensitive dye

Sato, Katsushige[1]; Momose-sato, Yoko[2] (*[1]Dept Hlth & Nutr Sci, Fac Human Hlth, Komazawa Women's Univ, Tokyo, Japan; [2]Dept Hlth & Nutr, Coll Human Enviro Studies, Kanto-Gakuin Univ, Yokohama, Japan*)

The functiogenesis of the embryonic central nervous system has long been unclear, because conventional electrophysiological means have several technical limitations. First, early embryonic neurons are small and fragile, and the application of microelectrodes is often difficult. Second, the simultaneous recording of electrical activity from multiple sites is limited, and as a consequence, response patterns of neural networks cannot be assessed. We have applied optical recording techniques with voltage-sensitive dyes to the embryonic central nervous system and provided a new approach to the analysis of the functiogenesis of the central nervous system. In this symposium, we present recent progress in optical studies on the embryonic central nervous system with special emphasis on development of the olfactory system. The studies clearly demonstrate the utility of voltage-sensitive dye imaging as a powerful tool for elucidating the functional organization of the vertebrate embryonic central nervous system.

(COI: No)

S43-2

Perinatal modulations of the respiration-related rhythmic activities by GABA and Cl^- co-transporters

Okabe, Akihito; Shimizu, Chigusa; Takayama, Chitoshi (*Dept. Mol. Anat., Sch. Med., Univ. of the Ryukyus, Okinawa, Japan*)

GABA is one of main inhibitory neurotransmitter in adult central nervous system but an excitatory neurotransmitter during early postnatal development. Such GABA action shift from excitatory to inhibitory is caused by decreasing of intracellular chloride concentration ($[Cl^-]_i$) which is determined by balance of K^+-Cl^- co-transporter-extrusion system (KCC2) and Na^+, K^+-$2Cl^-$ co-transporter-accumulation system (NKCC1). Role of GABAergic transmission in regulation of medullary respiration-related rhythmic activity (RRA) perinatally, however, is yet to be determined. Here, we examined how GABA and chloride co-transporters contribute to RRA during development in hypoglossal nucleus (12N) where inspiratory neurons reside. We recorded extracellular RRA in medullary slices obtained from embryonic day (E) 16 to postnatal day (P) 7 mice. RRA was induced by soaking slices in artificial cerebrospinal fluid (aCSF) containing 8 mM-K^+. Mean numbers of RRA were significantly increased from E16 to P0 but there were no significant changes in RRA during postnatal development. Application of GABA significantly decreased frequency of RRA on E16 but increased it after P3, whereas application of DIOA, a KCC2 blocker, significantly increased frequency of RRA on E16 but significantly decreased it after P1. In addition, dense KCC2 immunolabeling was seen in 12N from E16 and P7. These results suggest that decreasing $[Cl^-]_i$ levels caused by increasing KCC2 levels in 12N could play important roles in regulating the frequency of RRA during development.

(COI: No)

S43-3

GABA and glycine evoke depolarizing responses in early neonatal rat CNS

Ito, Susumu; Cherubini, Enrico (*[1]Grad Sch Emerg Med Sys, Kokushikan Univ, Tokyo, Japan; [2]Euro Brain Res Inst, Rome, Italy*)

GABA and glycine are the main inhibitory neurotransmitters in the adult CNS. Both GABA and glycine bind to receptors coupled to chloride channels. In adult neurons, the level of intracellular chloride is maintained at relatively low levels and E_{Cl^-} is below the resting membrane potential (V_m). Therefore, GABA and glycine hyperpolarize the membrane and inhibits neuronal firing through an inwardly directed flux of chloride. In neonatal animals, intracellular chloride is relatively high, and E_{Cl^-} is above V_m. Therefore, GABA and glycine depolarizes the membrane through an outwardly directed efflux of chloride. To be excitatory, GABA- and glycine-mediated membrane depolarization should reach the threshold for action potential generation. The intracellular chloride concentration is under control of two main cation-Cl^- co-transporters the NKCC1 and KCC2 that import and export $[Cl^-]_i$, respectively. The unbalance between these two transporters is responsible for the high $[Cl^-]_i$ found early in postnatal life. The developmentally up-regulated expression of the K^+/Cl^- co-transporter KCC2 is responsible, toward the end of the first postnatal week, for the shift of GABA and glycine from the depolarizing to the hyperpolarizing direction. In the immature hippocampus, the synergistic action of glutamate and GABA, both depolarizing and excitatory, triggers coherent network oscillations, the so-called giant depolarizing potential or GDPs. GDPs associated calcium transients are instrumental for enhancing synaptic activity at emerging GABAergic and glutamatergic pathways.

(COI: No)

S43-4

Optical assessment of ontogenic origin of vertebrate cardiac pacemaker functions : A cultured multiple-hearts study

Sakai, Tetsuro[1,2]; Kamino, Kohtaro[2] (*[1]Dept. Systems Physiol. Univ. of the Ryukyus Grad. Sch., Okinawa, Japan; [2]Tokyo Med. Dent. Univ., Tokyo, Japan*)

To elucidate the functional organization of cardiac pacemaker, we have used the early stage chick embryos with multiple-hearts which were made experimentally in whole embryo culture and examined the spatial gradient of intrinsic ryhthmicity using optical methods. The embryos were cut microsurgically through the tissue of the anterior intestinal portal at the 5- to early 7-somite developmental stage. Spontaneous electrical activity in 4 to 6 segmented hearts, during the 7- to 10-somite stages of development, were monitored simultaneously by means of multiple-site optical recordings of membrane potential activity, using a voltage-sensitive. Each segment of the heart exhibited its own inherent rhythmicity. In quadruple-hearts, the order of the rhythmicity was often [left-caudal segment] > [right-caudal segment] > [left-cephalic segment] > [right-cephalic segment]; the heart rate in the left-caudal segment was often faster than that in the other segments. These findings strongly emphasize the concept that, in the early phases of cardiogenesis, the formation of a regional gradient of pacemaker activity (i.e. a spatial gradient of intrinsic rhythmicity) results in the functional self-organization of the pacemaking area. And, in intact embryonic chick and rat hearts, we confirmed that this concept agrees the early process of the functional organization of the cardiac pacemaker that we recorded optically.

(COI: No)

Symposium 44

Impacts of active experience on brain morphology and function

(March 22, 17:30～19:00, Room F)

S44-1

Maternal experiences improve spatial learning through hippocampal neural plasticity

Furuta, Miyako; Fukushima, Atsushi; Funabashi, Toshiya; Akema, Tatsuo (*Department of Physiology, St. Marianna University School of Medicine, Kawasaki, Japan*)

Maternal experiences consist of a series of events including pregnancy, delivery, lactation and rearing. We questioned if those events can alter the behavior and the neural system of the mother rats. Behavioral changes during postpartum period are well documented in rats. The main factor is considered to be a drastic reduction of estrogen levels after delivery. In this study postpartum rats that resumed estrus cycle after weaning, and nulliparity controls at the same age, were subjected to Morris water maze and Y-maze tests for spacial learning assessments. In electrophysiological study, we used a protocol for inducing long-term potentiation using whole-cell recording to pair low-frequency synaptic stimulation (270 pulses, 10 Hz) with a depolarizing voltage-clamp pulse (1.5 min duration). Furthermore, the expression level of GluR1 and R2 was analyzed in the hippocampus by western blot using synaptosome fraction. Improved performance of primiparous rats was found in Y maze. The expression of GluR2 level was increased in the hippocampus of primiparous rats. Taken together, reproductive experience could change hippocampal function leading to improve spatial learning.

(COI: No)

S44-2

Rearing in enriched environment induces beneficial alterations in the central nervous system and emotional behavior

Urakawa, Susumu[1]; Ono, Taketoshi[1]; Nishijo, Hisao[2] (*[1]Dept Neurophysiotherapy, Grad Sch Med Pharmaceu Sci, Univ Toyama, Japan; [2]Dept System Emotional Science, Grad Sch Med Pharmaceu Sci, Univ Toyama, Japan*)

Early life experiences can modulate development of the neuronal network and modify various behaviors. Rearing in an enriched environment (EE) is generally believed to facilitate enhanced motor, sensory, and cognitive stimulation and also provide relatively increased social interaction than a standard environment (SE; housing conditions in conventional laboratory cages). In our previous reports, EE rats showed various alterations in central nervous system and behavioral outcome. The direct striatal injection of neuro-toxin caused neuronal cell-loss and subsequently occurred cell-replacement. However, EE ameliorated lesion-induced motor dysfunction and increased the number of migrating cells in the lesioned-striatum. These results suggest that EE induces beneficial effects in neuronal cell-replacement and recovery from motor dysfunction. In addition, we reported that EE significantly affected emotional responses and the number of parvalbumin-positive inhibitory neurons in the amygdala. The EE rats showed a decrease in anxiety-like behavior in the open field and high performance in a beam walking test. Compared with SE rats, EE males showed decreased sexual activity. The number of parvalbumin-positive neurons in the basolateral amygdala was increased by EE, correlated with performance in the beam walking behavior. The results suggest that EE induced behavioral plasticity, which might be mediated through its effects on parvalbumin-positive neurons.

(COI: No)

S44-3

An increase/decrease in physical activity influences structural and functional adaptive changes in the hippocampus

Nishijima, Takeshi; Kamidouzono, Yoshika; Ishiizumi, Atsushi; Kita, Ichiro (*Dept Human Health Sci, Tokyo Metropolitan Univ, Tokyo, Japan*)

The hippocampus is a highly plastic part of the brain that adaptively responds to levels of physical activity. A higher level of physical activity is a key factor in promoting structural and functional improvements of the hippocampus. The hippocampus has a functional gradient along its dorso-ventral axis; the dorsal part of the hippocampus plays a key role in spatial learning and memory, whereas the ventral hippocampus is involved in regulating emotional behaviors. Importantly, behavioral studies indicate that exercise can not only improve spatial learning and memory but also exert anxiolytic and antidepressant effects in rodents. Recently, we have shown that long-term exercise induces delta-FosB expression, a marker for chronic neuronal activation, and enhances neurogenesis throughout the dorso-ventral axis of the hippocampus. These findings support the current understanding that exercise is an effective non-pharmacological intervention that can improve multiple hippocampal function. It is also important to understand how a reduction in physical activity, i.e. a lack of positive experience, deteriorates hippocampal function. We found that the forced physical inactivity, by cessation of voluntary wheel running, was anxiogenic and impaired hippocampal neurogenesis. Although further studies are needed to confirm our findings, our results propose a new hypothesis that physical inactivity can be a risk factor for stress-induced mood disorders, which may be in part caused by the reduction of hippocampal function.

(COI: No)

S44-4

Neuroendocrine correlates of maternal behavior in humans and its developmental changes during pregnancy to motherhood

Nishitani, Shota; Doi, Hirokazu; Takamura, Tsunehiko; Shinohara, Kazuyuki (*Dept. Neurobiol. Behav., Grad. Sch. Biomedical Sci., Nagasaki Univ. Nagasaki, Japan*)

Maternal behavior has been recognized as to be essential for child development and mental health. To investigate the biological vulnerability to child rearing problems, researchers have been devoted to identify the neural correlates in humans. Although substantial amount of studies have showed possible neural correlates of maternal behaviors, there is little agreement on the link between the neural correlates and hormones so far. In voles, however, whole picture of molecular substitutes for maternal behavior has been shown. Comparing molecules and genes between prairie and mountain voles, it has been established that maternal behavior are closely associated with oxytocin receptor (OXTR). However, the influences of the genetic variants of OXTR on the neural activity associated with maternal behavior have yet to be revealed. In the present study, we show the genetic variants influences on the prefrontal activations measured with near-infrared spectroscopy for mothers who viewed video clips of their own child smile and other unfamiliar child smile. On the other hand, another important aspect to be noted is the phenomenon that changes in hormone levels during pregnancy to motherhood seem to make females' brain maternal. To investigate this phenomenon, we here show our preliminary studies of the developmental changes of the maternal prefrontal activations in response to children's facial expression discrimination task during pregnancy to motherhood. No potential conflicts of interest were disclosed.

(COI: No)

Symposium 45

The time in Anatomy and Physiology

(March 22, 17:30～19:00, Room G)

S45-1

Age related decline in reproductive functions and circadian rhythms

Nakamura, Wataru (*Lab. Oral Chronobiol. Grad Sch Dent, Osaka Univ, Osaka, Japan*)

In mammals, the estrus cycle is regulated by dual control of the hormone system and circadian rhythms via the hypothalamus-pituitary-gonadal axis (HPG-axis). The suprachiasmatic nucleus (SCN) is the main pacemaker of circadian rhythms, and coordinates the daily rhythms of various physiological functions and behaviors. In this study, we found that middle-aged Cry1- or Cry2-deficient female mice, which had shortened and lengthened circadian rhythms, respectively, had markedly lower pregnancy success rates compared to middle-aged wild-type (WT) mice. While young adult Cry -deficient female mice showed regular estrus cycles and normal reproductive functions from conception through to birth, middle-aged Cry-deficient female mice showed extended, irregular estrus cycles. These results suggested that the reproductive function of Cry-deficient female mice declines due to the early onset of aging-associated changes. Surprisingly, we found that if middle-aged Cry-deficient female mice with irregular estrus cycles and reduced pregnancy success rates were reared in a light/dark environment similar to their particular circadian cycle, the regularity of the estrus cycle improved and pregnancy success rates increased significantly. These results showed that the disorganization between the 24-h environmental rhythm and the circadian clock rhythm is a direct cause of the early aging-like decline in the reproductive function observed in Cry-deficient female mice.

(COI: No)

S45-2

Dynamic expression of Notch ligand Dll1 during development

Shimojo, Hiromi[1]; Isomura, Akihiro[2]; Ohtsuka, Toshiyuki[2]; Miyachi, Hitoshi[2]; Kageyama, Ryoichiro[1,2] ([1]*iCeMS, Kyoto Univ, Kyoto, Japan;* [2]*Inst. for Virus Research, Kyoto Univ, Kyoto, Japan*)

Cell-cell communications play an important role in cell fate determination. Notch signal is transmitted by cell-cell interactions and regulates formation of various tissues. Upon activation of Notch signaling, Notch effector gene Hes1 is activated and represses the expression of Dll1. It generates heterogeneous cell populations by lateral inhibition during neural development, whereas it forms homogeneous cell populations by synchronization during somitogenesis. However, how Notch signaling produces such opposite outcomes remains unclear. We previously found that the expression of Notch signal components oscillate with a period of 2-3 hr in neural progenitors. These results suggest that the regulation of Notch signaling is more dynamic than previous thought. To reveal the significance of Notch signal dynamics in various developmental events, we visualize the expression of Dll1 protein during neural development and somitogenesis. Live-cell imaging revealed that the expression of Dll1 protein oscillates in phase between neighboring cells during somitogenesis. By contrast it oscillates out of phase between neighbors during neural development. Cell-cell interactions dynamically change in both events, but the dynamics between neighboring cell is differently controlled, depending on tissue types. These results suggest that dynamics of Notch signaling regulate the formation of homogeneous and heterogeneous cell population by controlling of phase of Notch signal oscillation between neighboring cells.

(COI: No)

S45-3

Inhibitory maturation regulates the critical period plasticity for binocular vision

Sugiyama, Sayaka (*Lab of Neuronal Dev., Grad School of Med. Dent. Sci., Niigata Univ. Niigata, Japan*)

Binocular vision is established in the primary visual cortex (V1) through activity-dependent competition in early postnatal life. During the critical period, monocular deprivation (MD) yields a strong shift in cortical responsiveness toward non-deprived eye concomitant with a rapid pruning of dendritic spines and later axonal remodeling, hence causes a permanent deficit in deprived-eye vision (amblyopia). Growing evidence demonstrates that distinct GABAergic circuits drive the critical period plasticity for binocular vision. Transfer of Otx2 homeoprotein into parvalbumin (PV)-cells activates this sensitive period in the visual cortex. Here, we show that genetic cascade induced by Otx2 underlies maturation of PV-cell circuits. Otx2 deletion results in reduction of mature PV-cells enwrapped by chondroitin sulfate (CS) proteoglycans. Our data shows that CS reduction by genetic deletion of one CS enzyme causes the impairment of PV-cell function and disruption of plasticity. Similarly, Otx2 induces specific expression of actin-remodeling factor for plasticity. Thus, once PV-cell internalizes Otx2, extracellular and intracellular machineries activated by this homeoprotein may cooperate together in maturation of PV networks hence the cortical plasticity.

(COI: No)

S45-4

The neural mechanism of vocal signal transmission beyond generations in songbirds

Abe, Kentaro[1,2] ([1]*Grad Sch Med, Kyoto Univ, Kyoto, Japan;* [2]*JST, PRESTO, Kawaguchi, Japan*)

During the evolutional history of life, organisms continuously replicate themselves through reproductive processes. Through such repetitive cycle of generation, an organism pass the information on to the next generation as an inherited genome. In this respect, modern humans are peculiar in their features that they can also accumulate and pass on non-genetic information beyond generations through social interactions. To study the biological basis of neural mechanisms that allow such kind of non-genetic passage of information, I have studied the individual development of the skills to vocally communicate with other in songbirds. Similarly to the acquisition process of language skills in humans, proper postnatal social experiences largely influence, and are critically necessary for, the acquisition of such skills in songbirds. Using a specific postnatal education paradigm concomitantly with transgenic technology in songbirds, we separately manipulate both intrinsic and extrinsic factors involved in the development of vocal skills. Taking this approach, we investigated the molecular mechanism of how social interaction stimulates the acquisition of postnatally learned behaviors, i.e., vocal performance. We found that efficient skill acquisition is executed by coordinating such intrinsic information and extrinsic information, and a transcription factor which shows a neural activity dependent gene transcription activity play an important role in this process. Our results provide an insight into the broader question of how social influences affect the postnatal acquisition of behaviors or inherited information.

(COI: No)

S45-5

Regulatory mechanism of biological rhythm and instinctive behaviors

Yamanaka, Akihiro (*Dept Neuroscience II, Inst Env Med, Nagoya Univ, Nagoya, Japan*)

Neurons form complex network which work as functional circuit to regulate behavior in the brain. Little is known about how these circuit functions to regulate behavior since it was impossible to control the activity of specific type of neurons among them. Recently developed techniques, optogenetics and pharmacogenetics (chemicogenetics) enables control the activity of specific type of neurons in the brain using light or chemical substances. These new techniques allow us to study the function of these network and behavior using the whole animal. Especially, instinctive behaviors such as feeding, drinking and sleep/wakefulness behaviors are exhibited only in the whole animal. To reveal its regulatory mechanism, in vivo study using whole animal is essential. I developed a series of transgenic mice line which allow us to easily apply optogenetics. These instinctive behaviors are regulated by neuropeptide containing neurons in the hypothalamus. The activity of these peptidergic neurons was acutely manipulated using optogenetics or pharmacogenetics to control instinctive behaviors. In this symposium, I will discuss about neural regulatory mechanism of these instinctive behaviors using our recent results.

(COI: No)

Symposium 46

Structure and function of the hippocampus: approach from molecule to neuronal network

(March 22, 17:30～19:00, Room H)

S46-1

Conversion mechanism of temporal Ca^{2+} code into persistent biochemical code during LTP

Hayashi, Yasunori (*RIKEN BSI*)

Upon induction of long-term potentiation (LTP), a transient Ca2+ signal through NMDAR is converted into a long-lasting increase in synaptic transmission and an enlargement of the dendritic spines. There must be a point within the signaling cascade where transient Ca2+ code is converted to a persistent biochemical code. Here, we identified a formation of a stable heterooligomer between CaMKII and TIAM1, a Rac specific guanine-nucleotide exchange factor (GEF), as the point of the conversion. The binding of TIAM1 on "T-site" of CaMKII prevents autoinhibition and locks CaMKII in an active conformation similarly to the action of T286 autophosphorylation, which, in turn, results in a persistent activation of TIAM1. This mutually activating complex persistently activate of Rac, which is required for the maintenance of sLTP. TIAM1/CaMKII can form a ternary complex with NR2B, through the dodecameric structure of CaMKII, thereby maintaining the active complex at the vicinity of NMDAR. In this way, CaMKII acts as a structural and signaling hub that, once activated by Ca2+, can persistently activating TIAM1 and possibly other the signal transduction molecules at the vicinity of the activated synapse.

(COI: Properly Declared)

S46-2

Large-volume analyses of synapse nanostructure by automated section-collecting system

Iwasaki, Hirohide[1,2]; Isshiki, Masaaki[1]; Ito, Aya Ishida[1]; Kashiwagi, Yutaro[1,2]; Okabe, Shigeo[1,2] ([1]*Grad.Sch.Med.Univ.Tokyo., , Tokyo, Japan;* [2]*CREST, JST*)

A huge number of neurons in the brain are connected each other via synapses and form functional circuits. There are three prevailed models of synaptogenesis, Miller-Peters model, Sotelo model and filopodia model. However, our recent imaging studies indicate the presence of other types of mechanisms that support synaptogenesis in either cortical interneurons or cerebellar parallel fibers. These observations suggest the importance of comprehensive structural analyses of synapse formation. Structural data of synapses can be obtained by either light microscopy or electron microscopy. Although light microscopic detection of spiny protrusions and clustering of postsynaptic molecules has been utilized as markers of excitatory postsynaptic sites, structural features of spine synapses at a nanometer-scale resolution can only be achieved by electron microscopic observation. Conventionally, structures of spine synapses are analyzed by the three-dimensional (3D) reconstruction of dendrites after transmission electron microscopy. However, 3D reconstruction of dendrites is a labor-intensive procedure and the image reconstruction is inevitably limited to a small volume. In order to obtain comprehensive data for the 3D morphology of spine synapses in a large tissue volume, new technologies are required. An approach that combines both light and electron microscopy by using automated section-collecting system, ATUM, is a possible solution for the comprehensive analyses of synapse nanostructure. The technical details of this correlative microscopic approach will be presented.

(COI: No)

S46-3

Hippocampal EEG dynamics of virtually locomoting mice

Katayama, Norihiro (*Biomodeling Lab, Grd Schl Info Sci, Tohoku Univ, Japan*)

The hippocampus plays important role in spatial recognition and navigation. There are many studies reporting that the rhythmic EEG at 6-12 Hz (theta rhythm) occurred in the hippocampus. The theta activity is strongly correlated with the speed of locomotion of the animal. In addition, the theta activity is modulated by several sensations such as vestibular sensation. However, contribution of visual feedback to the theta activity has not been well disclosed. In this study, we investigated the relationship between the locomotion speed and the hippocampal theta rhythms of mice freely behaving in a virtual environment under head-restrained condition. We would like to show some data suggesting the contribution of sensory modalities to the hippocampal theta EEG activities.

(COI: No)

S46-4

Recent advances in anatomical research on the perineuronal net in the hippocampus

Jinno, Shozo (*Dept Dev Mol Anat, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan*)

Recent studies have suggested that the perineuronal net (PNN), a specialized extracellular matrix structure, and parvalbumin (PV), an EF-hand calcium-binding protein, are involved in the regulation of neural plasticity. Here, we aimed to quantitatively estimate the relationship between the two plasticity regulators, PV and PNN, in the hippocampus of young adult mice. Dual fluorescence staining for PV and *Wisteria floribunda* agglutinin (WFA; a broad PNN marker) showed that a substantial population of PV-positive (PV+) GABAergic neurons were PNN-negative (PNN-). Optical disector analysis demonstrated that there were fewer PNN+ neurons than PV+ neurons. The ratio of PNN expression in PV+ neurons was generally lower in the dendritic layers than in the principal cell layers, while the ratio of PV expression in PNN+ neurons was effectively 100%. The mean PV fluorescence was significantly higher in PNN+/PV+ neurons than in PNN-/PV+ neurons. Cumulative frequencies for single-cell PV fluorescence indicated that intensely stained PV+ neurons tend to be enwrapped by PNNs, while weakly stained PV+ neurons are likely to lack PNNs. We digested the PNNs by a unilateral injection of chondroitinase ABC (chABC) into the dorsal CA1 region. Although the densities of PV+ neurons remained unchanged, the PV fluorescence declined 7 days after chABC injection. Quantitative real-time polymerase chain reaction analysis demonstrated a reduction in PV mRNA expression following chABC injection. These findings indicate that the presence or absence of PNN affects the relative PV expression in GABAergic neurons in the hippocampus.

(COI: No)

S46-5

Local control of axonal excitability of hippocampal mossy fibers

Kamiya, Haruyuki (*Dept Neurobiol, Grad Sch Med, Hokkaido Univ, Sapporo, Japan*)

Axons are the sole outputs of the neurons, and carry neuronal information reliably to the target cells. Although axonal excitability has been shown to be modulated by subtle changes in the local micro-environment, detailed mechanisms and consequences of local control of axonal excitability were rarely tested. It has been demonstrated that bath application of low concentration of kainate (an agonist of kainate receptors) or muscimol (an agonist of $GABA_A$ receptors) enhances the excitability of hippocampal mossy fibers. In this study, we attempted to localize the sites of actions of kainate and muscimol. For this purpose, we adopted a quantitative focal application of these agonists to the distal axons of the mossy fibers. Unexpectedly from digital nature of propagation of action potentials along the axons, the size of presynaptic fiber volleys, the compound action potentials recorded extracellularly, increased by local application of low concentration of kainate to the distal axons of mossy fibers. These effects might reflect mild depolarization of either presynaptic or postsynaptic membranes, or both, since application of the solution containing a slightly higher concentration of potassium ions showed similar effects. Application of muscimol also enhanced presynaptic fiber volleys due to the excitatory effect of presynaptic $GABA_A$ receptors, possibly by the higher intracellular concentration of chloride ions within mossy fiber axons. We also carried out computer simulations of propagation of action potentials along the realistic model of mossy fibers. The mechanisms underlying modulation of presynaptic fiber volleys will be discussed.

(COI: No)

Symposium 47

New streams in researches knitted with neurophysiology and stem cell histology

(March 22, 17:30～19:00, Room I)

S47-1

Cell cycle analysis of endogenous neural precursors in the adult mouse brain after brief seizures

Mori, Tetsuji[1,2]; Wakabayashi, Taketoshi[2]; Hirahara, Yukie[2]; Takamori, Yasuharu[2]; Koike, Taro[2]; Kurokawa, Kiyoshi[2]; Yamada, Hisao[2] (*[1]Sch. Med. Tottori Univ., Yonago, Japan; [2]Kansai Medical Univ., Hirakata, Japan*)

Endogenous neural precursors reside in the subvendymal zone (SVZ) lining the lateral ventricle and the subgranular zone (SGZ) of the hippocampus throughout the life of mammalian. Many studies show that neurogenesis in these regions is enhanced by various stimuli, such as stroke and status epilepticus. Although there are many studies about the responses of SGZ precursors to epileptic seizures, few have examined effects on SVZ precursors. In this study, we focused on the responses of adult SVZ precursors to brief generalized clonic seizures induced by a single administration of pentylenetetrazole (PTZ), a commonly used chemoconvulsant. PTZ-induced brief seizures are much milder than status epilepticus, eliciting no obvious neuronal cell death. We found that brief seizures immediately resulted in cell cycle inhibition of SVZ precursors. This initial inhibition was followed by reduced cell cycle length and enhanced cell cycle re-entry after the first round of mitosis (20 hours after PTZ administration), leading to precursor pool expansion. However, the expansion of the precursor pool was transient. On the other hand, SGZ precursors showed different responses to the PTZ-induced seizures. The precursor pool of the SGZ transiently expanded three days after PTZ administration without obvious cell cycle inhibition. These results suggest that adult neurogenesis is susceptible to excessive neuronal excitation, and neurogenesis in the adult SVZ is more tightly regulated than that in the adult SGZ.

