Poster Presentations

Day 3

（March 23, 12:15～13:30）

P3-001～P3-019	Neuronal projection
P3-020～P3-065	Neurohistochemistry, Neurochemistry
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P3-118～P3-135	Motor function
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P3-207～P3-248	Neurological disorders, Neuropathophysiology
P3-249～P3-269	Others of Neuroanatomy, Neurophysiology, Neuronal cell biology
P3-270～P3-302	Behavior, Biological rhythm
P3-303～P3-336	Gross anatomy
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P3-001

Glutamatergic circuits in the song system of zebra finch brain determined by gene expression of vGluT2 and glutamate receptors

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The songbird brain has a system of interconnected nuclei that are specialized for singing and song learning. Electrophysiological findings indicate a role for the glutamatergic neurons in the song system. Vesicular glutamate transporter 2 (vGluT2) is considered to be a specific biomarker of glutamatergic neurons in birds. Neurons receiving glutamatergic afferents express mRNA of ionotropic glutamate receptor subunits. This study examined expression of vGluT2 and glutamate receptor subunit mRNAs in nuclei of the song pathways of male zebra finch brain by in situ hybridization. VGluT2 mRNA was revealed high density of expression in the song nuclei, namely HVC, lateral magnocellular nucleus of the anterior nidopallium, and robust nucleus of the arcopallium. Area X did not show expression of vGluT2 mRNA. Nuclei in the descending motor pathway (dorsomedial nucleus of the intercollicular complex and retroambigual nucleus) were expressed vGluT2 mRNA. Target nuclei of vGluT2 mRNA-expressing nuclei showed hybridization signals for mRNAs of ionotropic glutamate receptor subunits. At least one of five subunit mRNAs (GluA1, GluA4, GluK1, GluN1, GluN2A) was expressed in song nuclei. The present findings support the existence of glutamatergic circuits in the song system in songbirds.

(COI: No)

P3-002

Prenatal valproic acid exposure induces aberrant distribution of spinal nerves in mice

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Background: Teratogenicity of anticonvulsant valproic acid (VPA) is well known and has a potential to induce congenital malformations, such as a neural tube defect, in fetuses whose mothers are treated with it during pregnancy. However, the detailed mechanism of VPA teratogenicity still remains unknown. Thus, we examined the effects of VPA on peripheral nerve fiber innervation during development using a mouse model.
Methods: A single dose of 400 mg/kg VPA was subcutaneously injected in pregnant ICR mice on any one gestational day (GD) 6 to GD 9. On GD 10, embryos were collected from the pregnant mice and quickly immersed into 4% paraformaldehyde/0.1M PB (pH7.2). Distribution of neurofilament-immunoreacitve nerve fibers in the embryos was examined using a whole-mount immunostaining technique.
Results: The whole-mount immunostaining was clearly detected as nerve fiber bundles that consist of both cranial and spinal nerves. There was no obvious change in distribution of cranial nerves. On the other hand, abnormal distributions of spinal nerves, such as a loss of a whole bundle and an intrusion into adjacent segments, were identified in prenatal VPA-exposed embryos. The incidence of aberrant spinal nerve was higher in the embryos exposed to VPA on GD 8.
Conclusions: We demonstrated that prenatal VPA exposure has a potential to induce aberrant distribution of spinal nerves and development of spinal nerve innervation could be more susceptible to VPA exposure on GD 8 in mice.

(COI: No)

P3-003

A newly-identified hypothalamic area enriched with perineuronal nets

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Perineuronal nets (PNNs) are specialized extracellular matrix structures in the adult brain that play important roles in regulating synaptic plasticity. In this study, we examined the formation of PNNs in the mouse hypothalamus using WFA lectin (a broad PNN marker), and found a previously unidentified region located between the fornix and paraventricular nucleus (PVN). We named this new region the "hypothalamic delta area" (HDA), referring to its triangular shape. DNA microarray experiments and histochemical studies identified at least two types of neurons in the HDA: enkephalin- and calretinin-positive neurons, both of which were GABA negative. Furthermore, the HDA was shown to have bidirectional neural connections with the lateral septum (LS) and intra-hypothalamic nuclei such as the ventromedial hypothalamic nucleus and dorsal part of the premamillary nucleus. We also confirmed enkephalinergic projections from HDA neurons to the LS, and inversely, calbindin-positive LS neurons as afferents to the HDA. c-Fos expression analysis revealed that the activity of HDA neurons were increased by emotional stressors such as open field test, restraint stress, and aggressive behavior, but not by the metabolic stressors such as fasting and dehydration. These results suggest that the HDA is a newly-identified hypothalamic area connecting the hypothalamus with the limbic system and has specific functions related to emotional stressors.

(COI: No)

P3-004

A new reporter rat line which conditionally expresses red fluorescent protein (tdTomato)

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Rats offer potential advantages of larger body size and progressed ability to accomplish complex behavioral tasks over mice. However, the conditional gene expression system has not been enough arranged despite of its importance in anatomy and physiology. Here we report a conditional reporter rats which express red fluorescent protein (tdTomato) under Cre/loxP recombination system. The effectiveness of conditional expression was verified by the following three evidences. (1) When Cre was expressed in striatum, hippocampus or cerebellum using AAV, each Cre-immuno-positive cells merged with tdTomato signals. (2) When Cre expression was targeted specifically in the cortical layer 2/3 using *in utero* electroporation at embryonic day 18 (E18), neurons in the layer 2/3 were visualized by tdTomato fluorescence. (3) When the double transgenic rats with *phox2b*-Cre and *floxed* tdTomato were examined at embryonic E12.5, tdTomato was expressed in neurons of several hindbrain regions that are involved in the autonomic nervous system as well as in neurons that are responsible for respiratory rhythm generation, and co-localzed with endogenous Phox2b proteins. The neuronal fiber projections were clearly visualized by the tdTomato signals. Our reporter rat would facilitate the neurophysiological studies and the connectomics of identified neurons which express Cre under a certain promoter.

(COI: No)

P3-005

In vivo calcium imaging of thalamocortical axons in mouse primary visual cortex

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The prominent aspect of primary visual cortex (V1) is the response selectivity to visual stimulus features. Layer 4, the major entrance layer of V1, receives main visual input from two thalamic nuclei, lateral geniculate nucleus (LGN) and lateral posterior nucles (LPN). Layer 4 excitatory neurons in mouse V1 possess high selectivity for stimulus orientation. Recent studies indicated that LGN axons targeting layer 1, another recipient layer of thalamic input, had sharp orientation selectivity (Cruz-Martin et al., 2014). However, it is still unknown whether layer 4 also receives orientation tuned input from thalamus. To answer this question, we performed in vivo two-photon calcium imaging of thalamocortical axons in mouse V1 and investigated their response selectivity to visual stimuli. For axonal calcium imaging, GCaMP6s, genetically encoded calcium indicator, was locally expressed in either LGN or LPN neurons by AAV-mediated method. Boutons of thalamocortical axon expressing GCaMP6s were clearly visible in layers 1 through 4 of V1 and we could record their response selectivity to visual stimuli. We found LGN axons arborizing in layer 4 had broad orientation selectivity, while LPN axons in layer 4 had mixture of sharp and broad orientation tuning. These results suggest that layer 4 neurons also receives orientation tuned input form LPN, while largely untuned input from LGN.