(COI: No)

S47-2

Neuronal migration for maintenance and repair of adult brain

Sawamoto, Kazunobu (*Grad.Sch.Med.Sci.Nagoya City Univ., Nagoya, Japan*)

Neuronal migration is an important process in brain development and homeostasis. It is not only a phenomenon of embryogenesis: it also occurs in the adult brain, following adult neurogenesis. In fact, throughout life, numerous new neurons generated by stem cells in the adult ventricular-subventricular zone (V-SVZ) take the long journey (millimeters to centimeters, depending on the species) to the olfactory bulb (OB) through the rostral migratory stream (RMS). In the adult rodent V-SVZ and RMS, new neurons migrate in chains through astrocytic tunnels. After reaching the OB, the new neurons migrate radially and decrease their speed to stop in their final positions. New neurons are recruited into the empty position generated by cell death and the sensory input promotes the reiterated use of the same positions by new neurons. This mechanism may contribute to the stability and plasticity of the adult brain during neuronal turnover. The neural stem cells in the adult V-SVZ also have the capacity to partially regenerate new neurons after various insults. After ischemic injury in rodents, the V-SVZ-derived new neurons migrate from the V-SVZ towards the injured site along blood vessels. In this talk, I will present recent studies on the mechanisms of neuronal migration occurring in the adult brain of various animals under physiological and pathological conditions.

(COI: No)

S47-3

NG2-expressing progenitor cells maintain neuronal function by controlling local environment in the central nervous system

Kataoka, Yosky[1,2]; Nakano, Masayuki[1,2]; Yamato, Masanori[1]; Tamura, Yasuhisa[1] (*[1]Cellular Function Imaging Team, RIKEN Center for Life Science Technologies, Kobe, Japan; [2]Dept Physiol, Grad Sch Med, Osaka City Univ, Osaka, Japan*)

Progenitor cells expressing chondroitin sulfate proteoglycan 4 (NG2-expressing progenitor cells) are ubiquitously distributed throughout the gray and white matter in the central nervous system of adult mammals. NG2-expressing progenitor cells have been known to show the proliferative activity and give rise to mature oligodendrocytes. We reported that the cell fate of progenitor cells is shifted from oligodendrocytes to astrocytes depending on depolarizing stimuli to the brain, indicating that the progenitor cells are involved in activity-dependent tissue remodeling. We here discuss a new concept that NG2-expressing progenitor cells maintain neuronal function by controlling the local immune response. The progenitor cells are known to receive direct synaptic inputs from neurons, and are often located adjacent to neuronal somata, suggesting functional interaction between the progenitor cells and neurons. Recently, we succeeded in rapid and selective ablation of NG2-expressing progenitor cells. The ablation showed neuronal cell death in the hippocampus with increased expression of pro-inflammatory cytokines and pro-apoptotic genes. In the animals, hippocampal neurons contained death receptors involved in the signaling pathway for apoptosis. These observations suggest that NG2-expressing progenitor cells maintain neuronal survival by regulating local environment including the immune system.

(COI: No)

S47-4

Morphologies and Functions of Olig2-positive cells in the adult brain

Wanaka, Akio; Tatsumi, Kouko; Okuda, Hiroaki; Morita, Shoko (*Sch. Med. Nara Med. U., Nara, Japan*)

Olig2 is a member of a basic helix-loop-helix transcription factor family and regulates differentiation of motor neurons and oligodendrocytes in the embryonic neural tube. Olig2-positive cells often co-express NG2-proteoglycan and persist to adulthood. The Olig2/NG2 cells in the adult brain are regarded as oligodendrocyte precursor cells (OPCs), which have the potential to differentiate into oligodendrocytes, astrocytes or neurons. We employed a double transgenic mouse that can mark the Olig2-positive cells with membrane-targeted EGFP. The genetic labeling revealed that Olig2-positive cells preferentially differentiated into astrocytes in the mechanically injured cerebral cortex, while they showed oligodendrocytic differentiation in the demyelinated corpus callosum of cuprizone-fed mice. During these genetic labeling experiments, we noticed that Olig2-positive cells did not always co-express NG2 proteoglycan. Especially, in the basal ganglionic nuclei, Olig2-single positive cells were predominant and positive for GFAP immunoreactivity. We allowed the double transgenic mice run voluntarily for three weeks and compared the morphological characteristics of the Olig2-positive cells before and after voluntary running in the Globus pallidus. The Olig2-positive astrocytes transformed to bushy astrocytes by elaborating their fine processes after three-week running. These findings suggested that Olig2-positive cells in the adult brain changed their morphologies in response to pathological and physiological conditions.

(COI: No)

S47-5

A role of immune cells on brain repair

Matsuyama, Tomohiro; Nakagomi, Takayuki; Doi, Akiko; Kawahara, Maiko; Sakuma, Rika (*Lab Neurogenesis, Inst Adv Med Sci, Hyogo Col Med, Hyogo, Japan*)

We have found a new type of endogenous neural stem cells induced by cerebral ischemia (Eur J Neurosci 2009;29:1842). These cells are derived from vascular pericytes and express hematopoietic markers (Stem Cells Dev 2011;20:2037). Brain pericytes are a key component of neurovascular unit. Here, using the ischemic pericytes (iPC) of mouse brain and the human brain pericytes cultured under oxygen glucose deprivation, we show that pericytes developed the stemness through reprogramming. The iPC revealed a complex phenotype of neuroangioblast in addition to mesenchymal properties and differentiated into neural and vascular lineage cells. These data indicate that under ischemia pericytes can be reprogrammed into multipotent stem cells to differentiate into all components of neurovascular unit, suggesting that iPC contribute to both neurogenesis and vasculogenesis. However, such reparative mechanisms are often disturbed by immune response in injured brain. We already have demonstrated that CD4 T cells serve as negative modulators in neurogenesis after stroke (J Neurosci Res 2010;88:2385). Glucocorticoid-induced tumor necrosis factor receptor (GITR), a TNF receptor superfamily expressed on activated CD4 T cells, has a key role on brain repair after stroke (Cell Death Differ 2012;19:756). GITR triggering on CD4 T cells increases brain inflammation and decreases the iPC. These observations indicate that activated T cells are major deteriorating modulators of both neurogenesis and angiogenesis. This suggests that blockade of the GITR interaction may be a novel immune based therapy in stroke.

(COI: No)

Symposium 48

New structural and functional logics governing electrical signal propagation

(March 22, 17:30～19:00, Room J)

S48-1

The cerebral cortex has another neural network as its basic structure: the dendritic reticulum formed by gap junctions

Fukuda, Takaichi (*Grad.Sch.Med.Kumamoto Univ., Kumamoto, Japan*)

A subpopulation of cortical GABAergic neurons that contain parvalbumin (PV) use not only chemical synapses but also gap junctions for their communication. Gap junctions are formed between dendrites and allow direct transmission of electrical signals with minimal delay, thus they are thought to facilitate synchronous neuronal activities. Though some technical reasons make it difficult to detect neuronal gap junctions in the brain, we have recently developed a method to overcome the difficulty. Analysis in the visual cortex has shown that the dendritic linkage was not that of a simple cell-to-cell connection but constituted the reticulum formed by multiple dendrites that came close together. Reconstruction of coupled neurons revealed the three linkage types. First, PV neurons in close proximity formed mutual connections through proximally located gap junctions. Second, vertical dendrites bridged somata located at some distance inside the same columnar space. The third type was observed between PV neurons located in neighboring columns. Importantly, in both type 2 and 3 linkages, at least one of the two somata was located within the distance less than 100 μm from the connecting gap junction. This arrangement and previous physiological data suggest a role of distant gap junctions that they transmit action potentials as depolarizing synchronous signals between inhibitory neurons in one direction, whereas gap junctions among clustered neurons might mediate bidirectional synchronous signals. These structures may drastically update our knowledge on organizing principles in the cortical circuitry.

(COI: No)

S48-2

Microglia and synapse interactions: microglial contribution for synapse formation during development

Miyamoto, Akiko[1]; Wake, Hiroaki[1,2]; Murakoshi, Hideji[3]; Eto, Kei[1]; Nabekura, Junichi[1,2] ([1]*Dept Homeostatic Develop, Natl Inst Physiol Sci, Aichi, Japam;* [2]*Dept Physiol, Sch Life Sci, SOKENDAI, Kanagawa, Japapn;* [3]*Supportive Center for Brain Research, Natl Inst Physiol Sci, Aichi, Japan*)

Microglia, which are the immune cells in the central nervous systems, are one of the glial cells. Because of this immune cell character, microglial functions at injured or pathological condition have been well studied. Over the last decade, it has been revealed that microglia also have some actions for synaptic function and connection during physiological condition using imaging and electrophysiological techniques. For example, microglia selectively contact onto synapses in intact brain and are also involved in circuit refinement via synapse elimination at ischemic penumbra region and developmental period, which may contribute to neural circuit reorganization. Recently, we also found that microglia induce filopodia which are precursor of spine during cortical development using in vivo two photon imaging technique. We observed that filopodia was formed at microglial contacted dendrite of L2/3 pyramidal cell. Injection of microglia activation inhibitor or their ablation induced by genetic manipulation decreased cortical spines in density. We also examined miniature EPSC frequency and it was significantly reduced in microglia ablated mice. Taken together, This finding suggests that microglia contributed to neuronal circuit maturation not only via synapse elimination but also via synapse formation during development.

(COI: No)

S48-3

A novel stromal cell network visualized by FIB/SEM tomography

Nakamura, Keiichiro[1]; Hagashi, Ryuhei[1]; Nguyen, Michael[2]; Lang, Richard[2]; Hirashima, Shingo[1]; Kanazawa, Tomonoshin[1]; Takeya, Mitsue[1]; Hayashi, Tokumasa[1]; Hashitani, Hikaru[3]; Ohta, Keisuke[1] ([1]*Kurume Univ. Sch.Med., Fukuoka, Japan;* [2]*Monash Univ., Melbourne, Australia;* [3]*Nagoya City Univ., Nagoya, Japan*)

Fibroblasts or stromal cells are a dominant cell type in the connective tissue of various organs, and are connected to one another by gap junctions to form cellular networks. However, it is not easy to understand the whole structure of those cells or their spatial relationships by light microscopy because of thinness of their processes, nor by electron microscopy as these processes extend well beyond a single thin section. The recent development of a novel electron microscopic technology, FIB/SEM tomography, enables us to establish 3D ultrastructure of these cells. Using FIB/SEM, we can obtain over 1000 serial images, these images are then aligned and the 3D cell structures reconstructed using computerized segmentation instrumentation. In the present study, we concentrated on visualizing stromal cells in the renal pelvic and seminal vesicle. We showed in both organs that stromal cells or fibroblasts, which are recognized by their long thin cytoplasmic processes in a single section, are cells with very broad, thin and wavy "sheet-like" cytoplasmic processes. They approach one another at some points along their edge to form an incomplete wall between the epithelial layer and connective tissue beneath it. The stromal cells in the muscular layer of the seminal vesicle form a honeycomb-like structure which encircle the smooth muscle bundles. Thus these structures seem to create spatial divisions to form functional units within tissues.

(COI: No)

S48-4

Ca^{2+}- and voltage-dependent activation of TRPM4 channel may account for abnormal automaticity

Inoue, Ryuji[1]; Hu, Yaopeng[1]; Zhu, Xin[2]; Numata, Tomohiro[1] ([1]*Dept Physiol, Grad Sch Med, Fukuoka Univ, Fukuoka, Japan;* [2]*Biomed. Info. Tech. Lab. the Univ. Aizu, Fukushima, Japan*)

In the heart, under some pathological conditions, abnormal automaticity, which emerges as ectopic and repetitive spontaneous depolarizations, often leads to tachyarrhythmias. In this study, we performed a simultaneous recording of membrane potential and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), and analyzed the antiarrhythmic effects of a TRPM4 channel blocker 9-Phenanthrol (9-PA) on spontaneous action potentials (APs) in cultured HL-1 atrial myocyte clusters. In the majority of spontaneously beating HL-1 clusters, the rate of AP firing was irregular, but in a small number of the clusters, a regular and slower AP firing pattern reflecting the pace-making activity of I_f current was observed. Both regular and irregular APs were synchronized with transient elevations of $[Ca^{2+}]_i$ and strongly accelerated by isoproterenol (Iso; 0.1 μM) or $BaCl_2$ (0.1mM) which reportedly inhibits an inward-rectifying K^+ current in HL-1 myocyte, with depolarization of resting membrane potential and increased basal $[Ca^{2+}]_i$ level. These changes were abolished by 9-PA (10 μM). Moreover, in myocytes depolarized by $BaCl_2$, 9-PA decreased the slope of pre-AP depolarization, diminishing the AP firing rate. Mathematical simulations based on an HL-1 AP model indicated that Ca^{2+}- and voltage-dependent activation of TRPM4 is responsible for Iso-induced depolarization and concomitant increase in AP firing. This mechanism might contribute to the pathogenesis of tachyarrhythmias such as non-reentrant atrial tachycardia.

(COI: No)

Symposium 49

Regulation of physiological functions by neuroactive steroid and its morphological foundations: Regulatory mechanism for GABA signaling

(March 23, 9:00～10:30, Room D)

S49-1

Morphological appearance of GABAergic neuroactive steroid-synthesizing enzymes

Tsuruo, Yoshihiro (*Dept Anat Cell Biol, Inst Health Biosci, Univ Tokushima Grad Sch*)

Neuroactive steroids are synthesized in neural tissues as well as peripheral endocrine organs. They have a variety of neuromodulatory actions by the interactions of classical nuclear receptors and also by the allosteric modulation of γ-aminobutyric acid type A ($GABA_A$) receptors. The neuroactive steroids, allopregnanolone (3α-hydroxy-5α-pregnan-20-one) and tetrahydrodeoxycorticosterone (3α, 21-dihydroxy-5α-pregnan-20-one) are endogenous potent positive allosteric modulators of $GABA_A$ receptor function, and they are involved in anxiolytic , sedative, analgesic and anticonvulsant actions by opening the GABA-gated chloride channel. These neuroactive steroids are synthesized from progesterone and deoxycorticosterone, respectively. The sequential catalysis of metabolism is mediated by the two enzymes: 5α-reductase (5α-R) and 3α-hydroxysteroid dehydrogenase (3α-HSD). Two isozymes of 5α-R are found in rodents and human, and 3α-HSD has four isoforms in human and only one type in rodents. The expression of 5α-R or 3α-HSD is shown immunohistochemically using the specific antibodies against these enzymes in rodent neural tissues as well as peripheral endocrine organs.

(COI: No)

S49-2

GABAergic signaling in the developing CNS-GABAergic neurons and $GABA_A$ receptors-

Takayama, Chitoshi; Shimizu, Chigusa (*Sch. Med., Univ of Ryukyu, Okinawa, Japan*)

In the adult central nervous system (CNS), gamma-amino butyric acid (GABA) is a predominant neurotransmitter. GABA is synthesized by glutamic acid decarboxylase (GAD), and packed into synaptic vesicles by vesicular GABA transporter (VGAT) in the axons terminals of GABAergic neurons. After released from presynaptic terminals by exocitosis, GABA binds to the $GABA_A$ receptors ($GABA_AR$) on the postsynaptic membrane, induces hyperpolarization of membrane potential, and is rapidly transported into the presynaptic terminals and glial processes surrounding the synapses by the GABA transporters (GAT). We have been investigating the development of these GABAergic systems in various regions in the CNS, such as cerebellum, cerebral cortex and spinal cord, by immunohistochemistry for GABA, GAD, VGAT, GABAAR , GATs. Commonly, GABAergic neurons appeared far before the synapse formation, and GABA may be released by non-exocitotic system. Before synapse formation, extrasynaptically released GABA binds immature types of $GABA_AR$, mediates depolarization of membrane potential, since intracellular Cl- concentration is low due to the lower expression of K^+, Cl^-, co-transporter2 (KCC2). Furthermore, the depolarization activates voltage dependent calcium channel, induces Ca^{2+} influx, and the excitatory action of GABA might be involved in morphogenesis of CNS. After synapses are formed, released GABA binds the mature types of $GABA_AR$, and is involved in inhibition of glutamatergic activity. In this symposium, we demonstrate the changes in the GABAergic system in the cerebellum and cerebral cortex.

(COI: No)

S49-3

Neurosteroid actions on GABA-A receptors: sites and mechanisms

Steinbach, Joe-Henry (*Dep Anesthesiol, Sch Med, Washington Univ, St Louis, USA*)

Potentiating neurosteroids increase the strength of neuronal inhibition by enhancing the activity of GABA-A receptors, increasing the response to lower concentrations of GABA and prolonging the synaptic current. Potentiation occurs because neurosteroids reduce the rate at which the open channel closes, while the affinity of the receptor for the neurotransmitter GABA is not changed. Potentiating steroids also enhance the response to allosteric activators of the GABA-A receptor that can open the channel but bind to sites that are different from the GABA-binding site. Overall, the basic mechanism is to affect receptor kinetics rather than receptor affinity for agonists.Potentiating steroids interact with the 1st membrane-spanning region (TM1) of the α subunit. When a single residue in this region is mutated potentiation by steroids (but not other drugs) can be removed. However, potentiation can be restored to the receptor by mutations that convert the TM1 of the β or γ subunit to the sequence in α. This observation indicates that it is not a unique property of the α subunit, or of the subunits adjacent to the α subunit that allows potentiation. The native α subunit is involved in binding both steroid and GABA itself. However, potentiation does not require that a single subunit bind both drugs since receptors can be constructed that lack one or the other site on selected subunits, and potentiation is preserved. These findings indicate that steroid binding affects the gating properties of the receptor as a whole, rather than changing the function of a single subunit.

(COI: No)

S49-4

Role of neuroactive steroids in adrenal medullary cells

Inoue, Masumi; Harada, Keita; Matsuoka, Hidetada (*Dept Cell and Systems Physiol, Sch Med, Univ Environ and Occup Health, Kitakyushu, Japan*)

GABA is present not only in the central nervous system, but also in peripheral tissues, and exerts a variety of physiological functions via two kinds of receptors, ionotropic GABAARs and metabotropic GABABRs. In the adrenal gland, one of GABA-synthesizing enzymes, GAD67, is expressed in adrenal medullary (AM) cells and GABA is stored in chromaffin granules, but not in synaptic-like microvesicles. GABAARs consisting of α3, β, and γ2 subunits are mainly expressed in AM cells, whereas the expression of GABA transporters (GATs), which are involved in the clearance of GABA at GABAergic synapses, are absent in the adrenal medulla. GABA induces a depolarization with the consequent enhancement of catecholamine secretion. The GABAAR function in AM cells is increased by allopregnanolone, a neuroactive steroid which is produced in the adrenal cortex. Allopregnanolone induces a leftward shift of the dose-response curve for GABA. The EC50 of GABA in the presence of 0.1μM allopregnanolone decreases to less than one-tenth of that in the absence. Furthermore, the expression of α 3 subunits is enhanced by glucocorticoids. These results indicate that adrenal steroids play an important role for the regulation of GABA-mediated functions in AM cells. (CO1:No)

(COI: No)

Symposium 50

Frontier on fatigue, autonomic nerve dysfunction, and sleep-rhythm disorder

(March 23, 9:00～10:30, Room F)

S50-1

Fatigue and its correlates of autonomic nervous system, sleep, and circadian rhythm disorders

Tanaka, Masaaki[1]; Ishii, Akira[1]; Watanabe, Yasuyoshi[1,2] (*[1]Dept Physiol, Osaka City Univ Grad Sch Med, Osaka, Japan; [2]RIKEN, Center for Life Science Technologies, Hyogo, Japan*)

Fatigue is defined as a condition or phenomenon of declined ability and efficiency of mental and/or physical activities, caused by excessive mental or physical activities, diseases, or syndromes; it is often accompanied by a peculiar sense of discomfort, a desire to rest, and reduced motivation, referred to as fatigue sensation. Acute fatigue is a normal condition or phenomenon that disappears after a period of rest; in contrast, chronic fatigue, lasting at least 6 months, does not disappear after ordinary rest. Chronic fatigue impairs activities and contributes to various medical conditions, such as cardiovascular diseases, epileptic seizures, and death. In addition, many people complain of chronic fatigue. For example, in Japan, more than one third of the general adult population complains of chronic fatigue. It would thus be of great value to clarify the mechanisms underlying chronic fatigue and to develop efficient treatment methods to overcome it. In this symposium, we would like to review data primarily from behavioral, electrophysiological, and neuroimaging experiments related to neural dysfunction as well as autonomic nervous system, sleep, and circadian rhythm disorders in fatigue. These data provide new perspectives on the mechanisms underlying chronic fatigue and on overcoming it.

(COI: No)

S50-2

Autonomic nerve alteration caused by fatigue in children and adolescents

Mizuno, Kei[1,2]; Joudoi, Takako[4] (*[1]Pathophysiol Health Sci, RIKEN Cent Life Sci Technol, Kobe, Japan; [2]Dept Med Sci Fatigue, Osaka City Univ Grad Med, Osaka, Japan; [3]Osaka City Univ, Cent Health Sci Innov, Osaka, Japan; [4]Dept Pediatrics, Kumamoto Univ Hosp, Kumamoto, Japan*)

atigue induces an alteration of autonomic nerve function. An enhancement of sympathetic nerve activity based on a decrease in parasympathetic nerve activity measured by electrocardiogram (ECG) and accelerated plethysmography (APG) is closely associated with fatigue in children and adolescents. In addition to the ECG and APG, we found that skin conductance response (SCR), which is an index of sympathetic nerve activity, is also sensitive for detecting the abnormality of autonomic nerve function in patients with childhood chronic fatigue syndrome (CCFS). These results suggest that autonomic nerve alteration is a physiological marker for fatigue severity and the intervention effect on recovery from fatigue. By using the SCR and plethysmography, we are now trying to evaluate their fatigability and ability of recover from fatigue by measuring the time series variation of autonomic nerve activity for comparatively prolonged time including resting and cognitive challenge conditions. In addition, we are focusing particularly on a stimulation of the parasympathetic nerve activity and performing an intervention study to alleviate fatigue is also performing in fatigued children and adolescents. To overcome pubertal chronic fatigue, multifaceted intervention studies in relation to lifestyle modification, environmental space, and food and drug are needed.

(COI: No)

S50-3

A role of sleep and circadian rhythm in the fatigue recovery

Tajima, Seiki (*Hyogo children's sleep and development medical research center, Kobe, Japan*)

Fatigue is an indispensable bio-alarm to avoid exhaustive state caused by overwork or stresses. Sleep is well known as one of the important factors to recover fatigue. Here we show the relationships between fatigue pathology and sleep disorders. Sleep deprivation has high impact on fatigue pathology, especially in childhood. Miike et al reported that accumulation of sleep deprivation during childhood caused biological clock desynchronization. The desynchronization was expressed as circadian rhythm disorders. Under those conditions, not only clock gene expression but also endocrine rhythm, energy metabolism, cognitive function and autonomic activity were disordered. Miike also reported pediatric chronic fatigue status was prevented by just thirty minutes increase in total sleep time.On the other hand, prolonged total sleep time, increased locomotion during sleep and sleep fragmentation were essential in adult chronic fatigue state. From the viewpoint of autonomic function, vagal tone suppression was related with chronic fatigue.Those findings show that regular sleep habit is important for recover from fatigue state, and also, autonomic dysfunction leads to insufficient fatigue recovery.

(COI: No)

S50-4

Neural mechanisms of fatigue

Ishii, Akira[1]; Tanaka, Masaaki[1]; Yamano, Emi[1]; Watanabe, Yasuyoshi[1,2] (*[1]Dept Physiol, Grad Sch Med, Osaka City Univ, Osaka, Japan; [2]RIKEN Center for Life Science Technologies*)

Fatigue is a common problem in modern societies. In Japan, more than half of the adult population reports experiencing fatigue. Fatigue is defined as difficulties in initiating or sustaining voluntary activities and unpleasant sensation that accompanies fatigue (i.e., fatigue sensation) plays an important role in biological alarm to take a rest to avoid disrupting homeostasis. Over-activation of the fatigue sensation may be involved in the pathophysiology of chronic fatigue. However, the neural mechanisms of the fatigue sensation are not well-understood. We performed several neuroimaging studies to clarify the neural mechanisms of the fatigue sensation using magnetoencephalography (MEG) with high temporal and spatial resolutions. We showed that the posterior cingulate cortex (PCC) is involved in the self-evaluation of the levels of fatigue: The equivalent current dipole in the PCC and the decrease in delta band power in the PCC were observed when the levels of physical and mental fatigue, respectively, were self-evaluated. In another MEG study, the PCC and other brain regions such as the dorsolateral prefrontal cortex and frontal pole were related to the decision to rest in the presence of fatigue. In addition, we demonstrated that fatigue sensation can be classically conditioned in human and that the PCC was involved in the neural mechanisms of the classical conditioning of fatigue sensation. Our findings may help clarify the neural mechanisms of fatigue sensation and increase our understanding of the pathophysiology of chronic fatigue.

(COI: No)

Symposium 51

Multilayered physiology-anatomy joint symposium for the cerebral cortical development and maturation

(March 23, 9:00～10:30, Room H)

S51-1

Nuclear traffic of neocortical progenitor cells under the influence of mechanical factors

Miyata, Takaki (*Nagoya Univ. Grad. Sch. Med.*)

The neuroepithelium (NE) or ventricular zone (VZ), from which multiple types of brain cells arise, is pseudostratified. In the NE/VZ, neural progenitor cells are elongated along the apicobasal axis, and their nuclei assume different apicobasal positions. These nuclei move in a cell cycle-dependent manner, i.e., apicalward during G2 phase and basalward during G1 phase, a process called interkinetic nuclear migration (INM). Although INM is observed in a wide variety of epithelia, pseudostratification in the developing mammalian brain, especially in the neocortical primordium, is the most extensive and persistent during development, suggesting that neocortical NE/VZ would be a good model to study how physical or mechanical issues or parameters, such as tissue volume, cell number, and cellular traffic/flow in a given space, may affect neural progenitors' behaviors. Recent experiments in which overcrowding was induced in mouse neocortical NE/VZ, as well as comparisons of neocortical INM between mice and ferrets, have revealed that the behavior of NE/VZ cells can be affected by cellular densification. A consideration of the physical aspects in the NE/VZ and the mechanical difficulties associated with high-degree pseudostratification is important for achieving a better understanding of neocortical development and evolution.