(COI: No)

P3-006

Projection from lateral habenula to trigeminal mesencephalic nucleus and its function

Ohara, Haruka; Tachibana, Yoshihisa; Sato, Fumihiko; Takeda, Rieko; Oka, Ayaka; Kato, Takafumi; Yoshida, Atsushi (*Dept. of Oral Anatomy and Neurobiology, Grad. Sch. Dent. Osaka Univ., Osaka, Japan*)

The lateral habenula (LHb) is implicated in disappointment and expectation of negative conditions such as stressful conditions, suggesting that it is also involved in motor control of food intake. The trigeminal mesencephalic nucleus (Vmes) neurons convey deep sensations from masticatory muscles and periodontal ligaments, and function in orofacial movements, especially jaw movements. Therefore, we examined whether LHb neurons activated by stress to the animals directly project to Vmes neurons in rats. After a retrograde tracer, FG, was injected into Vmes, many neurons were labeled bilaterally in both the lateral part (LHbL) and medial part (LHbM) of LHb. After injections of an anterograde tracer, BDA into LHb, axon fibers and terminals were labeled bilaterally in Vmes. Some BDA-labeled terminals contacted the cell bodies of Nissl-stained Vmes neurons bilaterally. After FG injections into Vmes and subsequent application of restraint stress, many c-Foss immunoreactive (ir) cells were observed bilaterally in LHb; the number of c-Foss-ir cells in LHbM was higher than that in LHbL bilaterally. A small number of FG/c-Fos double labeled neurons were found bilaterally in LHb; the number of double labeled neurons in LHbM was slightly higher than that in LHbL. The ratio of double labeled neurons to FG labeled neurons in LHb was higher than that found in control cases with FG injections into Vmes but no restraint stress. This study suggested that LHb neurons activated by stress directly project to Vmes neurons.

(COI: No)

P3-007

Central processing of masticatory muscle sensation

Fujio, Takashi; Sato, Fumihiko; Tomita, Akiko; Ikenoue, Etsuko; Haque, Tahsinul Md.; Yoshida, Atsushi (*Dept. of Oral Anatomy and Neurobiology, Grad. Sch. Dent. Osaka Univ., Osaka, Japan*)

The masticatory muscle sensation is involved in the orofacial movements. This sensation is conveyed to the supratrigeminal nucleus (Vsup) by the trigeminal mesencephalic nucleus neurons in cats. However, little is known about how to identify the Vsup and about the central processing of the sensation through the Vsup. To address these issues, we used neuronal tract tracing and electrophysiological recording techniques in the rat. After application of cholera toxin subunit B to the masseter nerve (MN), we found anterogradely labeled axon terminals in almost entire area of the Vsup, which was cytoarchitectonically identified. The Vsup was also identified electrophysiologically by recording responses to electrical stimulation of the MN and the passive jaw-jerk; no fast responses were recorded after electrical stimulation of the lingual nerve. After injections of biotinylated dextranamine into the Vsup, anterogradely labeled axon terminals were found contralaterally in the caudo-ventromedial part of the ventral posteromedial nucleus (VPMcvm) and bilaterally in the paracentral nucleus in the thalamus. The VPMcvm was also identified electrophysiologically by recording responses to electrical stimulation of the MN and the passive jaw-jerk. After Fluorogold injections into the VPMcvm, retrogradely labeled cells were found contralaterally in the Vsup and in the dorsomadial margin of the trigeminal principal nucleus adjacent to the Vsup. These findings have for the first time demonstrated features of the central processing of muscle sensation.

(COI: No)

P3-008

Analysis on the inter-areal axon projection in the mouse neocortex

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Cerebral neocortex integrates different sensory inputs with internal status to elicit an appropriate behavior. Direct neuronal connections between functional areas within a cerebral hemisphere should take an important part in this process. Long association fibers (LAFs) are the long-range connections between distant areas located in different cortical lobes and recent studies have reported that the LAFs are disturbed in the mental/developmental diseases like schizophrenia and autism spectrum disorders, suggesting the importance of LAFs in cognitive functions. However, the detailed axonal structure of long association neurons (LANs) that constitute the LAFs and how its final structure is established during cortical development are yet to be revealed. To study the structure and development of the LANs, we identified the mouse genes preferentially expressed in LANs compared to callosal neurons, which connect bilateral hemispheres via corpus callosum, by microarray analysis. We confirmed that some of the candidate genes obtained were indeed expressed in LANs in the primary somatosensory area (S1), by double labeling with in situ hybridization and retrograde tracing from primary motor cortex (M1) to the S1. Using the promoters of these LAN-specific genes and tissue clearing methods combined with deep brain imaging, we visualized the entire axon structure of individual neurons at different time point of the development.

(COI: No)

P3-009

Features of projecting neurons of the nucleus of the tractus solitarius

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The caudal nucleus of the tractus solitarius (cNTS) contains many neurons in the dorsal region (the subpostremal and dorsolaretal subnuclei) and the ventral region (the commissural and medial subnuclei) that innervate other brain regions, but information regarding their somal size and distribution remains incomplete. Here we labeled projection neurons in the cNTS with a retrograde tracer, the B subunit of cholera toxin (CTb), and studied their somal distribution and size in relation to their projection sites, including the parabrachial nucleus (PB), periventricular nucleus (PVN), central nucleus of the amygdala (CeA), and periaqueductal grey (PAG). Major findings include: 1) The PB projecting cNTS neurons were located in the subposremal region and the dorsomedial subnucleus. Their somal size was relatively small. 2) The PVN projecting cNTS neurons were preferentially localized around the tractus solirarius in the medial subnucleus. Their somal size was relatively large. 3) The CeA projecting cNTS neurons were preferentially localized just above the dorsal motor nucleus of the vagus nerve in the ventral region. Their somal size was medium. 4) The PAG projecting cNTS neurons were localized around the border between the medial and commissural nuclei in the ventral region. Their somal size was medium. Our findings, in combination with results of previous studies showing a spatial laminar segregation of neuronal populations: a dorsal group of high excitation and a ventral group of balanced excitation and inhibition, suggest that neuronal processing mechanisms in the cNTS might be different in relation to efferent projection system.

(COI: No)

P3-010

Gustatory pathways from the parabrachial nuclei to the ventral part of the caudate putamen via the caudal part of the intralaminar thalamic nuclei in rat brain

Iwai, Haruki; Kuramoto, Eriko; Yamanaka, Atsushi; Goto, Tetsuya (*Grad. Sch. Med. & Dent. Sci. Kagoshima Univ., Kagoshima, Japan*)

Background: The medial parabrachial nucleus (MPB) and external part of the medial parabrachial nucleus (MPBE) relaying gustatory information in the rat have been reported to project to the caudal part of the intralaminar thalamic nuclei. The intralaminar thalamic nuclei are known to project to the caudate putamen (CPu), however, it is unclear where the caudal part of the intralaminar thalamic nuclei projects within the CPu. The objective of this study was to determine morphologically their brain areas.
Methods: We visualized the parabrachio-thalamo-striatal pathways with anterograde and retrograde tracers.
Results: The MPB or MPBE projected to the ventrocentral part of the CPu via the caudal part of the oval paracentral thalamic nucleus; to the ventrolateral part of the CPu via the ventrolateral part of the parafascicular thalamic nucleus; to the ventromedial part of the CPu via the ventrocaudal part of the central medial thalamic nucleus, ventromedial part of the parafascicular thalamic nucleus, and retroreuniens area; and to the most ventral part of the CPu via the parvicellular part of the posteromedial ventral thalamic nucleus.
Conclusions: We demonstrate that the ventral part of the CPu receives projections from the caudal part of the intralaminar thalamic nuclei. Since the ventral part of the CPu has been reported to involve in food intake and jaw movement, our results suggest that gustatory information from the MPB and MPBE affects oral function within the ventral part of the CPu.

(COI: No)

P3-011

Analysis of calcitonin gene-related peptide (CGRP)-expressing neurons in the peripeduncular nucleus during lactation

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During lactation, suckling stimulus is conveyed to the hypothalamus via the brain stem and causes various behavioral and endocrinal changes, such as suppression of reproductive function. In the present study, we purposed the identification of neurons conveying suckling stimulus from the brain stem to the hypothalamus in lactating rats. We found significant increase in the expression of calcitonin gene-related peptide (CGRP) alpha in the brain stem in lactating rats using the quantitative RT-PCR analysis. CGRP-immunoreactive (ir) neural cell bodies were found in the peripeduncular nucleus of the mesencephalon (PP) and the fibers were found in the ventromedial nucleus of the hypothalamus (VMH) and caudal part of caudate-putamen (CPu) in lactating rats. The ratio of cFos-ir to the CGRP-ir neurons in the PP in suckling-stimulated mother rats was greater than that in non-stimulated control. After ipsilateral injection of biotinylated dextran amine (BDA; anterograde tracer) into the PP, BDA-labeled fibers were found in the CPu and VMH. Moreover, after injection of Fluoro-Gold (FG; retrograde tracer) into the CPu, FG-labeled cell bodies were found in the PP and positive for CGRP. On the other hand, FG injection into the VMH also caused labeling for several PP neurons but not positive for CGRP. These results suggested that CGRP neurons in the PP projecting to the CPu play a key role in conveying suckling stimulus to various brain regions during lactation.

(COI: No)

P3-012

Projection pattern of geniculocortical afferents in mouse visual cortex

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The visual information is received at the retina and carried to the visual cortex through the dorsal lateral geniculate nucleus (dLGN) of thalamus. In primates and carnivores, the dLGN contains mainly three types of neurons projecting to the cortex, which have distinct characteristics in physiological property, dendritic morphology and axonal projection pattern in the cortex. In mice, however, it has not been established yet whether there are multiple types of projection neuron in the dLGN. Although three types of projection neuron were reported in mouse dLGN based on their dendritic morphology (Krahe et al., 2011), it remains unknown whether they have different axonal projection patterns in the cortex. While the dLGNs of primates and carnivores are segregated into anatomically distinct layers which contain specific types of neurons, the dLGN of mice does not have such laminar organization. Thus, multiple types of neurons are intermingled in mouse dLGN, making it difficult to examine the projection patterns of a particular type of neurons with conventional neuronal tracers. We established a method to visualize the entire axonal and dendritic structures of a few neurons in dLGN using the *in vivo* local electroporation technique (Ohmura et al., 2014). Using this novel technique, we found that mouse dLGN neurons show several distinct patterns of axonal projection in the cortex. These results suggest that the dLGN of mouse contains multiple types of projection neurons which represent parallel visual pathways.

(COI: No)

P3-013

Functional architecture of the gloss selective regions in the monkey inferior temporal cortex

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We have previously reported that there exist neurons that selectively responded to specific range of gloss in the inferior temporal (IT) cortex of the monkey (Nishio et al., 2012), and these gloss selective neurons encode perceptual gloss parameters (Nishio et al., 2014). Gloss selective neurons were concentrated in a restricted region extending 2-3 mm in the lower bank of the superior temporal sulcus (STS) in the central IT cortex. To understand the cortical processing related to the generation of gloss selective neurons, in the present study, we injected a small amount of retrograde tracer (CTB Alexa 555) in the lower bank of STS where gloss selective neurons were clustered. We observed retrogradely labelled neurons were distributed in several regions posterior to the injection site including area V4 and the posterior IT cortex. Labelled neurons were most densely observed within the STS, in particular at the lip of the STS in the posterior IT cortex, but we also observed clustering of labeled cells in the lateral surface of the IT gyrus dorsal to the anterior end of the posterior middle temporal sulcus (PMTS). These regions seem to correspond to the regions where an image of shiny object evoked strong responses in our previous fMRI experiment (Okazawa et al., 2012). These regions may from a network that is specially related to the processing of gloss information in IT cortex.

(COI: No)

P3-014

Analyses on the candidate receptors for the axon collateralization of the developing corticospinal tract

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Axon collaterals are involved in the coordination of neural activities required for the higher brain function. During development, corticospinal tract extends interstitial axon collaterals from their main shaft toward the multiple targets. Several lines of evidence suggest that a diffusible collateral-inducing factor(s) is released from the target. However, the molecular entities and mechanisms that induce and elongate the axon collaterals are mostly unknown. We selected candidate ligands for the collateral induction over the corticospinal tracts by choosing the molecules that were strongly expressed in their targets, such as the pontine nuclei and the superior colliculi. In addition, with the aid of the public database, we finally listed 105 candidate receptors. To test whether these receptors are truly involved in the axon collateralization, individual receptor in the layer V neuron of the cortex was disrupted using the RNAi interference. So far, we identified several potential candidates that are involved in collateral formation: knockdown of 10 candidate receptors resulted in poor collateral formation to the pontine nuclei, whereas that of 4 candidate receptors enhanced collateral formation.

(COI: No)

P3-015

Roles of Runx1 in muscle innervation of mouse embryonic hypoglossal neurons

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Runx1, runt-related transcription factor, plays important roles in the cell type specification and axonal projections of the nociceptive dorsal root ganglion (DRG) neurons. In our previous study, we found that Runx1 was expressed in the ventrocaudal part of hypoglossal nucleus (nXII) at embryonic day (E) 17.5. In *Runx1*-deficient mice, areas immunoreactive for vesicular acetylcholine transporter (VAChT; a presynaptic marker of motor axon terminals) were reduced in the vertical and transverse tongue muscles, whereas those in the genioglossus (GG) muscle were increased, suggesting that some hypoglossal neurons switch their targets in the absence of Runx1. In the present study, to address this possibility, we examined hypoglossal neurons using retrograde labeling. Cholera toxin B subunit (CTB) was injected into the GG muscle of E17.5 embryos, and the localization of CTB-labeled motoneurons was examined. The distribution of CTB-labeled hypoglossal neurons in *Runx1*-deficient mice was similar to that of control mice, indicating that Runx1 deficiency did not alter axonal projections to the GG muscle. We also examined the expression of two markers of cranial motoneurons, c-Met and c-ret. Althouth Runx1 regulates c-Met or c-ret expression in DRG neurons, Runx1 deficiency did not change the expression of these motoneuron markers. Thus, it is unlikely that the altered axonal projection from nXII in *Runx1*-deficient mice is associated with c-Met or c-ret dysfunction.

(COI: No)

P3-016

Intrinsic projections of the retrosplenial cortex in the rabbit: Projections to dysgranular area 30

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The retrosplenial cortex (RS) is an essential structure for discriminative avoidance learning in the rabbit. RS consists of areas 29a, b, and c, and area 30, but the connectivity between these areas has not been studied yet. Here, we studied afferent projections to area 30 from other areas within RS, with the retrograde tracing method. Eleven male New Zealand White rabbits weighing 2.5-3.0 kg were used. Under anesthesia, a single iontophoretic injection of cholera toxin B subunit (CTB) was made in a various rostrocaudal level of area 30. After 7 days, the rabbits were perfused with a fixative, and their brains were cut into sections, which were treated immunohistochemically to visualize retrogradely labeled cells. Injection of CTB into part of rostral area 30 labeled cells over the rostral one-third of area 30 and in area 29c. Injection into the mid-rostrocaudal part labeled cells distributing over the middle two-thirds of area 30 along the rostrocaudal axis and in area 29b. Injection into part of caudal area 30 labeled cells over the caudal one-third of area 30 and in area 29b. These labeled cells occurred in layers 2-6. Contralateral labeled cells occurred in layers 2 and 5 and superficial layer 6 of areas 30 and 29b/29c at the rostrocaudal level of each injection site. These results suggests that the rostral and caudal parts of area 30 may function independently, and mid area 30 may integrate information from the rostral and caudal parts of RS.