(COI: No)

S51-2

Molecular and cellular mechanisms of corticogenesis based on the structure of radial glia

Osumi, Noriko; Kikkawa, Takako (*Dep. of Dev. Neurosci., Grad. Sch. of Med., Tohoku Univ., Sendai, Japan*)

The cerebral cortex is one of the most complex structures in the central nervous system. The primordium of the cortex consists with neural stem/progenitor cells called radial glia (RG) that are gradually proceeding proliferation and differentiation in the ventricular zone (VZ). Pax6 transcription factor is specifically expressed in the RG cells, and the expression disappears in the cortical neurons, although strong and persistent expression of Pax6 is detected in some neurons in the olfactory bulb, thalamus, amygdala, and cerebellum. Expression of Pax6 continues in adult neural stem/progenitor cells throughout life. Pax6 is weakly expressed in astrocytes, and involved in their maturation. In primates, expression of Pax6 is not only seen in RG in the VS but also in the outer radial glia (oRG) in the outer subventricular zone (OSVZ). It would be interesting to know how Pax6 functions in these primate specific oRG. Multiple and cell-type specific functions of Pax6 are governed by various downstream molecules of Pax6. These include cell adhesion molecules, markers for RG (Fabp7/BLBP and fucosyltransferase synthesizing CD15/LewisX), transcription factors (Dmrta1 and Foxp2), centrosomal proteins (ninein), and RNA-binding protein FMRP. In an evolutionary point of view, one of the important roles of Pax6 is to promote long basal processes of RG and oRG. Fabp7, a fatty acid binding protein, is actually working to make such fine processes in the rodent cortical primordium. Subcellular localization of these molecules seems to be tightly associated with mechanisms of corticogenesis.

(COI: No)

S51-3

Mechanisms of cerebral corticogenesis by migrating neurons

Nakajima, Kazunori (*Keio Univ. Sch. Med., Tokyo, Japan*)

Cortical neurons form the cortical plate (CP) in an inside-out manner, in which the late-born neurons located more superficially than the early-born neurons. Reelin, a glycoprotein secreted in the marginal zone (MZ), is crucial for this layering.
To clarify the Reelin function in vivo, we expressed Reelin ectopically in the developing cortex, and found that Reelin caused the leading processes of migrating neurons to assemble in the Reelin-rich region, which in turn induced their cell bodies to form cellular aggregates around Reelin. The late-born neurons migrated past their predecessors toward the central Reelin-rich region within the aggregates, resulting in a birthdate-dependent inside-out alignment even ectopically.
In the intermediate zone (IMZ) and CP, neurons migrate along the radial fibers (locomotion). When the leading process reaches the MZ, the soma moves rapidly towards the top of the CP, while the tip of the process remains attached to the MZ (terminal translocation). We found that the outermost region of the CP is packed with immature neurons, and named this region the primitive cortical zone (PCZ). Sequential in utero electroporation experiments suggest that the Reelin-Dab1-Crk/CrkL-C3G-Rap1-dependent switching of the migratory mode from locomotion to terminal translocation plays critical roles for the neuronal entry into the PCZ. This cascade then modulates neuronal adhesion by activating integrins, leading to the eventual birth-date-dependent layering in the neocortex. Recent analyses of the Reelin-dependent cell adhesion using mathematical modeling will also be discussed.

(COI: No)

S51-4

Epigenetic regulation of reciprocal connectivity between clonal cortical neurons

Yoshimura, Yumiko (*National Inst Physiol Sci, Okazaki, Japan*)

In the neocortex, each neuron connects to a relatively small number of neighboring neurons in a highly specific manner. Previously, it is reported that radially aligned neurons derived from the same radial glial progenitor cell preferentially establish synaptic connections in postnatal cortex. Here we show that neurons within the same layer establish cell-lineage-dependent synaptic connections, and that connection specificity is predetermined by epigenetic regulation during embryonic development. To visualize clonal neurons, we generated chimeric mice by injecting induced pluripotent stem cells marked with a fluorescent protein into wild-type mouse blastocysts. We conducted dual whole-cell recordings from presumed clonal or non-clonal neuron pairs within a layer 4 barrel in cortical slices. During postnatal development, there was a transient increase in the probability of connection for clonal neuron pairs compared with that for non-clonal neuron pairs, after which many of the connections in the clonal pairs changed from being one-way to reciprocal. This high degree of reciprocity of connections between clonal cells was abolished in clonal cells lacking DNA methyltransferase 3b (Dnmt3b), which regulates gene expression. This connection specificity was also absent in clonal neurons lacking clustered protocadherins (cPcdhs); individual neurons express various isoforms of these cell-adhesion proteins, in a Dnmt3b-dependent manner. Our findings suggest that the Dnmt3b-mediated epigenetic regulation of cPcdh expression enables clonal neurons to establish cell-lineage-specific reciprocal connections during postnatal development.

(COI: No)

S51-5

Interplay between innate circuits and neuronal activity in the formation of orientation selectivity in visual cortex

Ohki, Kenichi[1,2] ([1]*Dept Mol Physiol, Kyushu U, Fukuoka, Japan;* [2]*CREST, Japan*)

Visual functions of cortical neurons are established by activity-independent and -dependent mechanisms. A recent study reported that the progeny of single cortical progenitor cells are preferentially connected in the postnatal cortex. Here we investigated whether clonally related cells have similar preferred orientation. We found that preferred orientations of clonally related cells are similar to each other, suggesting that cell lineage is involved in the development of response selectivity in the cortex. However, not all clonally related cells share response selectivity, suggesting that cell lineage is not the only determinant of response selectivity, and later postnatal activity-dependent processes may affect the final selectivity of neurons. Here, we examined the roles of neuronal activity in the development of orientation selectivity. We used genetic silencing of cortical activity starting before the formation of orientation selectivity. Despite a strong suppression of both spontaneous and visually evoked activity throughout development, orientation selectivity of neurons in the visual cortex forms and matures normally. After the orientation selectivity matures, the distribution of the preferred orientations of neurons is reorganized. We found that this process requires spontaneous activity, but not visually evoked activity. Thus, the initial formation and maturation of orientation selectivity is largely independent of neuronal activity, and the inital selectivity is subsequently modified depending on neuronal activity.

(COI: No)

S51-6

Activity-dependent neural circuit formation in the developing cortex

Yamamoto, Nobuhiko (*Graduate School of Frontier Biosciences, Osaka University*)

How genetic and environmental factors are involved in neuronal circuit formation is one of the most intriguing issues in neuroscience. The thalamocotical (TC) projection is a suitable system to investigate this problem. Sensory TC axons form branches primarily in layer 4 of the corresponding cortical area from the onset. However, complexity of TC axon branching is known to be modified by neuronal activity including sensory-evoked and spontaneous firing. We studied the molecular mechanism of TC axon branching by molecular screening, in vitro analysis, gene transfer techniques and genetically manipulated animals. Our result shows that the netrin family member, netrin-4, promotes TC axon branching via activity-dependent expression in the developing cortex. Similarly, BDNF can also act as an activity-dependent branch-promoting factor in the later developmental stages. Sema7A also promotes TC axon branching, but its expression is at the earliest period and activity-independent. Taken together, multiple branch-regulating molecules are expressed at the different developmental stages and are likely to contribute to TC axon branching in distinct context.

(COI: No)

Symposium 52

Structure and dynamics of the motor-related neuronal circuit in brain

(March 23, 9:00～10:30, Room I)

S52-1

Precise relationship among input-output connections, somatotopic representation and zebrin stripes in the cerebellum

Sugihara, Izumi (*Dept Systems Neurophyisol, Grad Sch, Tokyo Med Dental Univ, Tokyo, Japan*)

The cerebellum is involved in adapting motor performance to sensory information that are conveyed by cerebellar input systems (mossy and climbing fiber axons). The cerebellar output system is comprised of Purkinje cell axons and neurons in the cerebellar nuclei. Morphology of single axons has suggested the projection patterns of these systems may be closely correlated with each other as well as with the zebrin stripes in the cerebellum. Nanoinjections of bidirectional tracers were made into somatotopically identified regions within the hindlimb C1 zone in copula pyramidis of the rat cerebellum. Injection sites were mapped relative to the zebrin II expression pattern and were correlated with the pattern of retrograde cell labeling within the inferior olive and the basilar pontine nuclei, and the distributions of labeled Purkinje cell terminals in the cerebellar nuclei. Zebrin bands were found to be related to both climbing fiber and mossy fiber inputs, to cortical representation of different parts of the ipsilateral hindpaw, and also to Purkinje cell terminal fields in the cerebellar nuclei. These findings indicate that there is precise topographic organization within the circuitry of input and output axons, which leads to a well-defined functional map in the cerebellar cortex and nuclei. The striped molecular expression pattern in the cerebellar cortex is tightly related with this organization. Kakenhi 25430032.

(COI: No)

S52-2

Manipulation of primate neural networks by means of modified viral vectors

Inoue, Kenichi; Takada, Masahiko (*Sys Neurosci Sec, Primate Res Inst, Kyoto Univ, Inuyama, Japan*)

Using a retrograde gene-transfer vector (NeuRet vector), we established two experimental system that permits pathway-selective cell ablation and pathway-selective gene expression, respectively. Using the former system, we attempted selective removal of the cortico-subthalamic "hyperdirect" pathway. After electrical stimulation in the motor-related areas, triphasic responses are usually detected in the internal segment of the globus pallidus (GPi). In the present study, the NeuRet vector expressing human interleukin-2 receptor α-subunit was injected into the STN of macaque monkeys. Then, immunotoxin injections were made into the SMA. In these monkeys, single neuron activity in the GPi was recorded in response to the SMA stimulation. We found that the early excitation was largely reduced with neither the inhibition nor the late excitation affected. In the latter system, we injected the lentiviral vector carrying the gene encoding tetanus toxin light chain gene downstream of the tetracycline-responsive element into the striatum, and then injected AAV vector carrying the tetracycline reverse-transactivator gene into the nigra. We observed that motor deficits were induced by doxycycline administration in the monkeys injected with the vectors. The present data indicate that the application of the NeuRet vector enables us to manipulate a particular neuronal population in primates.

(COI: No)

S52-3

Neuronal activities in the motor thalamus of Parkinsonian rats: Rate vs Pattern

Nakamura, Kouichi C.[1,2]; Sharott, Andrew[2]; Vinciati, Federica[2]; Tanaka, Takuma[3]; Mallet, Nicolas[2]; Magill, Peter J.[2] ([1]*Grad Sch Med, Kyoto Univ, Kyoto, Japan;* [2]*MRC ANU, Oxford Univ, Oxford, UK;* [3]*Interdiscipl Grad Sch Sci Eng, Tokyo Inst Technol, Tokyo, Japan*)

The cerebral cortex, basal ganglia and thalamus together form looping circuits that are critical for movement. Some cardinal movement difficulties of Parkinson's disease (PD) are associated with the abnormal neuronal oscillations, particularly at beta frequencies (13-30 Hz), in the cortex and basal ganglia. Neurons of the motor thalamus are key mediators of basal ganglia influences on cortical motor processing. The motor thalamus is parcellated into two major zones; the 'basal ganglia-recipient zone' (BZ) and the 'cerebellar-recipient zone' (CZ). To address the key issue of how their activities might be disturbed in PD, we recorded the spontaneous firing of identified BZ and CZ neurons under anesthesia in dopamine-intact rats as well as PD model rats prepared by unilateral 6-hydroxydopamine (6-OHDA) lesion of midbrain dopamine neurons. In the PD rats, we found no evidence of pathologically reduced firing rates in either zone of motor thalamus. However, the tight coupling of firing of BZ neurons to cortical slow oscillations was phase-shifted by ~100 degrees. During cortical activation, many BZ neurons, but not CZ neurons, exhibited abnormal beta oscillations in the PD rats. Moreover, blockade of BZ thalamocortical activity by local GABA infusion swiftly abolished the ongoing beta oscillations in the cortex. We conclude that the thalamocortical substrates of movement difficulties in PD are more closely related to abnormal firing patterns than altered firing rates.

(COI: No)

S52-4

Functional thalamic inputs to the primary motor cortex during voluntary movements

Tanaka, Yasuyo H[1,4]; Tanaka, Yasuhiro R[1,5]; Wake, Hiroaki[2]; Hira, Riichiro[1,5]; Kondo, Masashi[1,4,5]; Masamizu, Yoshito[1,5]; Kawaguchi, Yasuo[3,5]; Matsuzaki, Masanori[1,5] ([1]*Div Brain Circuits, National Institute for Basic Biology, Okazaki, Japan;* [2]*Div Homeostatic Development Dept Developmental Physiol, National Institute for Physiological Sciences, Okazaki, Japan;* [3]*Div Cerebral Circuitry, National Institute for Physiological Sciences, Okazaki, Japan;* [4]*Physical and Health Edu, Gradu School of Edu, The Univ of Tokyo, Tokyo Japan;* [5]*JST, CREST, Saitama, Japan*)

Thalamocortical (TC) pathways drive information processing in the cerebral cortex. In the primary motor cortex (M1), TC inputs are known to carry information of the basal ganglia and the cerebellum to finally give an impact on the corticospinal neurons, which send motor signals into the spinal cord. However, it remains unknown how TC pathways drive M1 to execute a voluntary movement. To address this issue, using two-photon microscopy and an adeno-associated virus that encodes GCaMP6 we visualized the neuronal activity of TC axons projecting to M1 during a self-initiated lever-pull task. We found that the TC axons showed sequential activities. Next, to test functional contribution of TC axonal inputs to M1, we perturbed the activity of channelrhodopsin-2-expressing TC axons during the lever-pull task by blue-light illumination on the cortical surface of M1. We found that the task performance of mice became worse during the photostimulation period. In addition, by a photostimulation mapping *in vitro* we found that TC axons directly innervated corticospinal neurons. These results suggest that TC axons send a critical signal to M1 to execute lever pulling.

(COI: No)

S52-5

Functional activity in motor cortex and striatum for voluntary movements

Isomura, Yoshikazu (*Brain Sci Inst, Tamagawa Univ, Tokyo, Japan*)

The primary motor cortex and its major subcortical target, dorsolateral striatum, play a crucial role in controlling voluntary movements as an entrance of the skeletomotor loop. The layer 5 pyramidal cells in the primary motor cortex send glutamatergic axons to the medium spiny neurons in the striatum, the only types of projection neurons, which send GABAergic outputs to other parts of the basal ganglia. The striatal projection neurons participate in either the direct pathway (expressing dopamine D1 receptors) or the indirect pathway (D2 receptors). These D1- and D2-expressing neurons should be excited and inhibited, respectively, by dopamine release from the substantia nigra neurons, encoding a reward prediction error. Many researchers believe that the activation of direct pathway enhances voluntary movements (like as an accelerator in a car), while the activation of the indirect pathway depresses them (as a brake). However, it remains unclear how differentially individual striatal neurons for the two pathways represent motor information, and whether the motor information is affected by reward expectation. Recently, we examined spike activity of single striatal neurons, which were identified juxtacellularly with in situ hybridization, and also of motor cortex neurons by multi-neuronal recordings, in the rats performing voluntary forelimb movements in a reward-expectable condition. Our observations suggest that striatal neurons for the two pathways may work coordinately to integrate motor and reward information, while motor cortex neurons may specifically process motor information.

(COI: No)

Symposium 53

Synaptic structure and (dys) function: How do synaptologists challenge brain disease?

(March 23, 13:30～15:00, Room C)

S53-1

Synapse protection as a novel therapeutic strategy for psychiatric diseases

Hayashi-Takagi, Akiko (*Lab of Structural Physiol, Grad Sch Med, Umin Univ, Tokyo, Japan*)

Drug discovery in psychiatry has been limited to chemical modifications of compounds originally discovered serendipitously. Therefore, more mechanism-oriented strategies of drug discovery for mental disorders are awaited. Schizophrenia (SZ) is a devastating mental disorder with synaptic disconnectivity involved in its pathophysiology. Reduction in the dendritic spine density is a major alteration that has been reproducibly reported in the cerebral cortex of patients with SZ. Disruptedin-Schizophrenia-1 (DISC1), a factor that influences endophenotypes underlying schizophrenia, has a regulatory role in the postsynaptic density in association with the NMDA-type glutamate receptor, and Rac1. Prolonged knockdown of DISC1 leads to synaptic deterioration, reminiscent of the synaptic pathology of SZ. Thus, we tested the effects of novel inhibitors to p21-activated kinases (PAKs), major targets of Rac1, on synaptic deterioration. PAK inhibitors prevented progressive synaptic deterioration in adolescence as shown by in vivo two-photon imaging and ameliorated a behavioural deficit in prepulse inhibition in adulthood in a DISC1 knockdown mouse model. The beneficial effects of synaptic protection by low-molecular weight compound, including PAK inhibitors reported here, may provide us with an opportunity for drug discovery in major mental illnesses with synaptic disturbance. Thus, in the last section of my talk, I will show the recently established high-throughput compound screening by the simultaneous measurement of key disease-related parameters such as glutamatergic synapse, palvalbumin interneuron, and oxidative stress.

(COI: No)

S53-2

CDC42EP4/septin-based perisynaptic glial scaffold that facilitates glutamate clearance

Ageta-Ishihara, Natsumi[1]; Yamazaki, Maya[2]; Konno, Kohtarou[3]; Nakayama, Hisako[4]; Abe, Manabu[2]; Hashimoto, Kenji[5]; Nishioka, Tomoki[6]; Kaibuchi, Kozo[6]; Miyakawa, Tsuyoshi[7,8]; Hashimoto, Kouichi[4]; Watanabe, Masahiko[3]; Sakimura, Kenji[2]; Kinoshita, Makoto[1] (*[1]Dept Mol Biol, Grad Sch Sci, Nagoya Univ, Nagoya, Japan; [2]Dept Cell Neurobiol, Brain Res Inst, Niigata Univ, Niigata, Japan; [3]Dept Anat, Grad Sch Med, Hokkaido Univ, Sapporo, Japan; [4]Dept Neurophysiol, Grad Sch Biomedical Sci, Hiroshima Univ, Hiroshima, Japan; [5]Cent Forensic Mental Hlth, Chiba Univ, Chiba, Japan ; [6]Dept Cell Pharm, Grad Sch Med, Nagoya Univ, Nagoya, Japan; [7]Cent Gene Anal of Behavior, NIPS, Okazaki, Japan; [8]Div System Med Sci, Fujita Health Univ, Toyoake, Japan*)

CDC42EP1-5/BORG1-5, a family of small GTPase effector proteins, interact with CDC42 or heterooligomers of septin GTPases in a mutually exclusive manner, but the physiological significance is unknown. We find that CDC42EP4 is expressed in the mouse cerebellar cortex exclusively in Bergmann glia, where CDC42EP4 localizes to specific membrane domains enwrapping dendritic spines of Purkinje cells. Proteomic analyses of cerebellar lysate indicate that CDC42EP4 is in complex with septin heterooligomers composed of SEPT2/3/4/5/6/7/8/10/11, and the glutamate transporter GLAST, but not with CDC42. In *Cdc42ep4*$^{-/-}$ mice, GLAST is physically dissociated from the septins, and GLAST signals on perisynaptic glial membranes are delocalized away from parallel fiber-Purkinje cell synapses. Patch-clamp analysis of *Cdc42ep4*$^{-/-}$ cerebellar slices reveals that the inward current to Purkinje cells following parallel fiber stimulation is mildly protracted after blocking AMPA receptor desensitization, and surges severely upon additional inhibition of glutamate transporters. These data indicate that glutamate clearance capacity in *Cdc42ep4*$^{-/-}$ Bergmann glia is compromised, which is partially compensated by two adaptive mechanisms. The glutamate intolerance manifests *in vivo* as motor learning defects in the balance beam test. Together, the unique phenotype indicates that CDC42EP4-dependent interaction with septins facilitates perisynaptic concentration and efficiency of GLAST to achieve sufficient buffering and clearance of glutamate, and motor coordination.

(COI: No)

S53-3

Epigenetic regulation of homeostatic synaptic plasticity under epileptic neuronal activity

Futai, Kensuke[1]; Mao, Wenjie[1]; Watanabe, Takuya[1]; Shen, Li[2]; Hock, Hanno[3]; Akbarian, Schahram[2] (*[1]BNRI, Dept Psychiatry, Univ Mass Med Sch, MA, U.S.A.; [2]Dept Psychiatry, Mount Sinai Sch Med, NY, U.S.A.; [3]Dept Medicine, Massachusetts General Hospital, MA, U.S.A.*)

Adjusting to runaway network excitation is critical to prevent neuronal cell death in epilepsy and requires rapid induction of a series of genes by synaptic activity. Understanding the regulatory mechanism underlying network excitation-dependent gene expression has broad implications, as chronic shifts in the excitatory and inhibitory balance (E/I balance) are found in many neurological disorders, including epilepsy, Autism Spectrum Disorders, Schizophrenia and Alzheimer's disease. The mechanisms of homeostatic synaptic plasticity, including downscaling of the strength of excitatory synaptic transmission in neurons, should play a critical role in preventing runaway excitation by maintaining the E/I balance. The expression of this plasticity requires neuronal activity-dependent gene transcription and protein synthesis. Gene expression, in turn, is affected by complex chromatin remodeling and histone modification machinery. Intricate post-transcriptional modifications of histones change the conformation of chromatin, and, thus, determine the active or inactive states of gene expression. These modifications are achieved by chromatin remodelers. However, the role of chromatin remodeling in homeostatic synaptic plasticity is largely unclear. In this session, I will discuss our recent results addressing the role of chromatin remodeler on homeostatic synaptic plasticity.

(COI: No)

S53-4

Molecular mechanisms for altered spine dynamics among ASD model mice

Fukumoto, Keita[1,2]; Tamada, Kota[1]; Tanaka, Shinji[3]; Okabe, Shigeo[3]; Takumi, Toru[1,2,4] (*[1]RIKEN Brain Science Institute., Saitama, Japan; [2]Grad.Sch. Biomed Sci. Hiroshima Univ., Hiroshima, Japan; [3]Grad.Sch.Med. Tokyo Univ., Tokyo, Japan; [4]CREST, JST*)

Autism spectrum disorder (ASD) is a neuropsychiatric disorder appeared in childhood when synaptic dynamics is highly active. Many CNVs (copy number variation) / SNVs (single nucleotide variation) of synaptic molecules are found in patients with ASD. In addition, model mice for ASD show a wide variety of abnormal synaptic changes in light of density, length and maturity, etc., but there have been no common abnormality among them.We generated a CNV mouse model for ASD (patDp/+) by chromosome engineering technique. patDp/+ mice in which the duplication of mouse chromosome 7 (corresponding to human 15q11-q13 region) is derived from paternal allele show abnormal social behavior. We have recently discovered the increased turnover rate of spines in the cerebral cortex of patDp/+ mice as a common endophenotype among mouse models of ASD. To identify the responsible gene(s) for the spine phenotypes, we systematically searched them using in vivo imaging by introducing each gene in the 15q11-13 region. We will present our progress on this screening and the implication of the candidate gene on spine functions.

(COI: No)

S53-5

Circadian genes, rhythms and biology of mood disorders

McClung, Colleen (*Univ Pittsburgh*)

Disruptions in sleep and circadian rhythms are one of the central features of several psychiatric diseases including bipolar disorder, major depression and addictive disorders. In fact, changes to the sleep wake cycle are one of the core symptoms used for diagnosis of these diseases. Moreover, several human genetic studies have identified polymorphisms in the genes that control circadian rhythms that associate with these disorders. However, the mechanisms by which circadian genes control mood, reward, motivation and anxiety remain unclear. Our lab has used mouse models to try to uncover some of the molecular and cellular mechanism by which circadian gene disruption leads to changes in reward and mood. Importantly, these behaviors can be normalized with both lithium and valproic acid treatment, adding face validity to this model. More recently, we found that these mice cycle throughout a 24 hour period with manic-like behavior becoming apparent during the light phase and the return to a euthymic-like state during the dark phase. To the best of our knowledge, this is the only mouse model which spontaneously switches mood-related states. This model allows us to probe the neurobiology and molecular mechanisms that underlie the switch to a manic state in bipolar disorder. These mechanisms, along with the mechanisms by which mood stabilizing drugs produce a therapeutic response in these mice will be discussed.

(COI: Properly Declared)

Symposium 54

Recent findings in development, function and disease of GABAergic neurons

(March 23, 13:30～15:00, Room D)

S54-1

The multi-faced GABA: role of GABA signaling in basic developmental processes in and outside the nervous system

Szábo, Gábor (*Institute of Experimental Medicine, HAS, Budapest, Hungary*)

GABA is present in the whole living kingdom from bacteria through yeast and plants to vertebrates with diverse, but also shared basic signaling functions.
In mammals, GABA is mostly recognized as the principal inhibitory neurotransmitter, however it is also present in a wide variety of peripheral tissues. During development both in the nervous system and other organs, GABA regulates basic processes including proliferation, differentiation and migration. The diverse action of GABA is underlined by the molecular complexity of its signaling components including the synthesizing enzymes (adult and embryonic GADs), receptors and transporters.
Here we describe the presence and the role of GABA signaling in undifferentiated ES cells that corresponds to the blastocyst developmental stage, where it modulates proliferation and differentiation through GABAA and GABAB receptors in an opposite way by regulating intracellular calcium levels. For the first time, we detected all GAD forms, a variety of GABA receptor subunits and both membrane and vesicular GABA transporters in the developing eye lens, where they are expressed in the fiber cells in a spatially and temporally regulated fashion. Using mouse models with genetically altered GAD levels and primary lens cultures, we determined that different GAD forms have distinct functions in fiber cell proliferation, differentiation and elongation. In these processes GABA also acts through intracellular calcium rise.
The developmental role of GABA signaling in the formation and migration of the GnRH neuronal system will also be discussed.

(COI: No)

S54-2

Subclass-specific expression of perineuronal nets around parvalbumin-expressing GABAergic neurons in the mouse hippocampus

Yamada, Jun (*Grad.Sch.Med.Sci., Kyushu Univ., Fukuoka, Japan*)

The perineuronal nets (PNNs) surround the soma of a subset of neurons, and is considered to play a critical role in regulation of neural plasticity. Seminal works using immuno/lectin histochemistry reported that PNNs were associated with parvalbumin-expressing (PV+) GABAergic neurons. Although later reports have shown that PV+ neurons consist of at least five subclasses (basket cells, axo-axonic cells, bistratified cells, oriens-lacunosum-moleculare (O-LM) cells and hippocampo-septal projection (H-S) cells), the relationship between PNNs and classification of PV+ neurons remains unclear. We clarify whether PNNs are associated with specific subclasses of PV+ neurons in the mouse hippocampus. To characterize PNNs, we used Wisteria floribunda agglutinin (WFA) lectin and the antibodies against aggrecan (core protein), hyaluronan and proteoglycan link protein 1 (HAPLN1; link protein) and Cat-315 epitope (chondroitin sulfate proteoglycan). The PNNs labeled by WFA were associated with PV+ basket cells. The PNNs defined by Aggrecan were found in basket cells, O-LM cells and H-S cells, while HAPLN1-positive PNNs and Cat-315-positive PNNs were associated with basket cells and H-S cells. These data provide compelling evidence that PNNs may critically regulate hippocampal neuronal activity via subclass specific expression in PV+ neurons.