(COI: No)

P3-017

Tyrosine hydroxylase (TH) immunoreactive fibers unsusceptible to the degeneration occurring in the zitter mutant rat originate from the dorsal tier of the substantia nigra compact part (SNC)

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The zitter rat is an autosomal recessive mutant rat derived from the Sprague-Dawley (SD) strain and these mutant rats show fine tremor and flaccid paresis progressing with aging as well as curled body hair and bent whiskers. The zitter mutant rat also exhibits the degeneration of the TH immunoreactive fibers in the striatum with aging. We reported previously that these mutant rats exhibited the region-specific vulnerability in the TH immunoreactivity with more severe in the dorsal striatum than in the ventral striatum (Ueda et al, Neuroscience, 2000). However little is known about why this region-specific vulnerability occur. To identify neurons projecting to the ventral striatum, we injected a retrograde tracer, fluorogold (FG) into the ventral striatum of normal rats and examined the location of FG labeled neurons and its neurochemical properties. We found that 1) many FG labeled neurons were present in a dorsal tier of the SNC but not in a ventral tier of the SNC and 2) all FG labeled neurons were TH immunoreactive neurons. These results suggest that TH immunoreactive fibers unsusceptible to the degeneration occurring in the zitter rat originate from the dorsal tier of the SNC and support our previous findings showing that the dopaminergic neurons in the ventral tier of the zitter mutant rat is more vulnerable than that in the dorsal tier (Ueda et al, Neurosci letters, 2005).

(COI: No)

P3-018

The local field potential in the forebrain by the optogenetic manipulation of serotonergic neurons in the raphe nucleus

Yoshida, Keitaro; Takata, Norio; Mimura, Masaru; Tanaka, Kenji F (*Department of Neuropsychiatry, School of Medicine, Keio University*)

Serotonin (5-HT) is a neurotransmitter involved in a wide range of brain functions as a modulation of multiple type of behaviors such as appetitive, emotional, motor, cognitive and autonomic. Optogenetics is an elegant tool to control neurotransmitter release with millisecond precision and cell type-specific resolution. We previously succeeded in generating transgenic mice that expressed a light-sensitive channelrhodopsin-2 variant ChR2(C128S) in serotonergic neurons. The optogenetic stimulation of serotonergic neurons in the dorsal raphe nucleus (DRN) caused a robust increase of serotonin release in the medial prefrontal cortex (mPFC). Furthermore, the activation of DRN enhanced patience for a future reward when the animal is deciding whether to keep waiting or to abandon the wait (Miyazaki et al, 2014 Curr Biol.). The ventral hippocampus is known to be important targeted region of serotonin as well, because several types of serotonin receptors (Htrs) such as Htr1a, 2a, 2c, and 7 are highly expressed. However, it is unclear how serotonin modulates the activities of targeted neurons in these forebrain structures. To clarify the effect of the serotonergic modulation on the neuronal activities in the forebrain structures with high temporal and spatial precision, we recorded local field potential (LFP) in prefrontal cortex and ventral hippocampus by a sixteen-channel silicon probe. Our preliminary results showed that the activation of serotonergic neurons by optogenetics evoked LFP response in the mPFC and ventral hippocampus.

(COI: No)

P3-019

Differential innervation of the efferent nerves in the rat testis

Maeda, Seishi; Kuwahara-Otani, Sachi; Tanaka, Koichi; Hayakawa, Tetsu; Seki, Makoto (*Hyogo College. Med., Nishinomiya, Japan*)

In the mammalian testes, autonomic efferent nerves are innervated via the superior and inferior spermatic nerves. These post-ganglionic neurons may be originated from the sympathetic chains, pre-vertebral ganglia and pelvic ganglia, however, the detailed distributions of these neurons are still unclear. To examine the distributions of testicular efferent neurons, retrograde tracer Fluorogold (FG) was injected into the testicular nerves in the rat. A microcapsule filled with 2% FG was inserted to the cut-end of the left testicular nerves. After 3 days, the autonomic ganglia and the brains were removed and serially frozen sections were made, then, FG-labeled cells were observed and counted in each ganglion. As the results, Labeled cells were distributed in the ipsilateral sympathetic ganglia (sympathetic chains: 74.8%; the prevertebral ganglia: 16.7%) and the contralateral ganglia (sympathetic chains: 8.7%). Only a few FG-labeled cells were found in the ipsilateral pelvic ganglia. No labeled cells were observed in the parasympathetic ganglia. Almost all FG-labeled cells were represented for tyrosine hydroxylase immunoreactivity. These results suggest that the neurons projecting to the testis via superior spermatic nerves may be all sympathetic and originated mostly from the neurons located in the ipsilateral sympathetic chains. Furthermore the distribution of these neurons may reflect the descensus of the testis and its vascular system during development.

(COI: No)

P3-020

Systematic analysis methods of in situ hybridization labeled cells

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It is meaningful to know more information about in situ hybridization (ISH) labeled cells, so we attempted to analyse those cells one more step. But owing to the tissue damage derived from ISH staining process, it was hard to utilize the same tissue following ISH for more analysis. Therefore, in order to avoid the influence of this tissue damage, we made some kinds of efforts. And we managed to perform this analysis. In consequence, we developed some techniques of analysis for ISH labeled cells. 1) Comparison of the distribution with the cells expressing other gene or protein : ISH double staining, ISH + immunohistochemistry (IHC). 2) Search for other expressing genes in those cells : laser microdissection of ISH labeled cells + single cell PCR. 3) Visualization of the morphology of those cells : DiI injection into ISH labeled cells. Combining these techniques, we analyzed ISH labeled cells systematically.

(COI: No)

P3-021

Large-volume optical reconstruction of brain tissue with a resolution of single synapses

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Branching pattern of dendrites and distributions of spine synapses along dendrites are basic information for structural analysis of neural circuits. Tissue sectioning techniques are usually applied for such analysis, but visualization of the entire dendritic arborization requires labor-intensive procedures, including acquisition, alignment, and reconstruction of a large number of sections. Possible deformation and destruction of structures by sectioning itself is an additional concern. CLARITY, a recently-developed technique of tissue processing, enables rapid access of exogenous antibodies, large volume imaging, and improvement of the optical property, which is suitable for the use of an objective lens with a high numerical aperture. This technique is suitable for wide range microscopic observation inside the tissue without sectioning. We report that the CLARITY technique can be applied to observe entire dendrite morphologies of cortical pyramidal neurons with a resolution of single spines. Additional immunohistochemistry of the sample enabled us to evaluate molecular compositions of each synapse and the extent of contact with glial processes. CLARITY technique applied to high-resolution imaging of dendrites should be useful in detecting morphological phenotypes in mouse models of neurological and psychiatric disorders.