(COI: No)

S54-3

Prenatal stress-induced selective deterioration of neurogenesis of parvalbumin-positive GABAergic neurons

Fukuda, Atsuo (*Dept Neurophysiol., Hamamatsu Univ Sch Med., Hamamatsu, Japan*)

Exposure to prenatal stress and mutations in GAD1, which encodes the γ-aminobutyric acid (GABA) synthesizing enzyme glutamate decarboxylase (GAD) 67, are both risk factors for psychiatric disorders. In addition, decrement of parvalbumin (PV)-positive GABAergic interneurons in the medial prefrontal cortex (mPFC) and hippocampus (HIP) has often been observed in schizophrenia patients. However, the relationship between these risk factors remains unclear. So we examined GAD67-green fluorescent protein (GFP) knock-in mice (i.e., mice in which the Gad1 gene is heterozygously deleted; GAD67+/GFP) that underwent prenatal stress from embryonic day 15.0 to 17.5 to address the interaction between Gad1 disruption and stress. Administration of 5-bromo-2-deoxyuridine revealed that neurogenesis of GFP-positive GABAergic neurons, but not cortical plate cells, was significantly diminished in GAD67+/GFP but not in wild type (GAD67+/+) fetal brains during maternal stress. Differential expression of glucocorticoid receptors by different progenitor cell types may underlie this differential outcome. Postnatally, the density of PV-positive, but not PV-negative, GABAergic neurons was significantly decreased in the mPFC, HIP and somatosensory cortex of GAD67+/GFP mice. By contrast, these findings were not observed in GAD67+/+ offspring. These results suggest that prenatal stress, in addition to heterozygous deletion of Gad1, could specifically disturb the proliferation of neurons destined to be PV-positive GABAergic interneurons (Uchida et al., Transl. Psychiatry 2014).

(COI: No)

S54-4

Glutamate decarboxylase deficiency displays schizophrenia-like phenotypes: a study using knockout mice

Yanagawa, Yuchio (*Department of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine, Maebashi, Japan*)

GABA is a major inhibitory neurotransmitter in the adult mammalian CNS. GABA is synthesized by two forms of glutamate decarboxylases (GAD65 and GAD67). Impairments in GABAergic neurotransmission are thought to be associated with the pathogenesis of psychiatric disorders such as anxiety disorder, epilepsy and schizophrenia. For example, postmortem brain studies of schizophrenia patients have revealed GAD67 reduction in parvalbumin (PV) neurons of the cerebral cortex and hippocampus. To develop a mouse model of the psychiatric disorders and to further elucidate the involvement of GABAergic dysfunction in the disorders at molecular level, we generated conventional GAD65 and GAD67 knockout (KO) mice. Whereas the GAD65 KO mice displayed increased level of anxiety and spontaneous seizures, the GAD67 KO mice died around birth probably due to cleft palate and respiratory failure. Considering the perinatal death of the conventional KO mice, we generated conditional GAD67 KO mice, in which GAD67 was deficient primarily in PV neurons, and named these KO mice PV-GAD67 mice. The PV-GAD67 heterozygous mice displayed schizophrenia-like phenotypes such as an increase in MK801-induced locomotor activity and a deficit in prepulse inhibition, indicating that GAD67 reduction is associated with the pathophysiology of schizophrenia. The approaches using our KO mice will be discussed.

(COI: No)

Symposium 55

New roles for biological clocks in homeostasis

(March 23, 13:30～15:00, Room F)

S55-1

Homeostatic regulation of the circadian clock system evaluated by in vivo whole body imaging

Tahara, Yu (*Dept. of Physiol. and Pharm., Sch. of Adv. Sci. and Engi., Waseda Univ.*)

The circadian clock systems in mammals, endogenous pacemakers located in the brain and periphery, organizes various biological activities, and are synchronized by the environmental factors, such as light-dark cycles, food, exercise, and drugs. Recently, we have developed new methodology to measure gene expression rhythms of peripheral clock by in vivo imaging system using PER2::LUCIFERASE knock-in mice. This method enables us to track the changes of circadian network among tissues in individual mouse for long-term. Using this, we focused on the stress response of the circadian clocks, and found that physical/psychological stress was a potent synchronizer of peripheral clocks. Stress stimuli induced time-of-day dependent phase-advance or -delay of clock gene expression rhythms in peripheral tissues, as well as in the hippocampus and cortex, but not in the suprachiasmatic nucleus, a central clock. Moreover, several days of stress exposure at beginning of the light period caused loss of oscillation and severe internal desynchronization of peripheral clocks among tissues. This response was produced through glucocorticoid and sympathetic nervous activations with acute/direct change of clock gene expressions in peripheral tissues. Additionally, this response could be habituated and disappeared by repeated stress exposures. Thus, our results newly demonstrated that acute stress caused severe change of peripheral clocks, and also stress was a strong entrainable factor for the peripheral clocks.

(COI: No)

S55-2

Seasonal rhythms in affective states and communication between brain and peripheral metabolism

Yasuo, Shinobu (*Fac. Agr. Kyushu Univ., Fukuoka, Japan*)

Mammals have adapted their physiological functions and behaviors to seasonal changes in environment by using photoperiod as a primary cue. Humans also exhibit seasonality in their mood, sleep, sociality, appetite, and energy balance, which may be a vestige of evolutionally adaptation mechanisms. Extreme seasonality causes seasonal affective disorder (SAD) marked by depression during specific seasons, generally winter. Symptoms of SAD also include hypersomnia, hyperphagia, weight gain, and carbohydrate craving, suggesting that metabolic changes associating with brain functions are underlying SAD. This hypothesis has not been addressed experimentally using laboratory rodents, because mice and rats are non-seasonal breeders and have been considered as inappropriate models of SAD. In recent years, we clarified that exposure of C57BL/6J mice to winter-like photoperiod induces high immobility in forced swim test, a depression-like behavior, and low preference for saccharine, a depression-related anhedonia, accompanying low levels of brain serotonin. Interestingly, these mice under winter-like photoperiod also exhibited fat accumulation, alteration of plasma composition of free amino acid levels, low glucose tolerance, and alteration of muscle physiology. Further, photoperiod regulated corticosterone release that is deeply associated with peripheral metabolism, and adrenalectomy abolished the photoperiod-dependent changes in depression-like behavior. In the presentation, involvement of communication between peripheral metabolism and brain functions in seasonal regulation of mood will be discussed.

(COI: No)

S55-3

Hypothalamic regulation of energy metabolism by feeding rhythm

Shiuchi, Tetsuya[1,2] ([1]*Dept. Integrative Physiology, the Univ. of Tokushima Graduate School, Tokushima, Japan;* [2]*PRESTO, JST, Saitama, Japan*)

Biological clock is modulated by not only light stimulation also feeding stimulation. It has been recognized that disturbance of feeding rhythm has been understood as a risk factor for development of insulin resistance because of mess up the timing of expression phase of clock genes especially in liver. In contrast, hypothalamic control of peripheral energy metabolism is an important regulatory system in whole body homeostasis, although effect of feeding rhythm on it has not elucidated. We separated C57BL/6J mice for 3 feeding scheduled groups; given lab chows freely during dark phase (ZT12-24, Control group), first 4-hour in dark phase (ZT12-16; Morning group), and last 4-hour in dark phase (ZT20-24, Evening group). Mice in Evening group showed impaired whole body insulin sensitivity despite the smaller food intake than that of Control group, while mice in Morning group showed normal insulin sensitivity. We observed that higher lipid accumulation, increased gene expression of fatty acid synthesis, decreased fatty acid oxidation and impaired insulin signals in skeletal muscle in Evening group compared to other group. These effects were not observed in liver. On the other hand, mRNA expression of agouti-related protein (AgRP) was increased in hypothalamus in Evening group. Inhibition of central AgRP expression by antisense oligo improved insulin resistance in whole body and skeletal muscle of Evening group. These results suggest that feeding rhythm like as ingestion only in the evening impairs insulin sensitivity in whole body and skeletal muscle mediated by hypothalamic AgRP.

(COI: No)

S55-4

Exploring the mechanism of homeostasis: A study on a common kinase regulating metabolism and circadian clock

Hayasaka, Naoto[1,2] ([1]*Yamaguchi Univ. Grad. Sch. Med., Ube, Japan;* [2]*PRESTO, JST, Kawaguchi, Japan*)

Circadian clocks are known to play a role in the regulation of homeostasis, and their impairment may lead to disorders such as metabolic, immune and neuropsychiatric diseases. We found salt-inducible kinase 3 (SIK3) to be an essential component of both metabolism and circadian clock. *Sik3*-deficient (*Sik3* KO) mice demonstrate severe defects in glucose, lipid, bile acid and vitamin A metabolisms, and over 90 % of homozygotes die postnatally. In addition, *Sik3* KO mice exhibit several abnormalities in circadian rhythms, e.g., significantly longer and unstable periods in behavioral rhythms, imperfect entrainment to light-dark cycles, and splitting of light-entrained and non-entrained (free-running) rhythms in behavior under light re-entrained condition. These data, along with a previous report indicating that SIK3 is evolutionally very well-conserved from nematodes to mammals, strongly suggest an indispensable role for SIK3 in homeostasis and survival. To address the question of whether SIK3 mediates an interaction between metabolism and circadian clock to maintain homeostasis, we generated conditional *Sik3* KO mice and found a link between circadian clock and metabolic homeostasis.

(COI: No)

S55-5

Insulin mediates feeding-induced circadian entrainment in liver and white adipose tissue

Sato, Miho[1]; Hayasaka, Naoto[2,3]; Akashi, Makoto[1] ([1]*Res Inst for Chronobiology, Yamaguchi Univ, Yamaguchi, Japan;* [2]*Yamaguchi Univ. Grad. Sch. Med., Ube, Japan;* [3]*PRESTO, JST, Kawaguchi, Japan*)

The circadian clock is entrained to environmental cycles. Although the light input pathway has been well characterized, the mechanism of feeding-induced phase adjustment remains unclear. Here, we focused on insulin as one of the endogenous molecules and report that insulin may be involved in feeding-induced entrainment in vivo. To help elucidate the in vivo roles of insulin, we used S961, which is a highly specific competitive peptide inhibitor of insulin. Subcutaneous injection of S961 inhibited feeding-induced immediate early expression of Per2 transcripts in liver. Also, attenuation in the circadian entrainment of the liver clock was statistically significant in S961-treated mice. In ex vivo culture experiments, insulin induced phase shift in liver and white adipose tissue. Furthermore, the direction of the phase shift was phase specific, phase delay during PER2-decreasing phase and phase advance during the increasing phase. These results suggest that insulin maybe an immediate early factor in feeding-mediated tissue-specific entrainment. In this talk, I would like to discuss the significance of the phase regulatory role of glucose-homeostasis-maintaining hormone.

(COI: No)

Symposium 56

Cutting-edge *in vivo* nano-imaging technologies

(March 23, 13:30〜15:00, Room G)

S56-1

Toward high-speed high-resolution 3D imaging

Mimori, Yuko Kiyosue[1]; Shimozawa, Togo[1,2] (*[1]Riken CDB; [2]Waseda Univ*)

In this talk, we introduce two advances in spinning disc confocal- and light sheet-based microscopic technologies.
1. Spinning disk confocal microscopy for thick specimens
Yokogawa Confocal Scanner Unit (CSU), containing a set of ~20,000 microlenses and confocal pinholes, offers high-speed multipoint scanning. But conventional model was not optimized for thick specimens. We improved this method using two-photon excitation and modified CSU with a larger pinhole interval. Our strategies dramatically improve higher-resolution intravital imaging of tissues and embryos. (Shimozawa, T. et al., PNAS, 110: 3399-3404, 2013)
2. Lattice light-sheet microscopy for isotropic 3D imaging
Recently, the use of scanned Bessel beams showed to create very thin light sheets (< 300 nm). With the latest model employing lattice light-sheet illumination to achieve faster scanning, we succeeded to track growth of tens of microtubules within the mitotic spindle and all of the chromosomes in 3D, demonstrating its suitability for 3D isotropic high-resolution live imaging. (Chen et al., Science, 2014, in press)

(COI: No)

S56-2

Fast and wide field-of-view live imaging of whole organism by light-sheet microscopy

Nonaka, Shigenori (*NIBB, Okazaki, Japan*)

Light-sheet microscopy is an emerging technology that uses thin sheet-shaped excitation light to illuminate the focal plane of a detection objective. This method is characterized by low bleaching and phototoxicity, deep penetration depth, and high-speed image acquisition. These features are extremely suitable for live imaging of whole organisms of submillimeter scale.We have applied this microscopy for long term and high time-resolution live imaging of gastrulating mouse embryos and freely moving *Amoeba proteus*. Recently we have developed a new two-photon light-sheet microscope that enables better penetration depth and wider field of view, by use of high pulse-energy femtosecond fiber laser.

(COI: No)

S56-3

Relationship between synapse nanostructure and its stability in the mouse neocortex

Tanaka, Shinji[1,2]; Iida, Tadatsune[1,2]; Iwasaki, Hirohide[1,2]; Okabe, Shigeo[1,2] (*[1]Grad. Sch.Med.UnivTokyo., Tokyo, Japan; [2]CREST, JST, Saitama, Japan*)

Two-photon microscopy has advantages over conventional microscopy in deeper tissue penetration of infrared light and less photo-damage. Application of this technique to living animals enables us to observe the dynamics of subcellular structures and molecules in vivo. However, due to the relatively large point-spread function of the infrared light focused by a water-immersion objective lens, the optical resolution of two-photon imaging is not optimal. In order to clarify the relationship between synapse nanostructure and its stability in vivo, new technologies of correlative microscopy should be developed. In vivo two-photon imaging of dendritic spines and postsynaptic molecules revealed that stabilization of spine synapses takes place during the early postnatal development. Most of spine synapses contacting the intracortical axons are highly dynamics until the third postnatal weeks. In contrast, spine synapses receiving thalamic inputs were larger and highly stable. To further characterize morphological details of the dynamic and stable spines, we performed retrospective analyses of synaptic structure imaged in vivo by using CLARITY, which enables rapid access of exogenous antibodies, large volume imaging, and use of an objective lens with a high numerical aperture. This technical development should be useful in analyses of morphological characteristics of both spine synapses and surrounding glial processes with a submicron resolution, which will lead to identification of parameters important for synapse stability.

(COI: No)

S56-4

In vivo visualization of sarcomere dynamics in the beating mouse heart

Kobirumaki-shimozawa, Fuyu; Fukuda, Norio (*Dept Cell Physiol, The Jikei Univ Sch of Med, Tokyo, Japan*)

A fundamental principle in cardiovascular science is that a change in myocardial length dramatically changes the heart's pump functions on a beat-to-beat basis (*Frank-Starling Law of the Heart*). Despite the importance of accurate measurement of sarcomere length in cardiomyocytes, such measurement has not been achieved in the beating heart *in vivo* under the physiological condition, due to technical difficulties. In the present study, therefore, we developed a high-speed high-resolution imaging system for myocardial sarcomeres in living mice. The spatial resolution in the measurement of single sarcomere lengths was 20 nm at 100 frames per second. This system enabled three-dimensional analyses of sarcomere dynamics during the cardiac cycle, simultaneously with electrocardiogram and left ventricular pressure measurements. Since the discovery of the *Law* by Frank and Starling at the turn of the 20th century, we for the first time directly quantified sarcomere length values in left ventricular myocytes *in vivo* under the physiological condition. Likewise, the left ventricular developed pressure was linearly correlated with the sarcomere length change between diastole and systole on the order of 100 nm, providing direct evidence for the tight coupling between sarcomere length and the heart's pump function *in vivo*. The present experimental system has a broad range of application possibilities for unveiling sarcomere dynamics in cardiomyocytes *in vivo* in health and disease.

(COI: No)

S56-5

High accuracy nano-imaging of cancer and peripheral artery disease with X-ray and fluorescence

Gonda, Kohsuke (*Dept Med Phys, Grad Sch Med, Tohoku Univ, Sendai, Japan*)

We have been developing the technologies for X-ray computed tomography (CT) or fluorescence imaging to clarify the mechanism and develop the diagnostic methods for cancer and arterial sclerosis. The gold nanoparticles coated with PEG chains were prepared as contrast agents for X-ray CT imaging. As the resolution of X-ray CT imaging is around several tens of micrometers, X-ray CT can visualize at levels ranging from small tissues to whole body by high penetrative power of X-rays. Quantum dots (QDs) is one of recently-developed fluorescence nanoparticles. To perform various biomedical fluorescence imaging, we are using modified QDs and own in vivo imaging system with spatial accuracy of 9 nm. Fluorescence imaging has the resolution with hundreds of nanometer and high quantitative sensitivity because the fluorescence signal intensity is proportional to the intensity of the photon excitation energy. However, as the fluorescence imaging was affected by optical scatter and absorption in cells or tissues, tissue permeability of fluorescence is not well. Therefore, fluorescence imaging is suitable to visualize at levels ranging from molecular to small or thin tissues. The technology integration of both advantages for X-ray CT and fluorescence imaging is thought to greatly contribute to development of medical imaging with high accuracy and highly-quantitative sensitivity at levels ranging from molecular to whole body. Here we introduce high accuracy imaging of cancer and peripheral artery disease with X-ray CT or fluorescence and discuss the technology integration of both imaging.

(COI: No)

Symposium 57

Neurogenesis from embryo to adult

(March 23, 13:30～15:00, Room H)

S57-1

Oscillatory Expression of bHLH Transcriptional Factors in Neural Stem Cells

Imayoshi, Itaru[1,2]; Kageyama, Ryoichiro[2] (*[1]The Hakubi Center, Kyoto, Univ., Japan; [2]The Institute for Virus Research, Kyoto, Univ., Japan*)

During neural development and in the adult brain, neural stem cells give rise to appropriate numbers of neurons, astrocytes and oligodendrocytes in the specific timing and places. Many important intrinsic and extrinsic factors regulating the fate determination of neural stem cells have been identified, however, how do these key factors or molecules regulate diverse responses of neural stem cells is not still unclear. The basic-helix-loop-helix factors Ascl1/Mash1, Hes1, and Olig2 regulate the fate choice of neurons, astrocytes, and oligodendrocytes, respectively; however, these factors are coexpressed by neural stem cells. How such fate determination factors behave in progenitors and differentiating cells remains elusive. In this study, we found, by time-lapse imaging, that these factors are expressed in an oscillatory manner by neural stem cells, and that one of them becomes dominant during fate choice. Furthermore, FACS sorting and differentiation assay of NPCs having various amount of bHLH factors revealed that neural stem cells had the differentiation biases at that time, and that differentiation biases were dynamically changing by oscillatory expression of bHLH factors. These results indicate that neural stem cells are dynamically changing their state by oscillatory expression of fate determinate factors. We propose new neural stem cell regulatory mechanism that oscillatory expression of bHLH transcriptional factors ensures self-renewable and multi-potent ability of neural stem cells.

(COI: No)

S57-2

From embryonic to adult neurogenesis in the hippocampus

Seki, Tatsunori (*Hist. Neuroanat. Tokyo Med. Univ., Tokyo, Japan*)

In most of brain regions, neurogenic stem cells occur only during embryonic and early postnatal stages, and disappear at adult stage. However, the hippocampus possesses stem cells to continue to produce dentate granule cells from embryonic to adult stages. The adult stem cells are astrocyte-like cells expressing glial fibrilar acidic protein (GFAP) and brain lipid-biding protein (BLBP), and thus are distinct from embryonic stem cells such as those of neocortical pyramidal cells that express BLBP, but not GFAP. Recently, we have found that embryonic dentate stem/progenitor cells are different from both the neocortical and the adult dentate stem/progenitor cells. The embryonic dentate stem/progenitors express GFAP, but not BLBP. The analysis using Gfap-GFP mice and time-lapse imaging revealed that the Gfap-GFP+ distinct cell population first appears in the VZ of the medial pallium at the dorsal edge of the fimbria. During the embryonic period, they form a migratory stream from the VZ to the developing dentate gyrus, and establish the proliferative zones in which Gfap-GFP+ progenitors produce granule cells. Before birth, the Gfap-GFP+ progenitors were mostly negative for BLBP. However, after birth the Gfap-GFP+ progenitors begin to express BLBP, and the number of Gfap-GFP+/BLBP+ progenitors rapidly increase. By P14 a half of progenitors became double positive for Gfap-GFP and BLBP. They were mostly seen in the subgranular zone, and had a radial process, a morphological trait of adult type progenitors. These results indicate that the property of the dentate progenitors cells is converted immediately after birth, and become adult type progenitors.

(COI: No)

S57-3

Stress that induces adult neurogenesis in the mouse neocortex

Tamamaki, Nobuaki (*Dept of MNS, Kumamoto Univ., Kumamoto, Japan*)

It is well known that a significant number of neurons are continuously produced in the two sites of the adult mammalian brain, the hippocampal dentate gyrus and the subventricular zone of the telencephalon. The dentate gyrus produces new granule cells to code new descriptive memory every day. The subventricular zone of the telencephalon produces new GABAergic neurons as a response to the turn-over of the olfactory receptor cells in the nasal epithelium. Therefore, the adult neurogenesis might be the phenomena generally induced by the stress added on the neuron progenitors.As the results of aging, accidents, and other factors, brain suffers from hemorrhage, ischemia, epilepsy, amyloid deposition, virus infection and physical damages. There pathological damage may be stress to the neocortical neuron progenitors hidden somewhere inside the cranium. However, as far as we searched many papers, hemorrhage or ischemia failed to generate new neurons with an axon. Therefore, we started to use the other stress, such as (repetitive electrical stimulation) kindling and virus infection. Kindling induced epileptic seizure at each stimulation and grew the pia-progenitor in the pia mater and arachnid membrane (the leptomeninges). Finally kindling induced a small number of excitatory neurons and GABAergic neurons with an axon. Moreover, elimination of either excitatory neurons or GABAergic neurons by DTA expression induced many BrdU-positive excitatory neurons or BrdU-positive GABAergic neurons, respectively.

(COI: No)

S57-4

Understanding the new neurons in the olfactory bulb within the large olfactory neuronal network

Yamaguchi, Masahiro (*Dept Physiol, Grad Sch Med, Univ Tokyo, Japan*)

New neurons are continually incorporated into the neuronal circuit of the olfactory bulb (OB) even in adulthood. The new neurons differentiate into granule cells (GCs), the major inhibitory neurons in the OB, and provide remarkable plastic potential to the neuronal circuit. In this talk I will explain how new GCs work within the large olfactory neuronal network that involves the OB and the olfactory cortex. Among new GCs, nearly half are incorporated into the neuronal circuit while the other half are eliminated by apoptosis. This cell selection is important for the refinement and fine tuning of the neuronal circuit. New GCs receive bottom-up olfactory sensory inputs from the external world, and also receive top-down inputs from the principal neurons in the olfactory cortex. The bottom-up sensory inputs activate a subset of new GCs, and top-down inputs from the olfactory cortex selectively eliminate new GCs which are not activated by the bottom-up inputs. Thus selection of new GCs is conducted by the integration of bottom-up inputs from the periphery and centrally-generated top-down inputs. Further, I will discuss possible roles of new GCs in the signal transfer between the OB and the olfactory cortex and the proper expression of olfactory behaviors.

(COI: No)

S57-5

Relationship between frontal cortical oligodendrocyte and mood

Hayashi, Yoshitaka[1]; Fuke, Satoshi[1]; Fuchigami, Takahiro[1]; Koyama, Natsu[1]; Tatebayashi, Yoshitaka[2]; Hitoshi, Seiji[1] (*[1]Dept Integrative Physiol, Grad Sch Med, Shiga Univ Med Sci, Shiga, Japan; [2]Dept Affective Disorder, Tokyo Metro Inst Med Sci, Tokyo, Japan*)

In the post mortem brain study, stereological methods were usually used to estimate the number of cells in the brain. However, stereological methods are laborious and intrinsically low throughput, taking typically long periods to complete a large study. We therefore developed a novel quantitative cell-counting method using a flow cytometer. We applied this method to frozen unfixed postmortem human brains of the frontopolar and inferior temporal cortex from patients with mood disorders (major depressive disorders and bipolar disorders) and normal controls. We found significant reductions of the number of oligodendrocyte in the frontopolar cortex of mood disorders. The reduction of oligodendrocyte in frontopolar cortex from patients with mood disorders suggests that the pathogenesis of mood disorders may involve some abnormalities in oligodendrocyte in the frontopolar cortex. To explore further the dynamics of cortical oligodendrocyte, we demonstrated the oligodendrogenesis in the brain from crab-eating monkeys (macaca fascicularis), and estimated the number of oligodendrogenesis in the frontal, temporal, and occipital cortex. We found that the number of oligodendorogenesis was larger in frontal cortex than other region. Furthermore, we try to establish a nonhuman primate model of cytokine-induced depression by using interferon-alpha. In the preliminary study, we observed abnormalities of behavioral and neuropathological changes in the depression model of monkey.

(COI: No)

Symposium 58

Neuronal circuit in the basal ganglia in terms of transmitters and receptors

(March 23, 13:30～15:00, Room I)

S58-1

Area-specific dopamine receptor expression of astrocytes in basal ganglia

Yamada, Katsuya; Nagatomo, Katsuhiro (*Dept Physiol, Hirosaki Univ Grad Sch Med, Aomori, Japan*)

Midbrain substantia nigra pars reticulata (SNr), the major output nucleus of the basal ganglia, receives dopamine via dendrites of dopamine neurons, of which cell bodies locate in the adjacent nucleus substantia nigra pars compacta (SNc). However, cellular elements, especially receptor expression profiles, to which such dendritically released dopamine targets, is yet to be identified completely. Here we show that processes, but not nuclei, of acutely dissociated SNr astrocytes express D1 dopamine receptors by immunocytochemical investigation. No significant D2 receptor expression was detected in SNr astrocytes. In contrast, D1-expressing and D2-expressing astrocytes were found in striatum, although no such expression was detected in astrocytes obtained from cerebral cortex in the same slice. Based on these findings with other information so far obtained, we propose a working hypothesis that astrocytes in basal ganglia express dopamine receptors in their processes in an area-specific manner.

(COI: No)

S58-2

The role of physiologically released dopamine in the striatum

Momiyama, Toshihiko (*Dept Pharmacol, Jikei Univ Sch Med, Tokyo, Japan*)

Dopaminergic neurons in the substantia nigra pars compacta (SNc) send their axons to medium spiny neurons as well as cholinergic interneurons in the striatum, regulating neuronal activities of these striatal neurons. Nigro-striatal dopaminergic pathway plays important roles in motor control through the interaction between dopamine (DA) and acetylcholine (ACh). One of the potential mechanisms underlying the motor control is synaptic transmission in the striatum. However, little information has been available on the DA receptor subtypes contributing to the synaptic transmission.In the present study, Whole-cell patch-clamp analysis was carried out in dopamine (DA) D2 receptor (D2R) knock-out (KO) mice to elucidate the function of this receptor in the regulation of GABAergic synaptic transmission onto striatal cholinergic interneurons. In slice preparation obtained from wild type mice, GABAergic inhibitory postsynaptic currents (IPSCs) showed frequency-dependent suppression, and the suppression significantly reduced in D2R KO mice. Contribution of N-type calcium channel was significantly reduced in the striatal cholinergic interneurons of the D2R KO mice compared with that in the wild type mice, where D2-like receptors and N-type channels are tightly coupled in the GABAergic transmission. These findings provide a concrete evidence for the physiological role of D2R in the regulation of GABAergic synaptic transmission onto striatal cholinergic interneurons, confirming the tight coupling D2R and N-type calcium channels in the regulation of GABA release.

(COI: No)

S58-3

Regional Difference in Network of rat basal ganglia

Fujiyama, Fumino[1,2]; Unzai, Tomo[1]; Mizutani, Kazuko[1]; Oh, Yoonmi[1]; Nakano, Yasutake[1]; Nagai, Wataru[1]; Takahashi, Susumu[1]; Karube, Fuyuki[1]
([1]*Grad.Sch.Brain Sci.Doshisha Univ., Kyoto, Japan;* [2]*CREST, JST*)

Motor coordination and reinforcement learning mechanisms have recently been proposed to work in the neural circuit of the basal ganglia. However, the mechanism has been difficult to confirm anatomically, partially because of the complex organization of the striatum. In particular, because the striosome/matrix compartments are highly irregular and cannot be visualized without processing such as immunostaining, identification of the exact input and output pathways is difficult to be analyzed. We recently elucidated the input/output organization of each compartment using a single neuron reconstruction with viral vectors expressing membrane-targeted fluorescent proteins and other tracing studies. With respect to the excitatory striatopetal afferents, the striatal compartments receive different lines of information from the not only the cortical areas but also the thalamic nuclei. As concerned with the intra- basal ganglia network, not only the striatum but also the globus pallidus showed the characteristic projection patterns toward the other nuclei in relation to the chemical subregions of basal ganglia. These findings revealed that the topographic organization of the striatum could be well correlated with both the cortico-basal ganglia-thalamic loops and the intra- basal ganglia network. It suggests that the specific input/output organizations make possible the precise tuning for the motor coordination and reinforcement learning.

(COI: No)

S58-4

Control of behavioral flexibility by striatal cholinergic interneurons

Kobayashi, Kazuto (*Dept Mol Genet, Fukushima Med Univ, Fukushima, Japan*)

Flexible switching of behaviors in response to changes in environments is essential for the survival of animals. This behavioral flexibility is mediated through the neural circuitry linking the prefrontal cortex and basal ganglia. In the present study, the role of striatal cholinergic interneurons in behavioural flexibility is addressed by eliminating selectively these neurons in transgenic rats by immunotoxin-mediated cell targeting. Elimination of cholinergic interneurons from the dorsomedial striatum (DMS), but not from the dorsolateral striatum, results in enhanced reversal and extinction learning, sparing the acquisition of place discrimination. In addition, gene-specific silencing of M4 muscarinic receptor by lentiviral expression of short-hairpin RNA also enhances the place reversal learning, whereas gene silencing of M1 muscarinic receptor does not affect the performance of reversal learning. These data indicate that DMS cholinergic interneurons play a key role in the inhibition of behavioural flexibility, mainly through the M4 muscarinic receptor.

(COI: No)

S58-5

Role of dopamine D1 receptors in the hippocampal dentate gyrus in the action of antidepressants

Nishi, Akinori (*Dept of Pharmacol, Kurume Univ Sch of Med, Kurume, Japan*)

Antidepressant drugs are widely used for the treatment of depression. Mechanisms of antidepressant action are not fully understood. Recently, a selective serotonin reuptake inhibitor, fluoxetine, is shown to induce functional changes in mature granule cells in the hippocampal dentate gyrus (PNAS 107:8434-8439, 2010), in addition to the facilitation of adult neurogenesis. The profiles of granule cell functions after chronic fluoxetine treatment resemble to those of immature dentate gyrus in alpha-CaMKII +/- mice, showing the increased excitability of granule cells, the decreased expression of calbindin, a marker of mature granule cells, and the increased expression of dopamine D1 receptors (Mol Brain I:6, 2008). We observed that chronic treatment of C57BL/6 mice with fluoxetine induced the expression of D1 receptors, but not other dopamine receptors, in granule cells of the dentate gyrus. The high expression of D1 receptors resulted in activation of cAMP/PKA signaling. In vivo microdialysis analysis revealed that the serotonin responses to a novel environment in the dentate gyrus were suppressed after chronic fluoxetine treatment due to high activity of D1 receptors. In behavioral studies, D1 receptor agonism was shown to enhance antidepressant action of fluoxetine in mice chronically subjected restraint stress. These findings suggest that the dopamine D1 receptor in the dentate gyrus plays a key role in the action of antidepressants, and is a therapeutic target of depression.

(COI: No)

Symposium 59

Physiological Model-Based Cardiovascular Diagnosis/Therapy

(March 23, 15:00～16:30, Room A)

S59-1

The monitoring and the clinical application of left ventricular arterial coupling (Ees/Ea)

Shigemi, Kenji[1]; Obata, Yurie[1]; Hayabuchi, Mitsuyo[1]; Takaku, Akiko[1]; Hamada, Toshihiko[2]; Okafuji, Kazuhiro[3]; Matsuoka, Satoshi[4] (*[1]Dept of Anesthesiol and Reanimatol, Fac of Med Sci, Univ of Fukui, Fukui, Japan; [2]Dept of Clin Lab, Univ of Fukui Hosp, Fukui, Japan; [3]Health Exam Ctr, Fukui-ken Saiseikai Hosp, Fukui, Japan; [4]Det of Integrative and Systems Physiol, Fac of Med Sci, Univ of Fukui, Fukui, Japan*)

The estimation of ventricular arterial coupling (Ees/Ea) is clinically useful for general anesthesia and critical care, since the cardiac performance, such as cardiac output and ejection fraction, depends on Ees/Ea, and the efficacy of energetic transfer from the heart to the artery is also related to Ees/Ea. We approximated the waveform of the left ventricular time varying elastance curve with two straight lines, and end-diastolic left ventricular pressure was supposed to be zero for estimating Ees/Ea with four non-invasive parameters: end-systolic arterial pressure (Pes), diastolic arterial pressure (Pad), pre-ejection period (PEP), and eject time (ET). In order to apply this estimation to clinical monitoring, we used mean arterial pressure (Pm) as Pes and cardio ankle vascular index (CAVI) as arterial elastance (Ea). Healthy individuals (2,675 males, 2,287 females), who visited the health examination center at Fukui-ken Saiseikai Hosp, were recruited to measure Pm, Pad, PEP, ET and CAVI with vascular screening system (VaSera VS-1500, Fukuda Denshi, Tokyo). Mean and SD values were as follows: Ees/Ea = 1.22 ± 0.61, Ees = 1.44 ± 0.58 (mmHg/ml). There are suggested that the estimated values vary widely and the accuracy of the values beyond the normal range are low.

(COI: No)

S59-2

Current clinical application and problems of central blood pressure estimation based on pulse waveform analyses

Miyashita, Hiroshi[1]; Katsuda, Shin-ichiro[2] (*[1]Jichi Med Univ Health Care Center, Tochigi, Japan; [2]Dept Cellular and Integrative Physiol, Fukushima Med Univ Sch Med, Fukushima, Japan*)

Central blood pressure (CBP) is increasingly recognized as an important cardiovascular risk marker in addition to peripheral blood pressure (BP). Because CBP cannot be directly measured noninvasively, it should be estimated from peripheral arterial pulses. Among various methods to estimate CBP, we have focused on 2 principal methods; 1) generalized pressure transfer function (GTF), 2) radial 2nd systolic peak pressure (SP2). Both methods are based on aorto-radial pressure wave transmission properties, which can be modeled as a single or parallel elastic tube. It was supported by a wave separation analysis of precise animal data of pressure wave transmission along the forelimb arteries. A frequency component analysis of simultaneous human central and radial artery pressure waveforms has shown that central augmentation peaks consisted predominantly of lower harmonic components, which might make SP2 approximate CBP. We have found equivalence of appropriately modified SP2- and GTF-based CBP estimates in humans and rabbits.

A comparison between Japanese and standard (Westerner's) GTFs suggested limitations of GTF. Though the difference between central and peripheral BPs is a perfectly dynamic phenomenon, absolute CBP levels largely depend on mean (static) BP levels. CBP estimation unexpectedly elucidated large inaccuracy of brachial cuff BP measurement that has been the only method to calibrate noninvasive CBP. The calibration issue is the biggest problem to be solved.

(COI: No)

S59-3

Directional sensitivity of the arterial baroreflex to pressure input and its implication in baroreflex activation therapy

Kawada, Toru; Shimizu, Shuji; Sugimachi, Masaru (*Dept Cardiovasc Dynamics, Natl Cereb Cardiovasc Ctr, Osaka, Japan*)

The arterial baroreflex is an important negative feedback system that stabilizes arterial pressure (AP) during daily activities. Although pulsatility of baroreceptor input pressure is known to affect the baroreflex response, whether the directional change in the pulsatility affects the baroreflex response remains unknown. The neural arc of the arterial baroreflex from pressure input to efferent sympathetic nerve activity (SNA) can be modeled by a derivative filter followed by a nonlinear sigmoidal component. This model predicts that a forward triangular wave (FTW) input (a ramp increase followed by an abrupt drop) is more effective to suppress SNA compared with a backward triangular wave (BTW) input (a ramp decrease followed by an abrupt increase). This prediction was examined in anesthetized Wistar-Kyoto rats. Carotid sinus baroreceptor regions were isolated from the systemic circulation, and carotid sinus pressure was changed according to FTW or BTW with a peak-to-peak amplitude of 40 mmHg at the same mean pressure. The BTW input increased the mean level of SNA compared with the FTW input. Mean AP was higher during the BTW input than during the FTW input. These results indicate that we may be able to suppress SNA and reduce AP more effectively using the FTW input. Recent investigations indicate that electrical stimulation of the carotid sinus baroreflex can be a therapeutic approach to drug resistant hypertension. Modulating the pulse train of the carotid sinus stimulation using FTW would be more effective for the treatment of hypertension.

(COI: No)

S59-4

Elevation of pulmonary input impedance in low frequency can worsen right ventricle-pulmonary artery coupling

Fuke, Soichiro; Kashihara, Yuya; Namba, Yusuke; Tanaka, Masamichi; Yumoto, Akihisa; Saito, Hironori; Sato, Tetsuya (*Dept Cardiology, Japanese Red Cross Okayama Hospital, Okayama, Japan*)

Background: The relationship between pressure reflection and right ventricular (RV) function is not well known.
Methods: Nineteen patients suspected of having pulmonary hypertension (PH) were enrolled. Patients with a mean pulmonary artery pressure (PAP) of >20 mmHg were included in the PH group (n=9; mean PAP, 28 ± 7 mmHg), and others were included in the normal group (n=10; mean PAP, 13 ± 2 mmHg). The central aortic pressure was recorded in 10 patients. PAP and flow velocity by using ultrasound was simultaneously recorded. Additionally, RV pressure and flow velocity were recorded. Aortic flow velocity was estimated with the triangular wave. The characteristic impedance (Zc) was calculated in a time-domain manner. The maximal RV hydromotive pressure was estimated by using an extrapolated sin curve fitted to the isovolemic pressure, and Ees/Ea was then calculated using these values.
Results: |Z1|/Zc was smaller in the normal group than in the central aorta (1.19 ± 0.45 vs. 1.91 ± 0.68, p=0.012). |Z1|/Zc and |Z2|/Zc were larger in the PH group than in the normal group (2.24 ± 1.09 vs. 1.19 ± 0.45, p=0.012; 1.48 ± 0.72 vs. 0.89 ± 0.31, p=0.028, respectively). Augmentation index was elevated and Ees/Ea was decreased to a greater extent in the PH group than in the normal group (37.7 ± 23.8% vs. 8.8 ± 11.2%, p=0.003; 0.65 ± 0.42 vs. 1.84 ± 0.69, p<0.001, respectively).
Conclusion: In PH patients, elevation of pulmonary input impedance in low frequency and pressure reflection may cause RV-PA decoupling.

(COI: No)

S59-5

Intraoperative transit time flowmetry: How can physiology and anatomy predict clinical outcomes after CABG ?

Une, Dai[1,2]; Kikuchi, Keita[1]; Endo, Yoshiki[1]; Matsuyama, Takayoshi[1]; Fukada, Yasuhisa[1]; Kurata, Atsushi[1] (*[1]Div Cardiovasc Surg, Yamato Seiwa Hosp, Kanagawa, Japan; [2]Div Cardiac Surg, University of Ottawa Heart Institute, Ottawa, Canada*)

Background: Intraoperative transit time flowmetry is helpful in detecting technical errors such as bypass twist and stenosed anastomosis by measuring the volume of bypass flow and flow patterns. Also, it was reported that lower mean graft flow was associated with mid-term graft failure. We evaluated optimal cut-off values of graft flow for 1-year graft failure, and its risk factors.
Methods: Sixty-five saphenous vein grafts were examined with transit time flowmeter and fluorescent angiography during isolated coronary artery bypass grafting. Then, all the grafts were evaluated with angiography 1 year after operation. Receiver operating characteristics analysis was performed to decide the best cut-off value.
Results: The 1-year patency of vein grafts was 65% although all the grafts were patent in operative room. The best cut-off value of mean graft flow was 31 ml/hr, and this was a significant risk factor. However, its sensitivity and specificity to detect 1-year graft failure was approximately 65%. There was no other predictor.
Conclusions: The diagnostic ability of transit time flowmetry is not good enough although it was the only significant predictor of 1-year graft failure. When graft flow volume is same, larger grafts have less shear stress with slower blood flow, as a result, more hyperplasia. Probably, surgeon may be able to predict 1-year to mid-term graft failure more accurately by considering the size of vein graft.

(COI: No)

S59-6

Total unloading of left ventricle by circulatory assist device minimizes the infract size in ischemia-reperfusion injury

Saku, Keita; Kakino, Takamori; Sunagawa, Kenji (*Dept Cardiovasc Med, Kyushu Univ, FUK, JPN*)

Backgrounds: Although LVAD has been extensively used both in acute and chronic heart diseases, how LVAD impacts on cardiac energetics as well as mechanics remains poorly understood. The pressure-volume area (PVA) of the left ventricle (LV) has been shown to be tightly coupled with myocardial oxygen consumption (MVO2). Theoretical analysis indicates that, the partial LVAD support (PARTIAL) where LV remains ejecting, reduces LV preload while increases afterload, and thus does not decrease much the PVA. In contrast, the total LVAD support (TOTAL) where LV no longer ejects, markedly decreases PVA, thereby MVO2. We hypothesized PARTIAL and TOTAL unloading would have major differences in the infract size in ischemia-reperfusion injury.
Methods: In 12 anesthetized dogs, we created ischemia by occluding major branches of the left anterior descending coronary artery for 90 min, reperfused for the following 300 min. We compared the infract size (normalized by the risk area) among 3 groups, no support (CONT), PARTIAL (LV output=LVAD flow) and TOTAL (no LV output).
Results: Mean arterial pressure did not differ among 3 groups, while left atrial pressure significantly reduced in TOTAL (CONT: 11±5, PARTIAL: 8±4, and TOTAL: -1±3mmHg, $p < 0.01$). LVAD significantly reduced the infarct size (CONT: 40±3.2, PARTIAL: 27.6±5.8, and TOTAL: 5.0±1.6%, $p < 0.01$). The reduction of infarct size was by far lager in TOTAL (-88%) than in PARTIAL (-31%). **Conclusions:** Total unloading of LVAD minimizes the metabolic demand and maximize the beneficial impact on ischemia-reperfusion injury.

(COI: No)

Symposium 60

Integrated approaches to understand the pathophysiology of dystonia and involuntary movement

(March 23, 15:00～16:30, Room C)

S60-1

Dystonia -definition and multifaced phenotype-

Hasegawa, Kazuko (*Dept Neurolgy, National Hospital Organization, Sagamihara National Hospital*)

Dystonia is one of the common neurodegenerative disorders, however, clinical diagnosis is difficult. Dystonia is currently defined as a neurologic syndrome characterized by involuntary, sustained, patterned, and often repetitive muscle contractions of opposing muscles, causing twisting movements or abnormal postures. This definition may be difficult or cumbersome to apply for neurologists faced with patients in the clinic, causing underdiagnose of dystonia in clinical practice. Numerous causes evoke dystonia exactly, so that classification of dystonia is based on etiological role, such as genetic, primary, according to neurodegenerative disorders. I will try to present here many face of dystonia by video, and its classification.

(COI: No)

S60-2

Dystonia model mouse deficient of Na-pump alpha3 subunit gene

Kawakami, Kiyoshi[1]; Ikeda, Keiko[2]; Chiken, Satomi[3]; Sugimoto, Hiroki[1]; Nambu, Atsushi[3] ([1]*Division of Biology, Center for Molecular Medicine, Jichi Medical University, Tochigi, Japan;* [2]*Biology, Hyogo Medical College, Hyogo, Japan;* [3]*Division of System Neurophysiology, National Institute for Physiological Sciences*)

ATP1A3 is the causative gene for rapid-onset dystonia parkinsonism (RDP) and alternating hemiplegia of childhood (AHC). Many of the mutations found in patients are substitution mutations and most of the positions of mutations are distinct between RDP and AHC. The mutations are mostly loss of function mutations and lead to decreased activity of Na, K-ATPase. We established a *Atp1a3* gene deficient mice and performed behavioural and electrophysiological analyses related to symptoms of RDP and AHC. $Atp1a3^{+/-}$ showed increased symptoms of dystonia when being administered kainate into cerebellum (Ikeda et al J. Physiol. 2013). $Atp1a3^{+/-}$ showed shorter stride length in chronically-stressed condition. We next evaluated neuronal activity of basal ganglia under non-anesthesia condition. The spontaneous discharge rate of GPi neurons were significantly lower in $Atp1a3^{+/-}$ compared with wild-type. The triphasic response pattern evoked by cortical stimulation in GPi and GPe in the wild-type mice was altered in $Atp1a3^{+/-}$. The changes of the firing pattern are similar to those observed in DYT1 dystonia (Chiken et al . J. Neurosci 2008). The inhibitory neurotransmission from molecular-layer interneuron to Purkinje cells in the developing cerebellum was enhanced. The contents of dopamine and its metabolite were reduced in $Atp1a3^{+/-}$. These observations suggest the usefulness of $Atp1a3^{+/-}$ as a model of RDP.

(COI: No)

S60-3

Mouse model of dystonia with sensory neuropathy

Takebayashi, Hirohide (*Grad. Sch. Med. Dent. Sci., Niigata Univ., Niigata, Japan*)

Dystonia is a disorder characterized by involuntary muscle contractions that cause slow repetitive movements or abnormal postures. Dystonia musculorum (*dt*) mouse is an inherited dystonia model mouse with sensory neuropathy. *Dystonin* (*Dst*) is a causative gene for *dt* mice, which encodes for a cytoskeletal linker protein. We had generated a novel *Dst* gene trap (Dst^{Gt}) mice, in which actin-binding domain-containing isoforms are disrupted. Homozygous Dst^{Gt} mice showed typical *dt* phenotypes with progressive neurological symptoms: severe motor disorders in their limbs and twisted postures. Electomyogram showed abnormal co-contractions of agonist and antagonist muscle in the homozygotes. In histological analyses, abnormal neurofilament accumulation was observed in both peripheral nervous system and central nervous system. Since this Dst^{Gt} allele is a multipurpose conditional allele, we will perform conditional knockout and rescue experiments to investigate neuronal circuits responsible for dystonia phenotype.

(COI: No)

S60-4

Dystonia, basal ganglia and cerebellum

Nambu, Atsushi (*Div System Neurophysiol, Natl Inst Physiol Sci, Okazaki, Japan*)

Dystonia is a neurological disorder characterized by sustained or repetitive involuntary muscle contractions and abnormal postures. Reduced spontaneous activity with bursts and pauses has been reported in both internal (GPi) and external (GPe) segments of the globus pallidus in dystonia patients, especially in generalized dystonia. Similar activity changes were reported in dystonia animal models as well. In addition, deep brain stimulation, i.e., high-frequency stimulation in the GPi ameliorates dystonic symptoms. These observations suggest that the origin of dystonia is in the basal ganglia, and indicate the following pathophysiology: In normal state, GPi outputs suppress unnecessary movements, whereas in dystonia, reduced GPi outputs cause increased thalamic and cortical activity, resulting in involuntary movements. On the other hand, a number of dystonia animal models have been reported to be of cerebellar origin. For example, Wriggle Mouse Sagami, which exhibits coactivation of agonist and antagonist muscles and abnormal postures, showed decreased spontaneous activity of cerebellar Purkinje cells, with no significant activity changes in the basal ganglia. Another example is ouabain injection into the cerebellar nuclei, which was reported to induce dystonic symptoms through the cerebello-thalamo-striatal pathway. In this symposium, I would like to discuss the contribution of the basal ganglia and cerebellum to pathophysiology of dystonia.

(COI: No)

S60-5

Electrophysiological hallmarks for dystonia

Darbin, Olivier E (*Dept Neurology, Univ. South Alabama, Mobile, USA*)

Dystonia is a movement disorder with sustained and involuntary muscle contractions causing abnormal postures, twisting and repetitive movements. Dystonia can be a primary condition or secondary to brain trauma, infection, poisoning or unusual physical activity. Treatments include therapeutics for peripheral targets to reduce muscular contraction (anticholinergic drugs, butolin toxin or muscle relaxant) or central targets to 'normalize' the activity in motor circuitry (deep brain stimulation, baclofen). In absence of gross neuro-anatomical hallmarks, the physiopathology of dystonia remains poorly understood and based from the analyses of either electro-encephalograph, neuro-signals collected per -operative during procedure for deep brain implantation and, more marginally, from animal models.Pathological findings include abnormalities at the levels of the cortices, brainstem and basal ganglia in motor and sensory territories. Physiological abnormalities include topographic des-organization in the cortico-basal ganglia loops and impaired sensorimotor integration. At the level of the basal ganglia, and the globus pallidus specifically, there are a decreased firing activity, slower/weaker oscillations and increased occurrences in pauses and bursts. Data from primate suggests also that striatal gabaergic control may contribute to dystonic-like movement and/or be a therapeutic target.However, integrated interpretation of these data has not been reached. The causal relationship of these abnormalities to dystonic movements remain unestablished. The lack of 'gold standard' animal model is an obstacle to identify the underlying mechanisms of dystonia.

(COI: No)

Symposium 61

Morphological and functional mechanisms and their dynamics in the multimodality of inhibitory neural system

(March 23, 15:00～16:30, Room D)

S61-1

Local Impermeant Anions Establish the Neuronal Chloride Concentration

Egawa, Kiyoshi[1,2] ([1]*Dept Pediatrics, Hokkaido Univ. Hospital;* [2]*Mass. General Hospital, Boston, U.S.A.*)

Neuronal intracellular chloride concentration $[Cl^-]_i$ is an important determinant of g-aminobutyric acid type A ($GABA_A$) receptor mediated inhibition and cytoplasmic volume regulation. Equilibrative cation-chloride cotransporters (CCCs) move Cl^- across the membrane, but accumulating evidence suggests factors other than the bulk concentrations of transported ions determine $[Cl^-]_i$. To investigate regulatory mechanisms of $[Cl^-]_i$, we measured neuronal $[Cl^-]_i$ in murine brain slice preparations expressing the transgenic fluorophore Clomeleon. Main results are as following. 1) somatic $[Cl^-]_i$ are negatively correlated with SYTO staining intensity, which corresponds to cytoplasmic nucleic acid proteins. 2) $[Cl^-]_i$are also inversely correlated with the amount of negatively charged extracellular matrix evaluated by alcian blue staining. 3) $[Cl^-]_i$ increased when a part of $[Cl^-]_i$, was released by gluconate or pyruvate. 4) Chondroitinase ABC, an inhibitor of extracellular matrix, also increased $[Cl^-]_i$. CCC inhibition had modest effects on $[Cl^-]_i$ and neuronal volume, but substantial changes were produced by alterations of the balance between $[A^-]_i$ and $[A^-]_o$. Therefore, CCCs are important elements of Cl^- homeostasis, but local impermeant anions determine the homeostatic set point for $[Cl^-]_i$ and hence, neuronal volume and the polarity of local $GABA_A$ receptor signaling.

(COI: No)

S61-2

Functional regulation of neuronal K^+-Cl^- cotransporter KCC2

Watanabe, Miho[1]; Iwata, Satomi[1]; Furukawa, Tomonori[2]; Kumada, Tatsuro[3]; Uchida, Taku[4]; Hirose, Shinichi[4,5]; Fukuda, Atsuo[1] ([1]*Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan;* [2]*Dept Neurophysiol, Hirosaki Univ, Hirosaki, Japan;* [3]*Dept Occupational, Tokoha Univ, Hamamatsu, Japan;* [4]*Inst Mol Pathomechanisms Epilepsy, Fukuoka Univ, Fukuoka, Japan;* [5]*Dept Pediatrics, Fukuoka Univ, Fukuoka, Japan*)

The neuronal K^+-Cl^- cotransporter (KCC2) is a membrane transport protein that extrudes Cl^- from neurons and helps maintain low intracellular $[Cl^-]$ and hyperpolarizing GABAergic synaptic potentials. Several neurological disorders are associated with decreases in the Cl^- extrusion capacity of KCC2 that result in increase of $[Cl^-]_i$ and subsequent hyperexcitability of neuronal networks. Despite the importance for plasticity of inhibitory transmission, less is known about the regulation of the intrinsic KCC2 activity. KCC2 consists of 12 transmembrane domains (TMDs) and flanked by two intracellular termini. Recently several groups have identified in the KCC2 molecule different regions and amino acid residues that regulate the KCC2 transport activity or its cell surface stability. Here, we showed novel regulatory site of KCC2 activity. Mutation of phenylalanine residue (F571I) located inside 10th putative TMD inhibited ion-transport activity. In contrast, mutation of leucine residue (L907V) located in C-terminus resulted in up-regulation of transport activity. But mutation of proline residue (P384A) located large extracellular loop between TMDs 5th and 6th or arginine residue (R592Q) located inside 11th putative TMD did not affect transport activity. These results suggest that F571 and L907 are critically residues involved in KCC2 activity.