(COI: No)

P3-022

Sema4D is involved in microglial polarization after cerebral ischemia

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Brain ischemia evokes microglial activation. There is increasing evidence that activated microglia are polarized to functional distinct phenotype: proinflammatory M1-like microglia and anti-inflammatory M2-like microglia. This phenomenon shows the importance of controlling microglial function in therapy of brain ischemia. Sema4D is a 150 KDa transmembrane and secreted-type semaphorin belonging to the classIV semaphorin subclass. Sema4D promotes some immune functions, such as activation of B-cells, dendritic cells and T-cells. Although microglia express Plexin B1 and CD72 which are receptors of Sema4D, the interactions of microglia and Sema4D remain unclear. Here, we show that influence of Sema4D on microglial phenotype after cerebral ischemia by permanent middle cerebral artery occlusion. Sema4D-deficiency inhibited microglial polarization to M1-like, whereas promoted to M2-like after cerebral ischemia. Although there was no change in the mRNA levels of polarization-related cytokines, ERK1/2 phosphorylation which is downstream of Plexin B1 was inhibited by Sema4D. These results suggest that Sema4D directly affects microglial polarization.

(COI: No)

P3-023

Interferon regulatory factor 7 participates in the M1-like microglial polarization switch

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Microglia are generally considered the immune cells of the central nervous system. Recent studies have demonstrated that under specific polarization conditions, microglia develop into two different phenotypes, termed M1-like and M2-like microglia. However, the phenotypic characteristics of M1-like- and M2-like-polarized microglia and the mechanisms that regulate polarization are largely unknown. In this study, we characterized LPS-treated M1-like and IL-4-treated M2-like microglia and investigated the mechanisms that regulate phenotypic switching. The addition of M2-like microglial conditioned medium (CM) to primary neurons resulted in an increase in neurite length compared with neurons treated with M1-like microglial CM, possibly because of the enhanced secretion of neurotrophic factors by M2-like microglia. M1-like microglia were morphologically characterized by larger soma, while M2-like microglia were characterized by long processes. M2-like microglia exhibited greater phagocytic capacity than M1-like microglia. These features switched in response to polarization cues. We found that expression of interferon regulatory factor 7 (IRF7) increased during the M2-like to M1-like switch in microglia in vitro and in vivo. Knockdown of IRF7 using siRNA suppressed the expression of M1 marker mRNA and reduced phosphorylation of STAT1. Our findings suggest that IRF7 signaling may play an important role in microglial polarization switching.

(COI: No)

P3-024

The change of Morphology and Distribution of Microglia in the postnatal developing mouse cerebellum

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Microglia have been considered as immune cells which are activated by pathological events, but recent analyses suggest that they also play crucial roles in postnatal development of the immature brain. However, distribution and morphology of microglia in the developing brain remain poorly understood especially in the cerebellum. In the present study, we morphologically examined developmental changes of microglia in the mouse cerebellum. Mice aged from postnatal day 5 (P5) to P60 were transcardially perfused and microglia were labeled by Iba1 antibody. Around P5, majority of microglia was distributed in white matter. The density of microglia in the white matter massively decreased until P13. In contrast, the density of microglia was stable and identical among the internal granular layer, the Purkinje cell layer and the molecular layer after P8, Taking increase in the cortical volume during postnatal development into account, these results suggest that microglia migrate from the white matter to the cortex from around P7. We found that morphology of microglia was also changed in parallel with the cortical migration. At P5, microglia in the white matter tended to have large bodies and poor branching processes. Those in the cortex had fine long processes and small somata after P13, which is similar to those in adult mice. Taken together, these results suggest that translocation and maturation of microglia massively proceed from around first postnatal week to P13 in the mouse cerebellum.

(COI: No)

P3-025

Immunohistochemical study of axonic satellite glial cells in rat DRG

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Initial segment of neuronal process of the dorsal root ganglion (DRG) neuron is covered by axonic satellite glial cells followed by myelinating Schwann cells (Pannese in 1960). However, after this report, there had been no report on axonic satellite glial cells. In the present study, we elucidated features of axonic satellite glial cells in 6 weeks old rats by immunohistochemistry and BrdU histochemistry. About 10 axonic satellite glial cells covered approximately 100 μm of initial segment of the neuronal process. Each of the glial cells covered one neuronal process. These glial cells locating near the neuronal cell body (proximal region) showed satellite glial cell markers, Kca2.3, and weak p75 immunoreactivity. Axonic satellite glial cells situated near myelianting Schwann cell (distal region) strongly showed p75 immunoreactivity, but not Kca2.3. Moreover, the glial cells in distal region were also immuno-positive for promyelinating Schwann cell marker, Oct-6. To examine their proliferation and differentiation, BrdU was injected and observed with time course. Two hours after BrdU administration, BrdU was observed in axonic satellite glial cells which are close to myelianting Schwann cells. Two weeks after administration, BrdU was detected in most proximal myelinating Schwann cells. These results suggest that axonic satellite glial cells are composed of some cell population, and that the glial cells locating in distal region are Schwann cell precursors.

(COI: No)

P3-026

Hedgehog Signaling Regulates the Morphogenesis of Schwann Cell in Specific Time Windows

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Although hedgehog (Hh) signaling is one of the key signaling for regulating the myelination in peripheral nerve system, detailed mechanism has not been elucidated. We previously reported that Hh signaling was received by Schwann cells (SCs) through the primary cilia, and facilitated the myelination. Importantly, the ratio of primary cilia-positive SCs gradually increased from the promyelinating phase to the initial stage of myelin sheath formation (Yoshimura and Takeda, 2012). These results indicate that the SCs autonomously determine the sensitive period of Hh signaling for myelin formation, and Hh signaling chiefly play a crucial role during the early stage of myelination. In this study, we determine the detailed function of Hh signaling in promyelinating stage of mouse Schwannoma cell line TR6Bc1. In promyelinating stage, SCs become quiescent and form the primary cilia. Furthermore, mature SCs migrate along axons and extend their processes. When Hh signaling was activated by Smo agonist, maturation and process formation were significantly facilitated. However, other steps, such as proliferation and migration, were not affected. These results demonstrate that Hh signaling is specifically received by SCs to form the bipolar morphology in promyelinating stage. To further establish the function of Hh signaling for morphological change in SCs, we are now trying to construct the Smoothened (Smo: effector of Hh signaling) knock out cells by CRISPR/Cas9 system.

(COI: No)

P3-027

Myelination at the peripheral-central transitional zone of developing chick vestibulocochlear nerves

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The eighth cranial nerve consists of the vestibular and cochlear nerves. Our previous study showed regional differences of myelination in the chick vestibular and cochlear nerves. In this study, an immunohistochemistry with antibodies specific to Schwann cell marker protein zero (P0), oligodendrocyte marker proteolipid protein (PLP) and myelin basic protein (MBP) were used to detail the myelination at the peripheral nervous system (PNS) and central nervous system (CNS) transitional zone of the vestibular and cochlear nerves in embryonic chicks. Embryos in 9-14 day eggs (E9-14) were prepared for the immunohistochemistry. In the vestibular nerve, the immunoreactivity of PLP and MBP was first observed in CNS at E10 and that of P0 and MBP in PNS at E11. In the cochlear nerve, the immunoreactivity of PLP and MBP was first observed in CNS at E10 and E11, respectively, and that of P0 and MBP in PNS at E13. And then, positive axons gradually increased. These observations suggest that the onset of the myelination in CNS is earlier than that in PNS of both the vestibular and cochlear nerves. Moreover, the myelination in the vestibular nerve should occur earlier than that in the cochlear nerve. It seems that the myelination of Schwann cells is preceded by that of oligodendrocytes and the development of the auditory function is by that of the vestibular function.