(COI: No)

S61-3

Taurine regulates the intrinsic properties of the neural progenitors as a ligand for GABAA receptors in the mouse developing neocortex

Tochitani, Shiro (*Res. Cent. Child Mental Development, Univ. Fukui, Fukui, Japan*)

Precise temporal regulation of the intrinsic properties of neural stem cells (NSCs) to produce the diverse type of neurons and glial cells underlies the formation of the complex structure of central nervous system. We obtained evidences that $GABA_A$ receptors participate in this regulatory machinery. Fetal exposure to positive allosteric modulators for $GABA_A$ receptors at E10-11 accelerated the transition from neuroepithelial cells to BLBP-positive radial glia, resulting in the changes in the timing of neurogenesis. Exposure to $GABA_A$ positive modulators at E10-E12 enhanced the differentiation into Satb2-positive upper-layer neurons and suppressed the differentiation into Tbr1-positive deep-layer neurons. Exposure to $GABA_A$ antagonists at the same embryonic stages caused the opposite effects in terms of these four properties of neural progenitors. Both GABA and taurine are known as endogenous ligands for $GABA_A$ receptors. Immunohistochemical analyses and HPLC quantification showed that taurine is dominant in quantity among the endogenous ligands for $GABA_A$ receptors in the developing cortex before E14. The E12 taurine transporter-knockout mouse cortices exhibited the phenotypes that well resembled those observed in the embryos exposed to $GABA_A$ antagonists. These results show that $GABA_A$ receptor activation principally by taurine plays a crucial role in the regulation of the properties of NPCs in the early phase of cortical development.

(COI: No)

S61-4

Characteristic development of GABAergic transmission in the mouse spinal cord

Kim, Jeongtae; Kosaka, Yoshinori; Takayama, Chitoshi (*Grad.Sch.Med., Ryukyu Univ., Okinawa, Japan*)

In the mammalian central nervous system, gamma-amino butyric acid (GABA) is a predominant inhibitory neurotransmitter, whereas it acts as an excitatory transmitter in the immature CNS, and may be involved in morphogenesis. We have investigated the ontogeny of the GABAergic transmission by immunohistochemistry for glutamic acid decarboxylase (GAD), GABA transporters (GATs), vesicular GABA transporter (VGAT), and potassium chloride cotransporter2 (KCC2) in the mouse cervical spinal cord. In this session, we present the developmental changes in GABAergic transmission. (1) Before synapse formation, GABA may be extrasynaptically released by non-exocytotic system, and be transported into the processes of radial glia or immature astrocytes by GAT-3. (2) In the ventral horn, GABAergic neurons appear on embryonic day 12 (E12), synapses are formed after E13, and increased in number after E15. Synaptically released GABA was removed by only GAT-3 on the processes of astrocytes. (3) In the dorsal horn, GABAergic neurons are localized after E13, synapses are formed after E17, and increased in number after birth. Synaptically released GABA is removed by GAT-1 into presynapse and GAT-3 into processes of astrocytes. (4) GABA may act as an excitatory transmitter for several days before GABAergic synapse were formed in the embryonic spinal cord. (5) During development, GABAergic synapses are decreased in the ventral horn, whereas glycinergic synapses are increased.

(COI: No)

Symposium 62

Functional roles of monoaminergic/cholinergic neurotransmitters in higher order behaviors

(March 23, 15:00～16:30, Room E)

S62-1

Functional roles of cortical cholinergic modulation in visual contrast detection behavior

Soma, Shogo (*Grad Sch Med, Osaka Univ, Osaka, Japan*)

The cholinergic neurons originating in the nucleus basalis of the basal forebrain (BF) innervate the entire cortical mantle, releasing acetylcholine (ACh) context-dependently to optimize various cognitive processes such as sensation, attention, learning and memory, and decision making. However, it remains unclear how the optimization of cortical processing is realized. One target of the cholinergic projections is the primary visual cortex (V1), and ACh regulates the V1 neuron's activities in many species. In this talk, I focus on the cholinergic modulation of visual information processing at the neuronal and behavior levels. Recently, we extracellularly recorded visual responses to drifting sinusoidal grating stimuli from V1 of anesthetized animals (monkey and rat) and tested the effects of ACh applied locally by microiontophoresis or widely by topical administration. ACh modulated the visual responses in V1 by controlling the response gain and improving signal-to-noise ratio. To examine whether such cholinergic regulations of visual information processing contributes to the visual performance in behaving animals, we trained rats to detect visual stimulus in a two-alternative forced-choice task combined with a staircase method. We found that contrast detectability was improved by the systemic injection of donepezil, a cholinesterase inhibitor, and impaired by the immunolesion of BF cholinergic neurons by 192 IgG-saporin. Therefore, ACh endogenously released in cognitive behavior controls the contrast detectability by modulating cortical visual information processing to meet the purposes of behavioral context.

(COI: No)

S62-2

Imaging and implications of dopaminergic neurons for movement disorder: "opposite sides of the same coin" in Parkinson's disease

Matsuda, Wakoto[1]; Furuta, Takahiro[2]; Nakamura, Kouich C[2]; Hioki, Hiroyuki[2]; Kaneko, Takeshi[2] (*[1]LIMS, Kyoto Univ, Kyoto, Japan; [2]Morphol Brain Sci, Grad Sch Med, Kyoto Univ, Kyoto, Japan*)

The recent developments in brain imaging have offered new insights into the morphology of dopaminergic (DA) neurons. In this symposium, we describe these new morphological measurement techniques and how they contribute to our understanding of movement disorder, especially of Parkinson's disease (PD). We present novel imaging techniques using Sindbis virus vectors that coded membrane-targeted green fluorescent protein (GFP) that reveal important new structural information concerning DA neurons. Detail morphological images of DA neurons derived from this new approach are used to elucidate the role of DA neurons in PD. First, we point out how the new images reveal how DA neurons have a massive axonal arborization in the striatum. This arborization is on a scale not previously known, and of a form that implies both a particular vulnerability and a redundancy in DA neurons. Second, we describe how the imaging results indicate that DA neurons innervate both the striosome and the matrix compartments of the striatum. This dual innervation has implications for reinforcement learning in the basal ganglia and for how normal behavior is driven and how it may be disrupted by Levodopa PD therapies. We conclude with a summary of how these results contribute to our understanding of PD.

(COI: No)

S62-3

Implications for the monoaminergic/cholinergic basis of impulsive behavior

Yoshioka, Mitsuhiro; Kimura, Iku Tsutui; Ohmura, Yu (*Dept Neuropharmacol, Grad Sch Med, Hokkaido Univ, Sapporo, Japan*)

Impulsive actions are often viewed as everyday normal behavior; however, excessive levels of impulsivity are associated with several psychiatric disorders, such as attention-deficit/hyperactivity disorder, schizophrenia, and borderline personality disorder. Moreover, it could be a risk factor for drug addiction, criminal involvement, and suicide. We focused on the three-choice serial reaction time task, which is one of the most appropriate and simple rodent model of impulsive-like action and is based on the human continuous performance test. We examined implications for the monoaminergic/cholinergic basis of impulsive behavior using pharmacological intervention such as administration of not only monoamine-related but also nicotinic acetylcholine receptor (nAChR)-related drugs. We have elucidated the mechanism of action in some of these drugs. For example, we demonstrated that a milnacipran, a serotonin/noradrenaline reuptake inhibitor, enhanced the control of impulsive action by activating dopamine D1-like receptors in the infralimbic cortex (IL), and that intra-IL infusion of a selective $\alpha 4\beta 2$ nAChR antagonist dose-dependently blocked nicotine-induced impulsive-like action.In this symposium, we introduce recent advances in this field and describe the role of not only nAChR-related but also monoamine-related brain mechanisms in modulating impulsive behavior. We also suggest several potential therapeutic drugs to address these mechanisms in impulsivity-related disorders and explore future directions to develop anti-impulsive drugs.

(COI: No)

S62-4

Serotonergic involvements of sociality and mental disorder: Molecular imaging study by PET in non-human primates

Onoe, Hirotaka (*Imaging Function Group, Cent Life Sci Tech, RIKEN, Kobe, Hyogo, Japan*)

Serotonin is involved in regulating emotional and social behaviors, and also formation of social behavioral traits in humans and other primates. It also appears to be one of the major players in mood and mental disorders such as the major depression. But exactly how remains an open question. The availability of positron emission tomography (PET) for human and nonhuman primates has enabled examination of the in vivo functions of specific neurotransmitter systems underlying social behavior. We established a PET imaging system for conscious macaque monkeys and also common marmosets, a small primate species noted for its high social tolerance and cooperative sociality. We used this method to examine the dopaminergic and serotonergic systems in the brain using [^{11}C]DASB and [^{11}C]AZ10419369 , which are highly selective to serotonin transporter (SERT) and serotonin 1B (5-HT1B) receptor, respectively. Using parametric images of binding potential (BP) values and behavioral scores determined by social test in marmosets, we processed on the statistical mapping to identify brain areas of which BP values of SERT which are tightly associated with social behavior. We also investigate recently that pharmacological anti-depressive action of ketamine on 5-HT1B receptor. Results demonstrate that molecular imaging of the brain can provide valuable information for understanding the neural bases of personality and anti-depressive action in nonhuman primates. All procedures of this study were approved by the Animal Care and Use Committee of Kobe Institute in Riken

(COI: No)

Symposium 63

Frontiers in sleep research

(March 23, 15:00～16:30, Room F)

S63-1

Spacio-temoporal cellular circuit profiling for the organism-level systems biology

Susaki, Etsuo A[1,2] ([1]*Dept of Systems Pharm., Grad. Sch. Med, Univ Tokyo, Tokyo, Japan;* [2]*Lab. for Synthetic Biology, RIKEN QBiC, Kobe, Japan*)

Circuit-level identification and analysis of neural networks in the brain will require the development of whole-brain imaging with single-cell resolution. To this end, we performed comprehensive chemical screening to develop a whole-brain clearing and imaging method, termed CUBIC (Clear, Unobstructed Brain Imaging Cocktails and Computational analysis). CUBIC is a simple and efficient method involving the immersion of brain samples in a chemical mixture which enables rapid whole-brain imaging with single-photon excitation microscopy. CUBIC is applicable to multi-color imaging of fluorescent proteins or immunostained samples in adult brains, and is scalable from a primate brain to subcellular structures. We also developed a whole-brain cell-nuclear counterstaining protocol and a computational image analysis pipeline which, together with CUBIC reagents, enable the visualization and quantification of neural activities induced by environmental stimulation. CUBIC enables time-course expression profiling of whole adult brains with single-cell resolution. We are also developing high-throughput, next generation-type mouse genetics by using "ES-mouse" technology. The combinations of CUBIC and ES-mouse genetics will provide basics for realizing the organism-level systems biology.

(COI: No)

S63-2

The role of PPARs and ketone body metabolism in the regulation of sleep homeostasis

Chikahisa, Sachiko; Sei, Hiroyoshi (*Dept Integ Physiol, Tokushima Univ, Tokushima, Japan*)

Sleep regulations are associated with energy metabolism. Sleep restriction leads to metabolic disorders such as obesity and diabetes, but the mechanisms that underlie its effects remain unclear. We have recently found that peroxisome proliferator-activated receptors (PPARs) are involved in the regulation of sleep homeostasis. PPARs are transcriptional factors belonging to the nuclear receptor family which relate to the regulation of glucose and lipid metabolism. Chronic treatment of bezafibrate, a PPARs agonist, advanced wake/sleep pattern, and enhanced the slow-wave activity (SWA) in non-rapid eye movement (NREM) sleep in mice. Plasma concentration of ketone bodies acetoacetate (AcAc) and β-hydroxybutyrate (BHB) were also affected by bezafibrate. Bezafibrate-treated mice showed a marked increase in plasma AcAc and decrease in BHB. Ketogenesis is modulated by the activity of PPAR α, one of the three PPARs isotypes, under the condition of low glucose availability. To investigate the specific effects of ketone bodies on sleep homeostasis, plasma concentration of ketone bodies were measured after sleep deprivation for 6 hours. Sleep deprivation increased plasma ketone bodies and increased mRNA expression of PPAR α and mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 (Hmgcs2), a rate-limiting enzymes of ketogenesis, in the hypothalamus and cortex. In addition, central injection of AcAc, but not BHB, increased SWA during NREM sleep and suppressed glutamate release. Our results suggest that central PPAR α and metabolism of ketone bodies (especially AcAc) play a role in the regulation of sleep homeostasis.

(COI: No)

S63-3

Novel classification and function of rat thalamic neurons on the basis of the single-cell labeling studies

Kuramoto, Eriko (*Dept. Oral Anat. and Cell Biol., Grad. Sch. Medical and Dental Sci., Kagoshima Univ., Kagoshima, Japan*)

The thalamus not only acts as a relay between subcortical areas and the cerebral cortex, but also appear to play an important role in regulating arousal and attention. Recently it has been proposed that thalamic neurons are divided into core and matrix neurons; core neurons are found principally in sensory thalamic nuclei, and project to middle layers of the cortex in an area-specific manner; matrix neurons project to superficial layers of cortex over wide areas (Jones 1998). In the present studies, we analyzed axonal arborization of the rat thalamic neurons project to the frontal cortex by a single neuron labeling method with a Sindbis viral vector, and tried to apply the core-matrix classification to our results. The mediodorsal (MD) and caudal part of the ventral anterior-ventral lateral nuclei (VA-VLc) neurons mainly projected to middle layers like core neurons, however, the cortical fields covered by axon fibers of single neurons are not restricted in a cortical area. Thus, there might be at least two subtypes in the core neurons, focal- and diffuse-type core neurons. In contrast, ventromedial (VM) and rostral VA-VL (VA-VLr) neurons sent their axon fibers predominantly to superficial layers of widespread cortical areas, indicating that these neurons are classified into the matrix type. The result suggests that, even when a small number of the VM or VA-VLr neurons are activated, many pyramidal neurons in widespread cortical areas would be activated through the apical dendrites, and may thus be associated with arousal or attentional mechanism.

(COI: No)

S63-4

Optical dissection of the sleeping cortex

Kanda, Takeshi[1]; Tsujino, Natsuko[1]; Ishii, Ryo[1]; Yanagisawa, Masahi[1,2] ([1]*International Institute for Integrative Sleep Medicine, Univ Tsukuba, Ibaraki, Japan;* [2]*University of Texas Southwestern Medical Center, TX, USA*)

Sleep is the neurophysiological process of the brain, regulated by the brain, and required for the brain. Despite of its importance, the physiological nature of sleep has not been well explored. In mammals, cortex is highly susceptible to sleep. In fact, electroencephalography (EEG)-based criteria is generally admitted to determine the states of sleep and wakefulness. During NREM sleep, EEG is dominated by slow-wave activity. The slow-wave activity in the EEG and the underlying variations in local field potentials (LFP) occur regionally on a macro scale. However EEG, LFP and unit recordings cannot estimate the precise location of recording areas and cells on a microscopic scale. To explore the spatio-temporal pattern of individual neuron activity in the cortex during sleep and wakefulness, using two-photon microscopy, we performed Ca^{2+} imaging in the layer 2/3 of primary motor cortex of naturally sleeping and awake mice. When mice were awake and running, Ca^{2+} dynamics was highly active and the temporal patterns were not coordinated between neurons. The transition to synchronized state of Ca^{2+} activity was observed when mice were falling asleep. The highest synchronization appeared for typical NREM sleep. The synchronicity of spontaneous Ca^{2+} signals among neurons did not depend on the distance between neurons on micrometer scale. Our results, taken together with other studies, suggest that cortical neuron activity synchronize temporally during sleep and that the synchronization is regional but not completely local.

(COI: No)

S63-5

Forward genetic approach toward the elucidation of sleep/wakefulness regulation

Funato, Hiromasa[1,2] ([1]*Univ Tsukuba WPI-IIIS, Ibaraki, Japan;* [2]*Toho Univ, Med, Sch, Tokyo, Japan*)

Although sleep is a ubiquitous animal behavior, the molecular mechanism of sleep homeostasis remains unknown. We performed high-throughput screening of ENU-mutagenized mice in order to identify genes regulating sleep/wake behavior. We have so far analyzed EEG/EMG data of more than 6,000 mutagenized male mice. We established several pedigrees showing heritable sleep/wakefulness abnormalities. Among them, the Sleepy mutant pedigree shows 30% reduction in 24-h wake time. To map a chromosomal region responsible for the sleep phenotype of Sleepy mutant mice, we performed a linkage analysis in N2 mice, obtained by backcrossing the mutagenized founder C57BL/6J male to C57BL/6N female mice for two generations. The analysis revealed a single peak with a LOD score of more than 20. Whole exome sequencing of mutants and wild-type littermates from the Sleepy pedigree identified a nucleotide change specific to Sleepy mutant mice within the mapped chromosomal region. The single nucleotide substitution abrogates a splice donor site of the gene that we termed Sleepy. RT-PCR analysis of the brain and liver mRNA found a short variant of Sleepy mRNA specific to Sleepy mutant mice. Functional analyses of the Sleepy gene are now underway.

(COI: No)

S63-6

Neural mechanisms for inducing fluctuations of autonomic nervous system during REM sleep

Koyama, Yoshimasa; Nishimura, Kunihiro; Haruyama, Naoto; Aoyagi, Takafumi
(*Dept Sci and Technol Fukushima Univ. Fukushima Japan*)

REM sleep is characterized by EEG desynchronization and muscular atonia. In addition to these tonic events, several phasic events such as rapid eye movement (REM) or fluctuation of autonomic nervous system occur during REM sleep. Blood pressure also displays large fluctuation during REM sleep. However, the mechanisms controlling it remains unknown. In the mesopontine tegmental area including laterodorsal/pedunculotegmental nuclei (LDT/PPT), some neurons become contentiously active prior to and during REM sleep and are considered to generate REM sleep, while considerable number of neurons showed phasic firing during REM sleep. About 44 % of neurons recorded in and around the LDT had a correlation with blood pressure fluctuation during REM sleep. Emotional changes during waking cause changes in autonomic nervous system including blood pressure. The amygdala is considered to be a center of emotion during waking. Since it is active during REM sleep, amygdala are hypothesized to be involved in blood pressure fluctuation during REM sleep. More than half of the amygdala neurons became active during REM sleep, but increase in firing occurred after the onset of REM sleep and the firing are mostly phasic. In about 39% of the amygdala neurons, their firing had a correlation with blood pressure during REM sleep. These results indicate that brainstem REM generation area and the amygdala are deeply involved in blood pressure fluctuation during REM sleep. Functional relations between two areas are discussed

(COI: No)

Symposium 64

Anatomical and physiological perspective of brain environment

(March 23, 15:00～16:30, Room H)

S64-1

Novel subtypes of astrocytes regulate neuronal excitability via release of gliotransmitters

Miwa, Hideki; Shibasaki, Koji (*Gunma Univ. Grad. Sch.of Med., Gunma, Japan*)

Astrocytes play active roles in the regulation of synaptic transmission. Neuronal excitation can evoke Ca^{2+} transients in astrocytes, and these Ca^{2+} transients can modulate neuronal excitability. While only a subset of astrocytes appears to communicate with neurons, the types of astrocytes that can regulate neuronal excitability are poorly characterized. We found that 20% of astrocytes in the brain express transient receptor potential vanilloid 4 (TRPV4), indicating that astrocytic subtypes can be classified on the basis of their expression patterns. When $TRPV4^+$ astrocytes are activated by ligands such as arachidonic acid, the activation propagates to neighboring astrocytes through gap junctions and by ATP release from the $TRPV4^+$ astrocytes. Following activation, both $TRPV4^+$ and $TRPV4^-$ astrocytes release glutamate, which acts as an excitatory gliotransmitter to increase synaptic transmission through group 1 mGluR. Our results indicate that $TRPV4^+$ astrocytes constitute a novel subtype of the population and are solely responsible for initiating excitatory gliotransmitter release to enhance synaptic transmission. In addition to $TRPV4^+$ subtype astrocytes, we identified that another unique subtype of astrocytes, which can release an inhibitory gliotransmitter. For the first time, we revealed that astrocytes transduce pre-synaptic signals to other post-synaptic signals. Our finding indicates that astrocytes are an important member of neural network. Especially, we identify the neuron-astrocyte-neuron, a sort of the triangle cell-cell communications.

(COI: No)

S64-2

Pathophysiological roles of TRPM2 expressed in the monocytic lineage cells

Kaneko, Shuji[1]; Shirakawa, Hisashi[1]; Nakagawa, Takayuki[2] ([1]*Dept Mol Pharmacol, Grad Sch Pharm Sci, Kyoto Univ, Kyoto, Japan;* [2]*Dept Pharm, Kyoto Univ Hosp, Kyoto, Japan*)

Several lines of evidence suggest that neuroinflammation mediated by the interaction between immune cells and neurons plays an important role in the pathogenesis of neuropathic pain. Transient receptor potential melastatin 2 (TRPM2) is a nonselective Ca^{2+}-permeable cation channel that acts as a sensor for reactive oxygen species. Using TRPM2-knockout mice, we examined the roles of TRPM2 expressed on immune and glial cells in neuropathic pain. TRPM2 deficiency attenuated pain behaviors in various kinds of inflammatory and neuropathic pain, but not in nociceptive pain models. In peripheral nerve injury-induced neuropathic pain models, TRPM2 deficiency diminished infiltration of neutrophils mediated through CXCL2 production from macrophages around the injured peripheral nerve and activation of spinal microglia. Also, in an in-vitro study with mouse primary microglia, lipopolysaccharide in the presence of interferon-γ activated TRPM2-mediated Ca^{2+} signaling and increased the downstream p38 MAPK and JNK signaling that resulted in elevated NO and CXCL2 production. Analysis of wildtype x TRPM2-knockout mixed-bone marrow chimeric mice revealed that TRPM2 plays an important role in the infiltration of peripheral immune cells, particularly macrophages, into the spinal cord, rather than into the injured nerves. The spinal infiltration of macrophages mediated by TRPM2 may contribute to the pathogenesis of neuropathic pain. We also discuss about the role of microglial TRPM2 in the aggravation of ischemic brain damage.

(COI: No)

S64-3

The roles of autophagy and lysosomal proteolysis for the maintenance of the normal environment in neuronal axons: lessons from the comparative analyses of cathepsin D- and Atg7-deficient Purkinje cells

Koike, Masato[1]; Uchiyama, Yasuo[1,2] ([1]*Juntendo Univ. Grad Sch. Med., Tokyo, Japan;* [2]*Juntendo Univ. Grad Sch. Med., Tokyo, Japan*)

We previously generated conditional cathepsin D (CD) or Atg7-deficient mice specifically in Purkinje cells (PCs). In both lines of mice, PCs underwent degeneration. Interestingly, CD-deficient PCs largely disappeared until 2 months of age, whereas Atg7-deficient PCs still survived at that time. Immunohistochemical observations exhibited that axonal spheroids and swellings in presynaptic terminals were more pronounced in Atg7-deficient PCs than in CD-deficient PCs. Electron microscopy also demonstrated that abnormal tubular vacuoles, nascent autophagosome-like structures, and membrane-bound and electron-dense granules accumulated within axons and presynaptic terminals of PCs in both lines of mice. Immnohistochmical analyses revealed that such spheroids and/or swellings in axon terminals of CD-deficient PCs are positive for both LC3 and Atg9A, a membrane protein essential for autophagy, while those of Atg7-deficient PCs are only positive for Atg9A. Moreover, immunoelectron microscopy using ultrathin cryosections confirmed that such tubular vacuoles are positive for Atg9A and inositol triphosphate receptor (IP3R), indicating that these abnormal tubular vacuoles are derived from the smooth endoplasmic reticulum (sER). From these results we propose the possibility that the origin of the autophagosomal isolation membrane in axons is derived from sER.

(COI: No)

S64-4

Microglial environment and fate of injured neurons

Konishi, Hiroyuki[1,2]; Kobayashi, Masaaki[1]; Kiyama, Hiroshi[1,2] ([1]*Nagoya Univ. Grad. Sch. of Med, Nagoya, Japan;* [2]*CREST, JST, Japan*)

Microglia continuously survey the microenvironment using their motile processes. They quickly respond to neuronal injuries and become activated. Activated microglia are assumed to have neurotoxic or neuroprotective effects; however, the signal determining how activated microglia affect the fate of neuronal cells remains largely unknown. We recently identified several molecules as crucial regulators of microglial neurotoxicity after neuronal injuries. In this symposium, we will present a transmembrane protein DNAX-activating protein of 12 kDa (DAP12) among those molecules. DAP12 functions as an adaptor protein by forming complexes with specific membrane receptor proteins such as triggering receptors expressed on myeloid cells 2 (TREM2), and transduces signals into the cytoplasm. Using a mouse hypoglossal axotomy model, we revealed that DAP12 was specifically expressed in activated microglia by the injury. The duration of microglial activation after nerve injury was decreased in DAP12-deficient mice, although cell morphology and total cell numbers were not affected at the activation peak. Furthermore, expression of M1-phenotype markers including pro-inflammatory cytokines was suppressed in DAP12-deficient microglia both in vitro and in vivo. Consequently, axotomy-induced motor neuron death was markedly prevented in DAP12-deficient mice. These results suggest that DAP12-mediated microglial activation following axotomy promotes pro-inflammatory responses, and thereby exacerbates neurotoxicity. Collectively microglia could be a potent regulator, which determines a fate of injured motor neurons.

(COI: No)

S64-5

A big channel in small glia as a promising molecular target for the treatment of opioid-induced hyperalgesia

Hayashi, Yoshinori; Nakanishi, Hiroshi (*Dept of Aging Sci and Pharmacol, Facl of Dent Sci, Kyushu Univ, Fukuoka, Japan*)

BK channels are the intracellular Ca^{2+} and voltage gated potassium channel. They are widely distributed throughout the nervous system to control neuronal excitability and neurotransmitter release. They are also expressed in electrically non-excitable cells such as cancer cells and immune cells, whereas little is known about their functions. Recently, we have found that large outward currents mediated by BK channels in the spinal microglia contribute to the initiation of neuropathic pain. In the present study, we have examined a possible involvement of microglial BK channels in opioid-induced hyperalgesia, because some evidence suggests the involvement of microglia in this event. Repeated morphine administration gradually enhanced pain sensitivity. At the same time, repeated morphine administration activated BK channels in microglia, but not in neuron, by generation of arachidonic acid and its metabolites through μ receptors. Morphine-induced hyperalgesia was significantly suppressed by BK channel inhibitor. The development of hyperalgesia was accelerated by intrathecal administration of morphine-primed wild-type, but not BK channel-deficient, microglia. Furthermore, the activation of BK channels promoted $P2X_4$ receptor trafficking to the cell surface of microglia. These results indicate that BK channels in the spinal microglia also play an important role in the development of opioid-induced hyperalgesia. Therefore, the BK channel is a potential molecular target for the treatment of both neuropathic pain and opioid-induced hyperalgesia.