(COI: No)

P3-028

Characterization of Olig2-positive astrocytes in the normal adult forebrain

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

Olig2, a basic helix-loop-helix (bHLH) transcription factor, persists in the central nervous system from embryonic to adult stages. In the adult stage, nearly all Olig2 positive cells co-express NG2 proteoglycan, and constitute a subpopulation of oligodendrocyte precursors (OPCs). So-called "adult OPCs" have abilities to self-renew and to differentiate. Our genetic labeling study in the adult brain revealed that Olig2 positive cells generate NG2 glia (OPCs), oligodendrocytes and astrocytes (Tatsumi et al., 2008; Islam et al., 2009; Okuda et al., 2009). Recently we found a new subpopulation of Olig2-positive cell in the gray matter of the adult forebrain. They are post-mitotic and GFAP-positive, but do not express NG2 proteoglycan. These Olig2-positive astrocytes are distributed widely in the adult brain with clustering in the basal ganglionic nuclei such as the globus pallidus (GP) and the substantia nigra pars reticulata. Both of these nuclei receive inhibitory GABAergic signals from the striatum and the GP respectively, suggesting that Olig2-positive astrocytes extend their fine processes around the inhibitory synapses. Assuming the tripartite synapses theory, Olig2-positive astrocyte may contribute to inhibitory transmission in the adult forebrain.

(COI: No)

P3-029

Role of pro-oligodendroblast antigen in oligodendrocyte differentiation

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The pro-oligodendroblast antigen (POA) that reacts with the pro-oligodendroblast-specific antibody O4 has not been identified biochemically. The O4 also reacts with sulfatide (HSO3-3-galactosylceramide) at the mature OL stage, but sulfatide synthesis at the pro-oligodendroblast stage is uncertain. In the present study, we showed by imaging mass spectrometry that sulfatide existed in restricted regions of the ventricular zone of the spinal cord at embryonic 13.5 mouse, where pro-oligodendroblasts first appear. At this stage, short-chain sulfatide with 20 carbon fatty acids was predominant, while long-chain sulfatide with 24 carbon fatty acids was dominant in adult spinal cord. We examined OL differentiation in cerebroside sulfotransferase (Cst) -null mice that lack sulfatides. The number of immature OLs at embryonic 14.5 in Cst-null mice was lower than that in wild type cervical spinal cord. However, the population of the immature cells in Cst-null mice increased rapidly and became comparable with that in wild type mice at embryonic 16.5. Moreover, in primary OL culture from embryo, significant decreasing in the number of immature OLs was shown at 3 day in vitro in Cst-null mice and it recovered to a normal level at 5 day in vitro. Together, these results demonstrate that POA is the sulfatide species with short-chain fatty acids and regulates the early OL development.

(COI: No)

P3-030

Yokukansan ameliorates glucocorticoid receptor protein expression in oligodendrocytes of the corpus callosum after stress exposure

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Major depressive disorder is probably the oldest and still one of the most frequently diagnosed psychiatric illnesses. Major depressive disorder is one of the leading causes of disturbances in emotional, cognitive, autonomic, and endocrine functions, affecting nearly 7% of the population in Japan. According to the large amount of information on depressive diseases that has been accumulated during recent years, patients with major depressive disorder show an enhanced biologic stress-response mechanism, especially a hyperactive hypothalamic-pituitary-adrenal (HPA) axis and high levels of circulating cortisol. Although dysregulation of the HPA axis by chronic stress is indicative of major depressive disorder, the molecular mechanisms and functional changes in the brain underlying depression are largely unknown. Recently, we reported that stressed mice with elevated plasma levels of corticosterone exhibit morphological changes in the oligodendrocytes of nerve fiber bundles, such as those in the corpus callosum. However, little is known about the molecular mechanism of GR expression regulation in the oligodendrocytes after stress exposure. In this study, by using water-immersion and restraint stress as a stressor for mice, we attempted to elucidate the GR regulation mechanism in the oligodendrocytes and evaluate the effects of Yokukansan, a *Kampo* medicine, on GR protein level regulation.

(COI: No)

P3-031

Oligodendrogenesis of hippocampal axon fibers in the fornix of adult mouse

Miyata, Seiji[1]; Fukushima, Shohei[1]; Furube, Eriko[1]; Nishikawa, Kazunori[1]; Ono, Katsuhiko[2]; Takebayashi, Hirohide[3]; Nakashima, Toshihiro[1] (*[1]Kyoto Institute of Technology, Kyoto, Japan; [2]Kyoto Prefectural University of Medicine, Kyoto, Japan; [3]Graduate School of Medical and Dental Sciences, Niigata Univ., Niigata, Japan*)

Oligodendrocytes are generated at late development to form myelin sheath for proper signal transmission and neuronal survival, recently oligodendrocyte progenitor cells (OPCs) are shown to distribute more or less evenly throughout the adult brains in whole life. The present study showed that proliferation of OPCs in the fornix, main axonal pathways of hippocampal neurons, was regulated coordinately with that of neural stem/progenitor cells (NSPCs) in the hippocampus after antidepressant treatment. A peripheral inflammatory stimulation with lipopolysaccharide (LPS) attenuated proliferation of OPCs in the fornix and NSPCs in the hippocampus, but conversely induced robust and transient proliferation of microglia. A microglia inhibitor minocycline suppressed proliferation of OPCs and LPS-induced attenuation of OPC, indicating that microglia play a fundamental role in regulating both basal proliferation of OPCs under normal condition and attenuation of OPC proliferation under inflammatory condition. The administration of vascular endothelial growth factor signaling inhibitor suppressed basal proliferation of OPCs and LPS-induced proliferation of microglia. In conclusion, this study indicates that microglia play crucial function in controlling OPC proliferation in the fornix of adult brains.

(COI: No)

P3-032

Hedgehog signal modulates the release of gliotransmitters from astrocytes

Okuda, Hiroaki; Tatsumi, Kouko; Morita, Shioko; Wanaka, Akio (*Nara Med. Univ, Nara, Japan*)

Hedgehog (Hh) signaling pathway is conserved in a wide range of species from drosophila to human and plays a key role in regulating organogenesis. The sonic hedgehog (Shh), a member of Hh family, is an essential factor for central nervous system development. Shh stimulates differentiation of neural stem cells into motor neurons or interneuron in the neural tube and proliferation of immature cells in cerebellum and retina. Hh signaling molecules are also found in the adult brain, implying that Hh signaling functions in the mature nervous system. In the present study, focusing the function of Hh signaling in the adult mouse brain, we first examined expression of Hh signaling molecules in the adult mouse brain by in-situ hybridization. Patched 1, which is a receptor of Hh family members, was expressed in S100beta positive astrocytes and Shh mRNA was expressed in HuC/D-positive neurons in the adult mouse cerebellum. These results suggest that Hh is involved in neuron-glia interaction. We further confirmed that the Hh signal molecules were expressed in cultured cerebellar astrocytes using RT-PCR. We next examined whether or not recombinant Shh N-terminal (rShh-N) activates Hh signaling pathway and regulates astrocytic functions in vitro. rShh-N treatment induced D-serine release and inhibition of Hh signaling pathway led to decrease in glutamate and ATP release from cultured cerebellar astrocytes. These findings suggested that Hh signal pathway modulates release of gliotransmitters and is related to neuro-glial interactions in the adult mouse brain.