(COI: No)

Symposium 65

Recent insight into molecules involved in food intake, stress and emotion

(March 23, 15:00～16:30, Room I)

S65-1

Alteration in RNA editing of serotonin 2C receptors is involved in alcohol drinking in mice

Tanaka, Masaki; Watanabe, Yoshihisa (*Kyoto Pref. Univ. Med., Kyoto, Japan*)

Serotonin 2C receptor (5-HT2CR) is a G-protein coupled receptor known to have various actions such as involvements in food intake, emotional behavior and drug addiction. We have recently demonstrated that 5-HT2CR is involved in the increased alcohol intake after chronic alcohol exposure in C57BL/6J strain. 5-HT2CR is also known to undergo mRNA editing that converts genomically encoded adenosine residues to inosines by adenosine deaminases acting on RNA (ADARs). We will present our data in the conference that alcohol preference in mice depends on the degree of 5-HT2CR mRNA editing in the nucleus accumbens (ACC), a crucial region for reward and addiction. We have recently demonstrated that 5-HT2CR in the ACC is involved in the increased alcohol intake after chronic alcohol exposure in C57BL/6J strain. After chronic alcohol vapor exposure for 20 days, C57BL/6J mice grew to take more alcohol voluntarily but C3H/HeJ and DBA/2J mice did not show significant changes. The frequency of 5-HT2CR RNA editing in the ACC of alcohol exposed mice was significantly increased in the C57BL/6J strain accompanied by the increase in the expression of 5-HT2CR mRNA, ADAR1 and ADAR2 but that was not observed in the C3H/HeJ nor DBA/2J strains. Then, we examined the mutant mice that express exclusively unedited type (INI) of 5-HT2CR mRNA in C57BL/6J strain and found that they did not exhibit the increase of alcohol intake compared with wild type after chronic alcohol exposure. Collectively, these results indicate that the alteration in 5-HT2CR mRNA editing in the ACC underlies the alcohol preference in mice.

(COI: No)

S65-2

Roles of the medial amygdala in the control of neuroendocrine responses to conditioned fear stimuli

Onaka, Tatsushi; Yoshida, Masahide; Takayanagi, Yuki (*Div Brain and Neurophysiol, Dept Physiol, Jichi Med Univ, Tochigi, Japan*)

Fear responses play important roles in maintaining homeostasis in response to threatening events. Conditioned fear stimuli induce freezing behaviour and neuroendocrine responses such as release of ACTH and oxytocin. During fear conditioning, information of a conditioned stimulus and an aversive stimulus converges in the basolateral amygdala. As a result, an initially neutral conditioned stimulus acquires aversive properties. The basolateral amygdala sends signals to the central amygdala, from which projections control expression of freezing behaviour. Selective lesions of the central amygdala have been shown to block expression of freezing behaviour while the lesions after conditioning have been reported not to impair release of corticosterone or prolactin. Thus, output nucleus of the amygdala remain to be determined concerning the control of neuroendocrine fear responses. Here, we examined excitotoxic lesions of the medial amygdala in the control of neuroendocrine responses to conditioned fear stimuli. We found that lesions of the medial amygdala impaired activation of medullary prolactin-releasing peptide (PrRP)-synthesizing neurons and blocked neuroendocrine conditioned fear responses. PrRP-deficient mice showed impaired neuroendocrine responses to conditioned fear stimuli. All these data suggest that medial amygdala-PrRP neuron pathway mediates, at least, in part neuroendocrine responses to conditioned fear stimuli.

(COI: No)

S65-3

Central circuit mechanism that drives stress-induced autonomic responses

Nakamura, Kazuhiro[1,2]; Kataoka, Naoya[1] ([1]*Career-path Promotion Unit for young Life Scientists, Kyoto Univ, Kyoto, Japan;* [2]*PRESTO, JST, Japan*)

Psychological stress induces increases in body temperature, heart rate and blood pressure. Although these stress-induced autonomic responses are commonly observed in many mammals, their central circuit mechanisms have been unknown. Recently, we have identified a hypothalamomedullary neural pathway that mediates stress signaling to drive sympathetic thermogenesis in brown adipose tissue (BAT), hyperthermia and tachycardia. This pathway involves direct glutamatergic transmission from the dorsomedial hypothalamus (DMH) to sympathetic premotor neurons in the rostral medullary raphe (rMR). Blockade of this neurotransmission with drug nanoinjections diminished BAT thermogenic, hyperthermic and tachycardic responses to social defeat stress, a sociopsychological stress model, in rats. Optogenetic stimulation of the neurotransmission from the DMH to the rMR elicited increases in BAT thermogenesis, heart rate and blood pressure, mimicking stress responses. Interestingly, our histochemical analysis revealed stress-induced activation of two populations of DMH neurons: ones projecting to the rMR and the others projecting to the paraventricular hypothalamic nucleus (PVH), a neuroendocrine center. These results indicate that the DMH functions as a hub for stress signaling, with monosynaptic projections to the rMR for the sympathetic stress responses and to the PVH for stress hormone release.

(COI: No)

S65-4

Structure-function insight on the melanin-concentrating hormone receptor 1

Saito, Yumiko (*Gras.Sch.Int.Arts & Sci, Higashi-Hiroshima, Japan*)

Melanin-concentrating hormone (MCH) is a cyclic neuropeptide that was originally discovered as a hypothalamic hormone which causes paling of teleost fish skin. It was later found that mammalian MCH is abundantly present in lateral hypothalamus as known to be the center of feeding behavior. MCH binds to and activates two G protein-coupled receptors (GPCR), MCHR1 and MCHR2 in goldfish, flounder, zebrafish, Xenopus tropicalis, and human. In rodents, MCHR1 is the sole receptor expressed and promiscuously couples to Gaq and Gai/o proteins. Activation of these various downstream pathways may contribute to the diverse array of physiological processes regulated by MCH. The recent progress using genetic and pharmacological approaches has confirmed that the MCH-MCHR1 system is involved in energy homeostasis and possibly emotional processing, and is an exiting new target for the treatment of obesity and certain psychological disorders. Therefore, the complex molecular and structural changes that provide receptor activation and signaling are the focus of intense research. In this symposium, I review the current knowledge regarding: i) the common and distinguishing structural features of MCHR1 for receptor activation and G protein selectivity and ii) the fine-tuning mechanism of MCHR1 activity by receptor-interacting molecules and the physiological significance.

(COI: No)

S65-5

Novel food-derived bioactive peptides acting on the nervous system

Ohinata, Kousaku (*Grad.Sch.Agri.Kyoto Univ., Kyoto, Japan*)

It is known that endogenous bioactive peptides are produced from precursor proteins after cleavage by specific proteases. Food proteins were not recognized as such precursor proteins; however, so far, a number of bioactive peptides released from food proteins have been found. These peptides sometimes act on the nervous system to modulate emotional behaviour or food intake even after oral administration.

We previously reported that dipeptide YL (0.3 mg/kg, p.o.) exhibited potent anxiolytic-like activity, comparable to diazepam. Recently, it was revealed that YL exhibits antidepressant-like activity, and increases neurogenesis in the hippocampus. Based on structure-activity relationship of YL-related peptides, we found several food-derived peptides with anxiolytic- and antidepressant-like activities. These YL-related peptides may activate serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ system.

It is known that a decline in food intake is observed with aging, which is termed "anorexia of aging". In aged mice, ghrelin resistance was observed; however, rubiscolin-6 (1.0 mg/kg), a δ opioid agonist hexapeptide YPLDLF derived from a major green leaf protein Rubisco, stimulated food intake oral administration. Rubiscolin-6 may stimulate food intake activating the prostaglandin D_2-NPY pathway, independently of the ghrelin system.

During studies on mechanisms of food-derived peptides, novel pathways of the nervous system were also identified, suggesting that these peptides are unique research tools for studying neural pathways.

(COI: No)

S66-2

Angiotensin converting enzyme 2 (ACE2) links Apelin and angiotensin systems in controlling heart function

Kuba, Keiji (*Dept Biochem Metabolic Sci, Akita Univ Grad Sch Med, Akita, Japan*)

Angiotensin converting enzyme 2 (ACE2) is a negative regulator of the renin-angiotensin system (RAS), to catalyze conversion of Angiotensin II to Angiotensin 1-7. Apelin is a second catalytic substrate for ACE2 and functions as an inotropic and cardioprotective peptide. While an antagonistic effect between the RAS and the Apelin systems has been proposed, the functional interplay between Apelin and ACE2 remains elusive. Here we show that ACE2 is significantly down-regulated in Apelin deficient mice. Metabolomic profiling of angiotensin peptides showed similar down-regulation of Angiotensin 1-7 in Apelin mutant and ACE2 knockout mice. Pharmacological or genetic inhibition of Angiotensin II type 1 receptor (AT1R) rescues the impaired contractility and hypertrophy of Apelin mutant mice, accompanied with restored ACE2 levels. Importantly, treatment with Angiotensin 1-7 rescues hypertrophy and heart dysfunction in Apelin knockout mice. Moreover, Apelin treatment up-regulates ACE2 expression in failing hearts, and Apelin, via activation of its receptor APJ, increases ACE2 promoter activity in vitro. Apelin treatment also increases cardiac contractility and ACE2 levels in AT1R knockout mice. These data demonstrate that ACE2 couples the RAS to the Apelin system, adding a novel conceptual framework of the Apelin-ACE2-Angiotensin 1-7 axis as therapeutics for cardiovascular diseases.

(COI: No)

Symposium 66

Update of Research on Cardiovascular Regulation by Angiotensin

(March 23, 16:30～18:00, Room A)

S66-1

Cross Talk between the Hypoxia Response System and Angiotensin II Receptor

Ichiki, Toshihiro (*Dep Cardiol, Harasanshin HP, Fukuoka, Japan*)

Background: Prolyl hydroxylase domain-containing protein (PHD) induces proteasomal degradation of hypoxia-inducible factor (HIF)-α, a transcription factor. Inhibition of PHD stabilizes HIF-α expression and increases the expression of target genes such as vascular endothelial growth factor. We examined the effect of PHD inhibition on the expression of angiotensin (Ang) II type 1 receptor (AT1R) and cardiovascular remodeling.

Results: Hypoxia (1% O_2), cobalt chloride ($CoCl_2$), a hypoxia mimetics that inhibits PHD, and knockdown of PHD2, a major isoform of PHDs, by siRNA reduced AT1R expression in vascular smooth muscle cells. Oral administration of $CoCl_2$ to mice treated with Ang II significantly reduced aortic AT1R expression and perivascular fibrosis of coronary artery.Mice with myeloid-specific deletion of PHD2 (MyPHD2KO) were generated, resulting in the accumulation of HIF-1α and 2α in macrophages. Cardiac interstitial fibrosis, macrophage infiltration and myocyte hypertrophy induced by L-NAME, a nitric oxide synthase inhibitor, and Ang II treatment were significantly ameliorated in MyPHD2KO mice. Left ventricular hypertrophy and dysfunction induced by L-NAME/Ang II treatment in control mice were not observed in MyPHD2KO mice.

Conclusions: PHD inhibition downregulates AT1R expression and attenuates profibrotic effect of Ang II. Phd2 deletion in myeloid lineage attenuates cardiac hypertrophy and fibrosis, which may be mediated by decreased inflammation- and fibrosis-associated gene expression in macrophage. PHD inhibition may be a novel strategy for ameliorating cardiovascular remodeling through AT1R suppression.

(COI: No)

S66-3

Regulation of L-type Ca^{2+} channels by angiotensin II type 1 receptor/β-arrestin-2 biased signaling through casein kinase 2 in immature cardiomyocytes

Kashihara, Toshihide; Nakada, Tsutomu; Guo, Xiaoguang; Yamada, Mitsuhiko (*Dept Mol Pharmacol, Shinshu Univ Sch Med, Matsumoto, Japan*)

Angiotensin II (AII) plays important roles in cardiovascular functions. In this study, we examined the effect of AII on L-type Ca^{2+} channels (LTCC), which play a pivotal role in cardiac excitation-contraction coupling. 2-hour treatment of AII (3μM) doubled LTCC activity in mouse neonatal ventricular myocytes (NVMC) and immotile mouse atrial cell line HL-1, but not in isolated adult ventricular myocytes (AVMC). An AT_1 receptor blocker, candesartan (10μM), but not an AT_2 receptor blocker, PD123319 (3μM), abolished the effect of AII in NVMC and HL-1. In HL-1, knockdown of β-arrestin-2 but not β-arrestin-1, G_q or G_{11} significantly inhibited the effect of AII. PKC inhibitor, Go6983 (0.5μM), also did not affect the effect of AII. It is reported that in cardiomyocytes, AII promotes the proteosomal breakdown of cyclin-dependent kinase inhibitor 1B (p27), thereby activating casein kinase 2α' (CK2α') associated with this protein. Indeed, knockdown of p27 caused AII-independent activation of LTCC whereas knockdown of CK2α' resulted in significant inhibition of the effect of AII on LTCC in HL-1. The expression of CK2α' was 7 and 4.5 times higher in NVMC and HL-1, than in AVMC, respectively. Furthermore, the inhibition of Src family tyrosine kinases, which promote p27 degradation, with bostinib (2μM) significantly inhibited the effect of AII on LTCC in HL-1. These results indicate that AT_1 receptor/β-arrestin-2/Src/p27/CK2α' strongly activates LTCC in immature but not adult cardiomyocytes.

(COI: No)

S66-4

Angiotensin II receptor blocker (ARB)-sensitive and insensitive remodeling in hearts of inherited DCM mice

Kurebayashi, Nagomi[1]; Odagiri, Fuminori[1,2]; Inoue, Hana[3]; Sugihara, Masami[1,2]; Suzuki, Takeshi[1,2]; Murayama, Takashi[1]; Shioya, Takao[4]; Konishi, Masato[3]; Morimoto, Sachio[5] (*[1]Dept Pharmacol, Juntendo Univ Sch Med, Tokyo, Japan; [2]Dept Cardiol, Juntendo Univ; [3]Dept Physiol, Tokyo Med Univ; [4]Dept Physiol, Fac Med, Saga Univ; [5]Dept Clin Pharmacol, Fac Med Sci, Kyushu Univ*)

Inherited dilated cardiomyopathy (DCM) is a progressive disease often results in sudden death (SD) or heart failure (HF). Although angiotensin receptor blockers (ARBs) have been used for the treatment of HF, the effects of ARB on postulated electrical remodeling in inherited DCM are not well known. We examined effects of candesartan (CAND), one of the ARBs, on structural and electrical remodeling in hearts of inherited DCM mice (TNNT2 ΔK210). DCM mice were treated with CAND from 1 month of age. Non-treated DCM mice showed cardiac enlargement with prolongation of QRS and QT intervals, and died at $t_{1/2}$ of 70 days. CAND greatly suppressed cardiac dilatation, prolongation of QRS and QT interval and SD with lethal arrhythmia, and dramatically extended lifespan of DCM mice. Expression analysis revealed that down-regulation of Kv4.2 (I_{to}), and Kv1.5 (IK_{ur}) in DCM was partially reversed by CAND. Interestingly, non-treated DCM heart had both normal-sized myocytes with moderately reduced I_{to} and IK_{ur} and enlarged cells with greatly reduced K^+ currents (I_{to}, IK_{ur} IK_1 and IK_{SS}). CAND treatment completely abrogated the emergence of the enlarged cells but did not reverse the I_{to}, and IK_{ur} in normal-sized cells. ARB-sensitive and insensitive remodeling were found in DCM hearts. CAND treatment suppresses severe electrical remodeling related to structural remodeling in inherited DCM.

(COI: No)

Symposium 67

Central functions of oxytocin: Basic and clinical neuroscience

(March 23, 16:30～18:00, Room C)

S67-1

Oxytocin as a therapeutic target for depression and other mental disorders

Matsui, Hideki; Matsushita, Hiroaki (*Dept Physiol, Grad Sch Med, Okayama Univ Med Sch, Okayama, Japan*)

Oxytocin (OT) acts as a neurotransmitter/neuromodulator to regulate a diverse range of central nervous system (CNS) functions, including emotional and social behavior. Clinical reports suggest OT to be a promising drug for psychiatric diseases such as depression, anxiety disorders and autism. A recent study found that sexual activity with a female induced the release of OT in the CNS of male rats. Moreover, a drug for the treatment of human with sexual dysfunction, sildenafil, induces enhancement of OT release from the CNS of mammals. Sildenafil is a selective inhibitor of PDE5 enzyme. In this study, we examined the effect of mating behavior on depression-related behavior in wild-type (WT) and OT receptor-deficient (OTR KO) male mice. The WT mice showed a reduction in depression-related behavior after mating behavior, but the OTR KO mice did not. Moreover, application of sildenafil reduced depression-related behavior in male mice. The antidepressant-like effect was absent in OTR KO mice. The activation of a MAP kinase cascade and subsequent enhanced phosphorylation of CREB in the hippocampus have been proposed as common mediators of antidepressant efficacy. Sildenafil increased the phosphorylation of CREB in the hippocampus. These results suggest mating behavior and sildenafil have an antidepressant effect through activation of OT signaling pathway.

(COI: No)

S67-2

Oxytocin projections regulate the spinal gastrin-releasing peptide system that controls male sexual function

Sakamoto, Hirotaka (*Grad.Sch.Nat.Sci. & Tech.Okayama Univ., Okayama, Japan*)

We previously demonstrated that the gastrin-releasing peptide (GRP) system in the spinal cord influences spinal centers promoting penile reflexes in rats. The paraventricular nucleus (PVN) of the hypothalamus contains the somata of oxytocin (OT) neurons that project to the posterior pituitary, from which OT is released into blood vessels. Additionally, a group of OT neurons in the PVN also projects to the spinal cord. Therefore, the hypothesis has been proposed that OT, which is transported by long descending paraventriculospinal pathways, activates proerectile spinal centers. However, the direct linkage of the neural circuit between the hypothalamic PVN and penile innervation remains uncharacterized. Hence, the purpose of this study is to reveal the function of OT in the brain-spinal cord neural network controlling male sexual function. First, we found that the axonal distribution of OT in the lumbar spinal cord exhibits a male-dominant sexual dimorphism in rats. Furthermore, OT binding and expression of the specific OT receptor were observed in the somata of spinal GRP neurons. Consequently, we studied the expression of phosphorylated ERK (pERK) in the GRP neurons after ejaculation. This revealed that pERK induction in the GRP neurons appeared to be specifically associated with ejaculation, suggesting that secreted oxytocin in the lumbar spinal cord activates the GRP neurons during male sexual behavior. Taken together, these results suggest that the hypothalamic OT projections mediate the GRP system in the lumbar spinal cord that controls male sexual function.

(COI: No)

S67-3

Roles of oxytocin in the control of emotional and social behaviors

Takayanagi, Yuki; Yoshida, Masahide; Onaka, Tatsushi (*Div Brain and Neurophysiol, Dept Physiol, Jichi Med Univ, Tochigi, Japan*)

Various stressful stimuli, including conditioned fear stimuli, electric foot shocks, or restraint stress, have been shown to activate oxytocin neurons and facilitate oxytocin release. We have demonstrated that noradrenergic neurons in the medulla oblongata enhance oxytocin release in response to some stressful stimuli. An administration of oxytocin reduces anxiety-related behavior and attenuates the HPA axis. These data suggest that oxytocin released in response to stressful stimuli might exert anti-stress actions. On the other hand, social stimuli also have been shown to activate oxytocin neurons although the neural pathways for activation of oxytocin neurons in response to social stimuli are largely unknown. Oxytocin release is facilitated by administration of secretin, which is implicated in the control of social behavior. We investigated whether secretin regulates social behavior via the oxytocin system. An intracerebroventricular injection of secretin activated supraoptic oxytocin neurons. Application of secretin facilitated oxytocin release dendritically in an *in vitro* preparation of the supraoptic nuclei. Furthermore, local application of secretin into the supraoptic nucleus facilitated social recognition and its action was blocked by an oxytocin receptor antagonist injected into the medial amygdala. These results suggest that secretin activates supraoptic oxytocin neurons, potentiates dendritic oxytocin release, and facilitates social recognition via the oxytocin/oxytocin receptor system.

(COI: No)

S67-4

Clinical study to develop oxytocin as a candidate for therapeutics of core symptoms in autism spectrum disorders

Yamasue, Hidenori (*Dept. Neuropsychiatry, Grad. Sch. of Med., Tokyo Univ., Tokyo, Japan*)

Autism spectrum disorders, which prevail as high as 1 in 100 individuals, currently have no established pharmacological treatment. Previous preliminary studies have suggested therapeutic effects of oxytocin on the disorders. The speaker's research team further explored neural evidence for the therapeutic effects of the neuropeptide by examining oxytocin's effects on socio-communicational deficits, which constitute the core symptoms of autism spectrum disorders. In our double-blind, placebo-controlled, crossover trial involving 40 high-functioning adults with autism spectrum disorders, intranasal administration of oxytocin behaviorally mitigates autistic deficits in understanding social communication contents, such as irony and humor, whose verbal and nonverbal information is conflicting, by recovering originally-diminished brain activities, enhancing functional connectivity and affecting neurochemical aspects of neuronal markers in the area. Based on the findings from the trial of single dose administration, we further conducted a trial of long-term treatment of intranasal oxytocin to further establish clinical application of it as a therapeutic for core symptoms of autism spectrum disorders. In the symposium, further discussion on such possibility would be expected.

(COI: No)

S67-5

Oxytocin signal molecules: Physiology and pathophysiology

Higashida, Haruhiro (*Research Center for Child Mental Development, Kanazawa University, Kanazawa, Japan*)

I demonstrated that CD38, a transmembrane protein with ADP-ribosyl cyclase activity, plays a critical role in mouse social behavior by regulating the release of oxytocin (OXT), which is essential for mutual recognition. When CD38 was disrupted, social amnesia (Jin et al., Nature, 2007) and less paternal nurturing behavior (Akther et al., Mol. Brain, 2014) were observed in CD38 knockout mice. CD38 knockout sires failed to retrieve their pups when they were reunited after cohabituated separation in a new cage for 10 min. CD38 knockout sires treated with a single subcutaneous injection of OXT partially rescued the retrieval events when co-housed with CD38 knockout sires treated with OXT. Next, we examined the effect of local expression of human CD38 in the nucleus accumbens (NAcc) in males via lentiviral infection. Pairs of knockout dams treated with OXT and sires expressing CD38 in the NAcc displayed more retrieval. A complete recovery was obtained both by sires with the expression of CD38 in the NAcc and with OXT administration. After identifying the families of the retrievers or non-retrievers, c-Fos expression in neuronal subsets in the mPOA, ventral tegmental area, NAcc and ventral palladium was much higher in the retriever sires when they isolated together with their mates in new cages. Finally, I discuss that single nucleotide polymorphysims in CD38 may be possible risk factors for autism spectrum disorder by abrogating OXT function and that some ASD subjects can be treated with OXT in preliminary clinical trials.

(COI: No)

Symposium 68

Diversity of serotonergic system in the brain - from development to aggression, reward and decision-making -

(March 23, 16:30〜18:00, Room E)

S68-1

Serotonin as a trophic factor for the development of the behavior

Shiga, Takashi (*Fac. Med., Univ. Tsukuba, Tsukuba, Japan*)

Serotonin (5-hydroxytryptamine, 5-HT) is a neuromodulator in the adult brain. 5-HT and its receptors appear early in the developing brain, and 5-HT acts as a neurotrophic factor. In addition, the abnormality of 5-HT system leads to psychiatric disorders, such as anxiety disorder and depression, and learning disability. We have reported that the treatment of fluoxetine, a selective 5-HT reuptake inhibitor, during the early postnatal period decreased both the anxiety-like behavior in the elevated plus maze and the depression-like behavior in the forced swim test in adult BALB/c mice. The same treatment also improved spatial learning in Morris water maze. These results suggest that 5-HT system during the development affect various behaviors after maturation. Interestingly, the fluoxetine treatment of C57 BL/6 mice did not change the stress response and the learning ability. These strain differences may be due to the higher amount of the brain 5-HT in C57BL/6 mice as compared with BALB/c mice. Among the 5-HT receptors, 5-HT1A receptor may be responsible for the regulation of anxiety, because the treatment of the BALB/c mice with 5-HT1A receptor agonist decreased the anxiety-like behavior. The role of 5-HT1A receptor is also supported by the experiments using the 5-HT1A receptor-KO mice. Because 5-HT1A receptor agonist treatment increased the depression-like behavior, this receptor may regulate negatively the depression. These results suggest that different 5-HT receptors may be involved in the anxiety and depression.

(COI: No)

S68-2

Serotonin and aggressive behavior: from laboratory animal to human

Ueda, Shuichi; Kai, Nobuyuki; Yamaguchi, Tsuyoshi; Ehara, Ayuka; Tachibana, Atsumichi (*Dokkyo.Med.Univ., Tochigi, Japan*)

Aggressive behavior is an instinctive and essential behavior in many mammalian species, and can be classified into two categories: predatory aggression and affective aggression by Moyer (1968). Predatory aggression is similar to human premeditated violence, which represents a planned behavior with low autonomic response, whereas affective aggression is similar to impulsive aggression, which is reactive and associated with high autonomic response. Serotonin (5-HT) is an important neurotransmitter and/or neuromodulator associated with a wide range of behaviors. In particular, a negative correlation between brain 5-HT activity and aggressive behavior has been studied. We previously reported that fetal 5-HT neurons transplanted into the rat hypothalamus restored inhibition of predatory aggression that have been induced by lesion with 5, 7-dihydroxytryptamine into dorsal and medial raphe nuclei. Reinnervation of 5-HT fibers in the lateral hypothalamus (LH) from the grafted neurons resulted simultaneously with a significant reduction of c-Fos expression in the LH neurons. These results indicate the possibility that 5-HT neurons regulate predatory aggression through the inhibition of the activity of the LH neurons. On the other hand, affective aggression is seemed to regulate by two monoamines, 5-HT and dopamine (DA), with opposing roles. Hyperactivity of DA system is associated with increased affective aggression, while 5-HT system inhibits affective aggression.