(COI: No)

P3-033

The roles of fatty acid desaturase on the differentiation of cultured neural stem cells

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Polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and arachidonic acid, are essential for the growth and functional development of the brain. In postmortem human brain tissues of Alzheimers disease, depressive disorder, and schizophrenia, decreasing PUFA levels and decreasing mRNA levels of enzymes for PUFA synthesis were observed. Moreover, there is a failure in cell proliferation and differentiation of neural stem cells (NSCs) in these diseases. We hypothesized that enhancement of PUFA synthesis in the brain is an important target to prevent and treat these diseases. The present study examined roles of PUFA synthetic enzymes on the differentiation of the cultured rat fetal NSCs. Addition of 1% fetal calf serum (FCS) increased the percentage of GFAP (an astrocyte marker) -positive cells. Addition of B27, a medium supplement that increases neuronal survival in primary CNS cultures, increased the percentage of Tuj-1 (a neuronal marker)-positive cells. FCS and B27 treatment increased the mRNA levels of stearoyl-CoA desaturase, delta-5 and delta-6 desaturases, and fatty acid elongase-5 via sterol regulatory element-binding protein (SREBP) 1c transcriptional activation. SREBP1c is known as a main regulator of PUFA synthesis. These results suggest that activation of PUFA synthesis is involved in promoting the differentiation of NSCs.

(COI: No)

P3-034

Protective effects of a novel nucleic acid analogue (COA-Cl) against oxidative damage in PC12 cells

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COA-Cl is a synthesized nucleoside analogue with the molecular weight of 284. We previously reported that it has angiogenic potency both *in vitro* and *in vivo*. We also found that COA-Cl promoted the synthesis and secretion of VEGF, the most robust pro-angiogenic growth factor in human fibroblast.

In this study, we investigated the neuroprotective effects of COA-Cl on H_2O_2-induced apoptosis in rat pheochromocytoma (PC12) cells. H_2O_2 (0-200 μM, 24 h) increased LDH release from PC12 with decrease in cell viability. However, treatment with COA-Cl (100-200 μM) significantly reduced LDH release and attenuated the decrease in cell viability dose dependently. In addition, immunoblot analysis showed that COA-Cl inhibited H_2O_2 -induced apoptosis by increasing the Bcl-2/Bax ratio. We also examined the ability of COA-Cl against oxidative damage caused by a neurotoxin 6-hydoroxydopamine (6-OHDA), which has been widely used to generate the experimental model of Parkinsons disease. COA-Cl showed the similar protective effects against the 6-OHDA induced oxidative stress to those against H_2O_2. Collectively, COA-Cl might be considered to be a promising neuroprotective agent against oxidative damage.

(COI: No)

P3-035

Prosaposin overexpression after kainic acid-induced neurotoxicity

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Excessive glutamate release plays a pivotal role in numerous neuropathological disorders, such as ischemia or seizure. We aimed to investigate whether intrinsic prosaposin (PS), a neuroprotective factor when supplied exogenously in vivo or in vitro, is up-regulated after the excitotoxicity induced by kainic acid (KA), a glutamate analog. In this study, PS immunoreactivity and its mRNA expression in the hippocampal and cortical neurons showed significant increases on day 3 after KA injection, and high PS levels were maintained after 3 weeks. The increase in PS, but not saposins, detected by immunoblot analysis suggests that the increase in PS-like immunoreactivity after KA injection was due to an increase in PS as a neurotrophic factor to improve neuronal survival. Furthermore, several neurons with slender nuclei inside/outside of the pyramidal layer showed more intense PS mRNA expression than other pyramidal neurons. These neurons were shown to be GABAergic interneurons in the extra- and intra-pyramidal layers. Several large neurons in the V layer of the cerebral cortex and the choroid showed very intense PS mRNA expression after KA injection. This study indicates that inhibitory interneurons as well as stimulated hippocampal pyramidal and cortical neurons synthesize PS for neural survival, and the choroid plexus is highly activated to synthesize PS, which may prevent excitotoxic neural damage. This study demonstrates axonal transport and increased production of neurotrophic factor PS after KA injection.

(COI: No)

P3-036

Abnormal lysosomes accumulating in Cathepsin D-deficient mouse neurons are targeted for selective autophagy through p62 and NBR1

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Cathepsin D (CD)-deficient mice show a new form of lysosomal accumulation disease with a phenotype resembling neuronal ceroid lipofuscinosis (NCL). In neurons deficient in CD, abnormal lysosomes called granular osmiophilic deposits (GRODs) and autophagosomes accumulated in the perikaryal regions. The present study shows that a part of GRODs were incorporated into double or multimembranous autophagosomes, while such autophagosomes with GRODs were not observed in neurons of mouse brains doubly deficient in CD and Atg7, in which autophagy cannot be executed. In these single knockout mice of CD and even in double knockout mice of CD and Atg7, p62 and NBR1, adaptor proteins of selective autophagy were co-localized with ubiquitin on the limiting membranes of GRODs by immunoelectron microscopy using the cryo-thin section-immunogold method. These results suggest that p62 and NBR1, together with ubiquitin, are involved in selective autophagy of GRODs in CD-deficient mice.

(COI: No)

P3-037

Quantitative analysis of development and aging of genital corpuscles in glans penis of the rat

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The development of genital corpuscle (GC) in human has previously been described by using classical histological methods such as methylene blue staining or silver impregnation. However, other species, including rats, have not been examined and a quantitative study of GC development is also lacking. This study report a quantitative evaluation of the development and aging of GCs in the rat glans penis using protein gene product 9.5 (PGP9.5) immunoreactivity as a neuronal marker. In addition, neural elements in the glans penis were studied by immunohistochemical staining for calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY). GCs were identified as corpuscular endings consisting of highly branched and coiled axons with many varicosities, which were immunoreactive for PGP9.5. GCs were also immunoreactive for CGRP and SP, but not for VIP and NPY. The results revealed that densities and sizes of GCs in the rat develop postnatally, reach the peak of development after puberty, and continue to exist until old age, in contrast to prenatal and early postnatal development of other sensory receptors of glabrous skin. These results suggest possibility that GCs develops under influence of sex hormone.

(COI: No)

P3-038

Translational machinery and protein synthesis in growth cones of rat dorsal root ganglion neurons; atomic force microscopic and fluorescence microscopic analysis

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Although the concept of local translation in neurons is widely accepted, there is a debate about whether axonal translation occurs. Herein, we analyzed the presence of ribosomal proteins in the growth cones of rat dorsal root ganglion (DRG) neurons, by immunofluorescence analysis. Actual protein synthesis was monitored by puromycin technology. Structural analysis was performed using atomic force microscopy (AFM). DRG neurons were prepared from embryonic rats and dissociated using trypsin. DRG neurons were resuspended in culture medium and plated onto dishes. They were maintained in DMEM containing CPT-cAMP to facilitate axon elongation and growth cone formation. Brain-derived neurotrophic factors were applied to induce translational activation under the presence of puromycin. After AFM observation, specimens were labeled with Alexa 488 phalloidin for actin filament staining, followed by anti-ribosomal protein P0/P1/P2 antibody. Immunofluorescence images revealed that actin filaments were distributed in the peripheral region and in the filopodia. The positive regions of ribosomal protein P0/P1/P2 were closely related to the distribution of actin filaments. AFM images showed that high regions of DRG tended to be rich in actin filaments and ribosomal protein P0/P1/P2, compared with low regions of DRG. These results are discussed in relation to locally-synthesized proteins and are related to the three-dimensional structure of DRG.

(COI: No)

P3-039

Expression of nitric oxide synthase (NOS) isoforms after peripheral nerve transection in mice

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The distribution and function of nitric oxide synthase (NOS) in various tissues and organs have been investigated. There are 3 isoforms of NOS: nNOS present in nerve cells, eNOS present in vascular endothelial cells, and iNOS synthesizing NO in response to cytokine stimulation. In this study, to clarify the action of NOS in neurodegeneration and nerve regeneration after transection of the sciatic nerve in mice, we immunohistochemically investigated NOS expression. The right sciatic nerve was cut in mice. The nerve was excised with the surrounding tissue and frozen sections were prepared. The sections were immunostained following the standard method and observed under a light microscope. Strong NOS positivity was detected in the nerve fiber stump on the central side after transection. Inflammatory cells infiltrating around the stumps on the central and peripheral sides were positive for iNOS on day 1, but the positivity level decreased after day 7. eNOS was positive in blood vessels on the central and peripheral sides of the stump from day 1. nNOS-positive nerve fibers were noted mainly on the central side of the stump, and then extended toward the peripheral side, suggesting that NO is involved in the nerve regeneration process.