(COI: No)

S68-3

Neuronal activity of dorsal raphe nucleus during reward schedule

Shidara, Munetaka[1,2] ([1]*Systems Neurosci, Facul Med, Univ Tsukuba, Tsukuba, Japan;* [2]*Kansei Behav Brain Sci, Grad Sch Compreh Human Sci, Univ Tsukuba, Tsukuba, Japan*)

Dorsal raphe nucleus is a major source of serotonin neurons which are related to emotion, appetite, stress, aggressive behavior, mental disorder, and so on. It is only recently that physiologists have begun to investigate the dorsal raphe's possible role in reward processing. Here, we examined whether dorsal raphe neurons showed differential activities between the conditions when the monkey could or could not predict reward availability and amount. We recorded from 98 single neurons in dorsal raphe of two monkeys during a multi-trial reward schedule task. In the task, the monkeys were required to perform a visual discrimination trial 1, 2 or 3 times (schedule) for obtaining liquid reward of 1, 2 or 3 drops. In the cued condition, the length and brightness of the cue indicated schedule progress and reward amount, respectively. In the random condition, the cue was randomly presented with respect to schedule length and reward amount, so that the monkeys could not predict the reward schedule and amount. We found the neurons encoding the information about schedule onset, reward expectation, reward outcome, and reward amount in the mean firing rates. Furthermore, information theoretic analysis showed that the temporal pattern of neuronal responses contained additional information about the schedule progress. These results suggest that the dorsal raphe neurons have important roles on reward information processing and, considering the diverse anatomical connection, possibly providing signals throughout the brain to coordinate persistent goal-seeking behavior.

(COI: No)

S68-4

Serotonin and patience

Miyazaki, Katsuhiko; Miyazaki, Kayoko W; Doya, Kenji (*Neural Computation Unit, OIST, Okinawa, Japan*)

Recent recording and pharmacological inhibition studies of serotonin neurons in the dorsal raphe nucleus (DRN) have shown that these neurons play roles in promoting actions for future rewards. Here we developed mice that express channelrhodopsin-2 in the serotonin neurons and showed that the selective activation of the serotonin neurons in the DRN enhanced the mice's patience in waiting for both the conditioned reinforcer tone at a tone site and the food reward at a food site. Optogenetic activation of DRN serotonin neurons while the mice waited for the tone by keep nosepoking at the tone site significantly reduced the number of tone wait errors. When serotonin neurons were activated during the variable delay periods when the mice waited for the food by keep nosepoking at the food site (3, 6, or 9 sec or infinity, i.e., omission), the reward wait errors were significantly reduced in the 9 sec waiting trials. In the reward omission trials, the waiting time of the mice was significantly longer (17.5 sec; mean) in the serotonin activation trials compared with the trials with no activation (12.0 sec). This effect was observed specifically when the animal was engaged in deciding whether to keep waiting and not due to motor inhibition. Control experiments showed that the prolonged waiting times observed with optogenetic stimulation were not due to behavioral inhibition or the reinforcing effects of serotonergic activation (Miyazaki et al., Curr Biol, 2014). These results indicate that the temporally precise activation of the serotonin neurons during waiting facilitates patience for delayed rewards.

(COI: No)

S68-5

Appetitive and aversive information coding in the primate dorsal raphé nucleus

Nakamura, Kae; Hayashi, Kazuko; Nakao, Kazuko; Noritake, Atsushi (*Dept. Physiol. Kansai Medial University Osaka Japan*)

There have been conflicting hypotheses about whether the central serotonergic system is involved in appetitive or aversive information processing. To reveal whether and how such opposing information processing can be achieved by single neurons in the dorsal raphé nucleus (DRN), the major source of serotonin in the forebrain, we measured the activity of these neurons while monkeys were conditioned in a Pavlovian procedure with two distinct contexts: an appetitive context where a reward was available and an aversive one where an airpuff was delivered. We found that single DRN neurons were involved in distinct aspects of appetitive and aversive information processing. First, more than half of the recorded DRN neurons discriminated appetitive and aversive contexts by tonic changes in activity. In the appetitive context, they then kept track of expected reward value indicated by the conditioned stimuli. Some of them also encoded an error between the obtained and expected values. In the aversive context, the same neurons maintained tonic modulation in their activity throughout the block. However, modulation of their responses to trial events depending on airpuff probability was not common. Taken together, these results indicate that single DRN neurons encode both appetitive and aversive information, but over differing time scales, relatively shorter for appetitive and longer for aversive. Such dynamic ranges of information processing performed by single DRN neurons may contribute to the integral role of the serotonergic system in decision making in different emotional contexts.

(COI: No)

Symposium 69

New trends for research on the regulatory mechanism of neuronal development

(March 23, 16:30～18:00, Room G)

S69-1

Roles of volume-regulated anion channels during neuronal migration in the developing brain

Akita, Tenpei; Furukawa, Tomonori; Fukuda, Atsuo (*Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan*)

Neuronal migration is the process regulating coordinated changes in neuronal morphology. Such morphological changes inevitably include the changes in neuronal cell volume both locally and globally, but how the cell volume during neuronal migration is regulated is not elucidated yet. Cell volume regulation is attained by regulating the net influx and efflux of solutes and water across the plasma membrane. As a pathway for anion flux during the volume regulation, the volume-sensitive outwardly rectifying (VSOR) anion channel is known to play a major role in almost all types of vertebrate cell. But its role in the developing brain remains to be investigated. We recently confirmed that VSOR anion channels indeed work in the neurons in the developing brain. The VSOR anion channel is permeable not only to Cl^- ions, but also to amino acids like glutamate, aspartate and taurine. Taurine is abundant in the developing brain and we recently found that taurine acts as a major endogenous agonist of $GABA_A$ receptors in the developing brain to control the speed of radial migration of the neurons, especially in the subplate region of the developing neocortex. We further confirmed that VSOR anion channels provide the pathway for taurine release to regulate the radial migration. Thus the VSOR anion channel plays dual roles; one is in cell volume regulation and another is in intercellular communication, to regulate neuronal migration in the developing brain.

(COI: No)

S69-2

Control of cerebellar granule cell migration by intrinsic programs through modulating Ca^{2+} and cyclic nucleotide signaling

Kumada, Tatsuro (*Dept Occup Ther, Tokoha Univ, Hamamatsu, Japan*)

In the developing brain, immature neurons migrate from their birthplace to their final destination. On the way to their destination, neurons changes the speed and direction of cell movement in a cell type and cortical layer-specific manner. External guidance cues allow migrating neurons to restrain and guide in local milieu, but little is known about the role of intrinsic signals in controlling neuronal migration. To elucidate this issue, we have investigated relationship between behavior of migrating neurons and second messenger signaling (specifically Ca^{2+} and cyclic nucleotide signaling) in cerebellar granule cell migration. Previously, we demonstrated that Ca^{2+} spike frequency can control the cortical layer specific alterations of granule cell migration and loss of Ca^{2+} spikes is required to complete neuronal migration. Interestingly, our results suggest that intrinsic programs can determine the timing of Ca^{2+} spike loss. Thus, we also examined the role of intrinsic signals in neuronal cell turning. Time-lapse imaging of individual granule cell movement in microexplant culture revealed that these cells periodically turned without cell-cell contact and in the absence of potential guidance cues. These observation revealed four distinct modes of cell turning. Remarkably, the occurrence of each mode of turning was differentially controlled by the orchestrated activity of multifarious signaling pathways through Ca^{2+} and cyclic nucleotide signaling. These results suggest that intrinsic programs provide prerequisite signals for neuronal migration.

(COI: No)

S69-3

Transcription factor Npas4 regulates the sensory experience-dependent development of dendritic spines in newborn olfactory bulb interneurons

Yoshihara, Sei-ichi[1]; Takahashi, Hiroo[1]; Nishimura, Nobushiro[1]; Kinoshita, Masahito[1]; Asahina, Ryo[1]; Hibi, Yoko[2]; Nagai, Taku[2]; Yamada, Kiyofumi[2]; Tsuboi, Akio[1]
([1]*Lab for Mol Biol of Neural System, Nara Med Univ, Kashihara, Japan;* [2]*Dep of Neuropsychopharm and Hosp Pharm, Nagoya Univ, Nagoya, Japan*)

Sensory experience regulates development in various brain structures, including the cortex and olfactory bulb (OB). However, little is known about the developmental role of sensory experience in the OB GABA-releasing inhibitory interneurons, such as granule cells (GCs). In this study, by *in situ* hybridization (ISH) screenings, we newly identified a transcription factor, *Npas4* gene, which is expressed in a subset of OB GCs following sensory experience. Then, we performed the gain- and loss-of-function experiments for *Npas4* in OB GCs, based on lentiviral injection. *Npas4* overexpression in newborn OB GCs increased the spine density even under sensory deprivation. Conversely, both *Npas4* knockdown and knockout resulted in a significant reduction in the spine density of OB GCs. In addition, by ChIP-seq plus ISH screenings, we identified, as a novel target of Npas4, an E3 ubiquitin ligase *Mdm2* gene, which is expressed at low levels in the wild-type OB but at higher levels in the *Npas4*-knockout OB. Proteomics analysis further revealed that Mdm2 ubiquitinates and degrades Dcx to reduce the dendritic spine density of OB GCs. Taken together, our findings suggest that *Npas4* regulates *Mdm2* expression to ubiquitinate and degrade Dcx for shaping the dendritic spines of OB GCs after sensory experience (Yoshihara *et al*, **Cell Reports**, 8, 843-857, 2014).

(COI: No)

S69-4

Roles of axon guidance molecule FLRT2 in development of vascular system

Yamagishi, Satoru[1]; Kubota, Yoshiaki[2]; Sato, Kohji[1] ([1]*Hamamatsu Univ. Sch. Med., Shizuoka, Japan;* [2]*Sch., Med., Keio Univ., Tokyo, Japan*)

Axon tracts and blood vessels course throughout the body in an elaborated and orderly pattern, often alongside one another. The mechanisms involved in wiring neuronal and vascular networks share the axon guidance molecules such as netrin/unc5, slit/robo, semaphorin/neuropilin, and ephrin/Eph. Recently we reported that the new axon guidance molecule FLRT2 repel upper layer neurons in order to suppress the radial migration during brain development. FLRT2 is cleaved at juxta-membrane region and bind to Unc5B/D receptors. Here we show that FLRT2 is involved in the formation of vascular system. FLRT2 mutants appear partial embryonic lethality with hemorrhage from mid- to late-gestation stage. Interestingly, at E9.5 the expression of adhesion molecule PECAM in endothelial cells in FLRT2 mutants is down-regulated. Furthermore, FLRT2 mutants also showed partial hypervascular phenotype during eye development. Finally, Unc5B-positive endothelial cells are repelled from FLRT2 in a stripe assay. These results suggest that FLRT2 plays important roles in vascular development not only as a repulsive guidance molecule but also as a regulator of the vascular integrity.

(COI: No)

S69-5

FLRT3 is a Robo1-Interacting Protein that Determines Netrin-1 Attraction in Developing Axons

Egea, Joaquim (*Univ. Lleida, IRBLLEIDA, Lleida, Spain*)

Guidance molecules are normally presented to cells in an overlapping fashion; however, little is known about how their signals are integrated to control the formation of neural circuits. In the thalamocortical system, the topographical sorting of distinct axonal subpopulations relies on the emergent cooperation between Slit1 and Netrin-1 guidance cues presented by intermediate cellular targets. However, the mechanism by which both cues interact to drive distinct axonal responses remains unknown.Here, we show that the attractive response to the guidance cue Netrin-1 is controlled by Slit/Robo1 signaling and by FLRT3, a novel coreceptor for Robo1. While thalamic axons lacking FLRT3 are insensitive to Netrin-1, thalamic axons containing FLRT3 can modulate their Netrin-1 responsiveness in a context-dependent manner. In the presence of Slit1, both Robo1 and FLRT3 receptors are required to induce Netrin-1 attraction by the upregulation of surface DCC through the activation of protein kinase A. Finally, the absence of FLRT3 produces defects in axon guidance in vivo.These results highlight a novel mechanism by which interactions between limited numbers of axon guidance cues can multiply the responses in developing axons, as required for proper axonal tract formation in the mammalian brain.

(COI: No)

Symposium 70

Activity-dependent regulation of myelinated nerve function and morphology

(March 23, 16:30～18:00, Room H)

S70-1

Molecular mechanisms of myelinated nerve formation and injury

Susuki, Keiichiro (*Boonshoft Sch. Med., Wright State Univ., Dayton, USA*)

The nervous system function in vertebrates depends on the interaction between neurons and glial cells forming myelin, a multi-lamellar structure surrounding axons. The action potentials are initiated at the axon initial segment, a highly specialized neuronal compartment in the proximal axon. The action potentials are then regenerated at the nodes of Ranvier, short gaps between two adjacent myelin segments, and propagate rapidly along the axon. Both axon initial segments and the nodes are characterized by highly accumulated molecular complex including voltage-gated ion channels. During development, the axon initial segments are intrinsically determined and assembled by the neurons through the restriction of the molecules by the distal axonal cytoskeleton. Nodes of Ranvier are formed by multiple mechanisms: interactions with extracellular matrix, paranodal diffusion barrier, and stabilization by the axonal cytoskeleton. Since axon initial segments and nodes of Ranvier are critical for the proper nervous system functions, the dysfunction and/or disruption of these domains lead to neurological symptoms. Recent evidences demonstrate that the altered expression or localization of molecules at axon initial segments and nodes are key contributors to the pathophysiology of various neurological and psychiatric disorders. Better understanding of mechanisms underlying myelinated nerve formation and injury will provide important clues to establish novel therapeutic approaches for currently intractable nervous system diseases.

(COI: No)

S70-2

Activity dependent myelination and impaired motor learning as the result of its disruption

Wake, Hiroaki (*NIPS, Okazaki, Japan*)

Myelin, a multilayered membrane insulation wrapped around the axons, increases axonal conduction velocity at least by 50 fold. Myelination around the axon is thought to be crucial for information processing by changing the timing of neural firing patterns during development and learning. Additionally, Stimulating myelination as a result of impulse activity in axons could enable myelin to be regulated by environmental experience, which could contribute to information processing in the brain. We have demonstrated that local translation of MBP mRNA in oligodendrocyte processes is initialized myelin formation at the site of connection between oligodendrocytes and axons depending on neural activity. These findings provide new insight into how myelination, and thus conduction velocity and function of neural circuits, can be regulated by nervous system activity. Then to consider how activity dependent myelination can be involved in information processing, we used myelin proteolipid protein 1 (PLP1) over expression mouse (PLP-tg). To understand the neural basis of the cognitive impairment caused by the reduction of the neural conduction velocity, we used two-month-old PLP-tg mice which have a slight reduction of conduction velocity and combined in vivo two photon microscopy with a motor learning task. GFP-based Calcium Calmodulin probe (G-CaMP) was induced by an adeno-associated virus (AAV) injection in layer 2/3 of the M1 cortex to enable detecting a difference in the firing pattern of neuronal activity with a lever pulling motor learning task.

(COI: No)

S70-3

Functional plasticity of white matter in the hippocampus

Yamazaki, Yoshihiko (*Dept Physiol, Yamagata Univ Sch Med, Yamagata, Japan*)

Structural plastic changes of white matter in the adult brain are received considerable attention in relation to normal cognitive function and learning. Oligodendrocytes and myelin can respond to neuronal activity with depolarization of membrane potential. We previously reported that repetitive depolarization of the oligodendrocyte increased the conduction velocity of axons it myelinated in rat hippocampus. These results indicate that white matter shows functional plasticity as well as structural plasticity and that the depolarization of oligodendrocytes is involved in the generation of functional plasticity. To investigate the functional plastic changes in the white matter, we used a mouse with channelrhodopsin-2 expression restricted to oligodendrocyte and examined the effects of oligodendrocyte depolarization on axonal conduction of action potentials. Using extracellular recordings of compound action potentials at the alveus of the hippocampus, we found that light-evoked depolarization of oligodendrocytes induced early- and late-onset facilitation of axonal conduction that was dependent on the magnitude of oligodendrocyte depolarization; the former lasted for approximately 10 min, whereas the latter continued for up to 3 h. Using whole-cell recordings from CA1 pyramidal cells and recordings of antidromic action potentials, we found that the early-onset short-lasting component included the decrease of conduction latency of action potentials. These modulatory effects of oligodendrocytes would promote synchrony among the axons, and may influence the information processing in the white matter.

(COI: No)

S70-4

Mitochondrial behavior in myelinated axons modulated by axonal electrical activity

Ohno, Nobuhiko[1,2] ([1]*Univ. Yamanashi, Yamanashi, Japan;* [2]*Nat. Inst. Physiol. Sci., Aichi, Japan*)

Myelination facilitates rapid propagation of axonal conduction by confining Na+-channel currents at nodes of Ranvier, and conserves ATP consumption needed to exchange axoplasmic Na+ for extracellular K+. To meet the energy demands of saltatory nerve conduction, myelination may alter transport and localization of axonal mitochondria, which is a major source of axonal ATP. The majority of axonal mitochondria are located at stationary foci which are distributed along the entire length of the axon, while a population of relatively small axonal mitochondria are translocated in both anterograde and retrograde directions. The transport and docking of axonal mitochondria are dynamic processes which can be modulated by axonal metabolic demands. In myelinated axons, stationary mitochondria are abundant in juxtaparanodal and internodal axoplasm where Na+/K+-ATPase as well as mitochondrial energy substrates are enriched. Upon increased axonal firing, motile mitochondria preferentially stopped near the nodes, and the stationary mitochondria can be increased in nodal axoplasm. The nodal stopping of motile mitochondria is mediated by increase of axoplasmic Ca2+, and perturbed when compact myelin formation is congenitally impaired. These results support the concept that myelination modulates mitochondrial behavior at the nodes to meet the metabolic demand of saltatory nerve conduction.

(COI: No)

S70-5

Spike initiation and conduction in auditory time-coding pathway

Kuba, Hiroshi[1,2] ([1]*Dept Cell Physiol, Grad Sch Med, Nagoya Univ, Nagoya, Japan;* [2]*JST PRESTO*)

Sound localization requires detecting a difference in sound arrival times between the two ears (ITD, interaural time difference). In birds, this calculation is made in a brainstem circuit that is composed of arrays of binaural coincidence detectors innervated with delay lines, which enables ITDs to be encoded as a place of active neurons within the circuit. Precise and reliable delivery of spikes to the coincidence detectors is critical for accurate ITD detection, and neurons in nucleus magnocellularis (NM) play the role. To accomplish their tasks, NM neurons are highly specialized in arrangements of the axon initial segment (AIS) and node of Ranvier, which are axonal compartments, accumulated with high density of voltage-gated Na channels and involved in initiation and conduction of action potentials, respectively. For example, length of the AIS is effectively coupled with synaptic inputs, which ensures precise and reliable initiation of spikes in individual neurons. On the other hand, inter-nodal length is known to differ along the axon in a region-specific manner; that is, the length is long outside the delay lines, while it becomes shorter at the delay lines, contributing to a fine regulation of conduction velocity. In this symposium, I will summarize our findings on these differentiations, and discuss how they are influenced by neuronal activity.

(COI: No)

Symposium 71

Regulation of appetite and energy metabolism by brain

(March 23, 16:30～18:00, Room I)

S71-1

Effect of GALP on lipid metabolism in the liver

Hirako, Satoshi[1]; Takenoya, Fumiko[2]; Kageyama, Haruaki[3]; Wada, Nobuhiro[1]; Shioda, Seiji[1] (*[1]Showa Univ. Sch. of Med., Tokyo, Japan; [2]Hoshi Univ. Sch. of Pharm. and Pharm. Scie., Tokyo, Japan.; [3]Faculty of Health Care, Kiryu Univ., Gunma, Japan.*)

Galanin-like peptide (GALP) is well known as a neuropeptide regulating feeding behavior and energy metabolism. In this study, we examined anti-obesity effect of GALP by focusing on lipid metabolism. Mice were i.c.v. injected saline or GALP, and removal of the liver and adipose tissue at 100 minutes after the administration of GALP. Then, we studied hepatic and adipose tissue lipid metabolism related gene expression by use of real-time PCR analysis. Next, to investigate the anti-obesity effect of chronic administration of GALP, mice were fed a high fat diet to induce obesity and were intranasal administrated of GALP for 2 week. The respiratory exchange ratio of GALP group was lower than that of the saline group. In the GALP group, fatty acid oxidation-related gene mRNA levels were increased in the liver. In the adipose tissue, the mRNA levels of HSL and ATGL, were increased in the GALP group. In chronic infusion study, the body weight gain was decreased by GALP treatment as compared with the control group. Hepatic triglyceride levels decreased and, fatty acid oxidation-related genes expression were increased in GALP group. The present study indicates that GALP stimulates the hepatic lipid metabolism and anti-obese effect of GALP may be caused by improvement of lipid metabolism in the liver. It is thought that GALP may be effective for treatment and the prevention for obesity and life-style-related diseases in the near future.

(COI: No)

S71-2

Paraventricular nucleus NUCB2/nesfatin-1 neuron is targeted by leptin and regulates feeding

Nakata, Masanori; Darambazar, Gantulga; Wang, Lei; Yada, Toshihiko (*Dept. Physiology, Division of Integrative Physiology, Jichi Medical University*)

Nesfatin-1, an anorectic peptide processed from nucleobindin-2 (NUCB2), is expressed in the hypothalamus including the paraventricular nucleus (PVN), the region serving as the integrative center for energy homeostasis. Central and peripheral injections of nesfatin-1 decrease food intake in rats and mice. Accumulating evidences suggest that the NUCB2/nesfatin-1 localized in PVN is an emerging new player in regulation of food intake and energy metabolism. In this study, we used adeno-associated virus (AAV) vectors encoding shRNA targeting NUCB2 (AAV-NUCB2-shRNA) and examined the role of PVN NUCB2/nesfatin-1 in feeding behavior. PVN-specific NUCB2 knockdown resulted in increases in food intake during light phase and body weight gain, without affecting energy expenditure. Moreover, anorexigenic ability of peripherally- and centrally-administered leptin was impaired in mice receiving AAV-NUCB2-shRNA. Leptin markedly increased NUCB2 mRNA expression in PVN in vivo and in vitro. Leptin induced Ca^{+} signaling in PVN NUCB2/nesftin-1-immunoractive neurons. These results demonstrate that the PVN NUCB2/nesfatin-1 physiologically regulates feeding and body weight and serves as the direct target for the anorexigenic action of leptin.

(COI: No)

S71-3

AMPK in the paraventricular hypothalamic nucleus regulates food selection behavior in mice

Okamoto, Shiki (*Endocrinol Metab, NIPS, Aichi, Japan*)

Hypothalamic AMP-kinase (AMPK) regulates feeding behavior in response to hormonal and nutrient signals. However, the effect of AMPK on food preference remains to be established. We found that refeeding after overnight fasting, which activates AMPK in the PVH, increased the selection of high carbohydrate diet (HCD) but decreased that of high fat diet (HFD) in mice. The effect of fasting was suppressed by expression of shRNA for AMPK alpha1 and 2 in the PVH with lenti virus. In contrast, expression of constitutively-active AMPK (CA-AMPK) in PVH neurons increased HCD selection. We examined the principle neurons in the PVH for the regulation of food selection behavior. Microinjection of CRH into the PVH was found to increase HCD selection, and expression of shRNA for CRH in the PVH blunted the HCD selection in response to fasting. Preferential expression of hM3Dq or CA-AMPK in CRH Cre neurons also increased the HCD selection. We found that the change in food selection was dependent on the AMPK-induced fatty acid oxidation (FAO) in the PVH. Furthermore, pharmacological activation of AMPK increased cytosolic [Ca2+] in CRH neurons isolated from the PVH, and the effect of AMPK was abolished with the expression of shRNA for AMPK or with the suppression of FAO. Diet-induced and genetically obese mice have been shown to prefer HFD. We found that the obese mice decreased AMPK and FAO activity as well as CRH mRNA expression in the PVH.Thus, our results suggest that AMPK-FAO system in CRH neurons in the PVH regulates food selection behavior for HCD and HFD.

(COI: No)

S71-4

Neuropeptide W (NPW) induced hypophagia is mediated via CRH neurons

Takenoya, Fumiko[1,2]; Hirako, Satoshi[2]; Wada, Nobuhiro[2]; Kageyama, Haruaki[3]; Shioda, Seiji[2] (*[1]Dept. Ex. Sports. Phys. Hoshi Univ. Sch. Pharm. Sci. Toky, Japan; [2]Dept. Anat. Showa Univ. Sch. Med, Tokyo, Japan.; [3]Dept. Nutrition, Faculty Health Care, Kiryu Univ. Gunma, Japan.*)

Neuropeptide W (NPW), which was isolated from the porcine hypothalamus, which belong to the G protein-coupled receptor family. Centrally administered NPW is known to suppress feeding behaviour and promotes to secret drenocorticotropin hormone (ACTH) and corticosterone. It is reported that NPW is involved in the regulation of the hypothalamus-pituitary-adrenal cortex (HPA) axis. The aim of this study was to ascertain the roles of NPW in feeding regulations axis via CRH neurons. We observed that NPW-containing axon terminals were make synapses on CRH cell bodies and dendritic processes in the PVN. Central infusion of NPW induced c-Fos expression in the PVN compered to saline injection to the mice, but not vasopressin- nor oxytocin-positive neurons in the PVN. To determine whether NPW regulates feeding behaviour via CRH neurones, the feeding behaviour of rats was studied following NPW i.c.v. injection with or without CRH antagonist pretreatment. The CRH antagonist canceled the NPW-induced anorexia. Moreover, using the CellKey system which enables comprehensive pharmacological evaluation of cell surface receptors, including GPCRs and tyrosine kinase receptors, using adherent and suspension cell lines and primary cells and have been shown to be characteristic of Gs, Gq, and Gi GPCRs of analysis. NPW response in cells on CellKey System was Gi axis. These results suggested that NPW mediated neuronal feeding pathway via CRH neurons in the PVN.

(COI: No)

S71-5

Clinical application of GLP-1 to obesity-related diabetes

Ueno, Hiroaki; Nakazato, Masamitsu (*Internal Med., Miyazaki Univ., Miyazaki, Japan*)

Glucagon-like peptide-1 (GLP-1) regulates diverse physiological phenomena such as insulin and glucagon secretion, gut motility, and appetite. We administered GLP-1 and saline during a test meal to healthy subjects and patients with type 2 diabetes to assess the role of GLP-1 in regulating glucose metabolism, gut peptides, appetite, and the autonomic nervous system. In both groups, GLP-1 administration significantly decreased plasma glucose and glucagon levels, and increased insulin and blood pressure. Feelings of fullness and hunger were similar between GLP-1 and saline in both groups. This GLP-1 test may be useful for predicting the effects of incretin-related drugs and assessing insulin secretion capacity; however, further studies are necessary.
We developed a novel device and GLP-1 compound for intranasal administration. Twenty-six patients with type 2 diabetes were enrolled in a double-blind placebo-controlled study. Intranasal GLP-1 or placebo was administered immediately before every meal for 2 weeks. The plasma peak concentration of active GLP-1 was 47.2 pmol/L, and Tmax was 8.1 min. The early phases of insulin and glucagon secretion were recovered and suppressed, respectively, in the GLP-1 group. Glycoalbumin decreased significantly after GLP-1 administration. Body weight and appetite were unchanged. Subjects exhibited no marked adverse events after using nasal GLP-1. Long-term application of the drug, including body weight reduction, should be evaluated in future trials.

(COI: No)