(COI: No)

P3-040

Localization of VIP immunoreactive and NPY immunoreactive neurons in the rat submandibrar ganglion

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Introduction: The submandibrar ganglion(SMG) have been considered to be involved in parasympathetic nervous system. In many physiological studies have been reported on account of SMG was not just relay nucleus, but also may have more complicated function. This study was intended to examine distribution of NPY immunoreactive and VIP immunoreactive neurons in SMG with a confocal laser microscope. Furthermore, ultrastructure of SMG was investigated in conventional optical and electron microscopes.
Material and Methods: Animals were fixed, SMG were removed, and frozen sections were cut at 20 micrometer thickness. Sections were treated for double-immunohistochemical demonstration of VIP and NPY and examined with a confocal laser microscope.
Results and Discussion: Although most of SMG neuron showed NPY immunoreactivity, neurons around of the hilum were negative. The ganglion neuron around of the hilum showed VIP immunoreactivity, and on the other hand NPY immunoreactive neurons were not obseved. No morphological difference was demonstrated between the neuron around the hilum and in the neuron of any other parts by electron microscope. Most of SMG neurons showed NPY immunoreactivity and VIP immunoreactive neuron were localized around the hilum. Conclusion was that SMG was related with the superior cervical ganglion.

(COI: No)

P3-041

Generation and characterization of a transgenic rat line expressing Venus under control of the gastrin-releasing peptide promoter

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We previously demonstrated that the sexually dimorphic gastrin-releasing peptide (GRP) system in the lumbosacral spinal cord mediates male sexual function, and this spinal system is developed and regulated by an androgen dependent manner. In parallel, it has been reported that the somatosensory GRP system in the spinal cord contributes to the regulation of itch specific transmission independently of the pain transmission without a sexual dimorphism. The purpose of this study is to establish the animal model that is able to efficiently analyze two different spinal GRP systems controlling male sexual function and itch sensation *in vivo*. Therefore, we generated the GRP-Venus transgenic (Tg) rat expressing Venus under control of the GRP promoter. We first observed the Venus fluorescence in the lumbosacral spinal cord of GRP-Venus Tg rats. Using immunohistochemistry, we also found that most GRP neurons in the lumbosacral spinal cord co-expressed Venus fluorescence. In addition, in GRP-Venus Tg females, a long-term androgen treatment significantly increased the number of Venus-positive neurons in the lumbosacral spinal cord. RT-PCR analysis further confirmed the expression of Venus mRNA both in the lumbosacral spinal cord and spinal ganglion. Thus, GRP-Venus Tg rat model appears to be a powerful tool for analyzing spinal GRP systems controlling male sexual function and itch sensation.

(COI: No)

P3-042

Expression of Trk-fused gene protein in the motor neurons of the rat corticospinal tract

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The *TRK*-fused gene (*TFG* in human, *Tfg* in rat) has originally found in cancer tissues as a part of fusion-oncoprotein in anaplastic lymphoma and mixoid chondrosarcoma. In the normal cells, studies were shown that TFG involves in ER-golgi protein secretion and the NF-kB pathway signaling. Although TFG mutations were found neurodegenerative diseases affecting motor and sensory functions in hereditary motor and sensory neuropathy with proximal dominant involvement (HMSNP), hereditary spastic paraplegias, and Charcot-Marie-Tooth disease type II (CMT2), a role of TFG in the nervous system remains unclear. We have previously produced an antibody against rat TFG and used it to localize TFG to selected neurons in specific regions in the rat brainstem. In the present study, we investigate in TFG immunoreactivity in the motor neurons in the brainstem and spinal cord of the rat corticospinal tract. We have identified the TFG-positive neurons in the parts of cranial motor neurons in the rat brainstem and motor neurons in the ventral horn of the spinal cord. Our TFG expression data provides better understanding of TFG in the motor system.

(COI: No)

P3-043

Masticatory muscle inflammation prolongs MAPK activation in the brainstem

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Object: The mechanism of myofascial pain syndrome (MPS) is little known. The aim of our study is to elucidate the mechanism of MPS, so we examined the effect of MAPK in the trigeminal subnucleus caudalis (Vc) activation induced by a noxious stimulation of the left masseter (LM).
Methods: The LM of Sprague Dawley rats (male, 250g, n=60) was stimulated as follows: 1) L-S6 (experimental) group; The rat's LM was injected with lipopolysaccharide 2 μg/kg (100 μl) on the 1st day of the experiment. On day 2, the same site was injected with 6% sodium chloride solution (S6, 100 μl, 5 times per 90 min). 2) S-S (control)group: The rat's LM was injected with normal saline (S, 100 μl) on the 1st day of the experiment. On day 2, the same site was injected with S (100 μl). Rats were allowed to survive for 1 day, 7 days or 14 days after the last injection. The masseter s and brainstems were dissected and cut with a cryostat (at 30 μm thickness). These specimens were investigated with anti-bradykinin receptor B2 (BKRB2, masseter) or anti-p-p38 MAPK (brainstem) enzyme labeled antibody method. The specimens were observed and evaluated using a light microscope mounted with a 3CCD digital camera system.
Results: The BKRB2-immunoreactive (IR) cells both groups were observed until 7 days after stimulation. In the experimental group, the p-p38 MAPK-IR cells were particularly observed in the Vc until 14 days after stimulation. However, the p-p38 MAPK-IR cells in the control group were little existed until 3 days.
Conclusion: The MAPK activation expression is activated by chronic pain.
(COI: No)

P3-044

Prosaposin and its receptors in the cerebellum after kainic acid injection

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Prosaposin (PSAP), a highly conserved glycoprotein, is a precursor of saposins A-D. Accumulating documents suggest PSAP to be a neurotrophic factor in vivo and in vitro that induces differentiation and prevents death in a variety of neuronal cells through the active region within the saposin C domain. Recently, GPR37 and GPR37L1 were recognized as PSAP receptors. In this work, we explored the variation in expression of PSAP and its receptors in Purkinje cells from the cerebellum by immunohistochemistry using rats injected with kainic acid (KA). The data show that PSAP was expressed in the cytoplasm of Purkinje cells and was markedly enhanced on days 3, 7, and 14 following KA treatment. Meanwhile, the expression of GPR37L1 was increased on days 1 and 3 but not on day 7 or 14 compared with rats receiving normal saline. In contrast, the expression of GPR37 was significantly diminished on days 7 and 14, in contrast to the expression pattern of GPR37L1. These findings indicate that PSAP protects Purkinje cells from damage induced by KA with the aid of its receptors, GPR37L1 and GPR37, and that these receptors have diverse effects during this process.
(COI: No)

P3-045

Deletion of *Crmp4* results in altered morphology and physiology in the olfactory bulb

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Collapsin response mediator protein 4 (CRMP4) is suggested to be involved in neuronal development. Since previous reports showing roles of CRMP4 were mainly performed *in vitro*, information about roles of CRMP4 *in vivo* is insufficient. And no physiological phenotypes in *Crmp4*-knockout (KO) mice have been reported, making it difficult to elucidate *in vivo* roles of CRMP4. Our previous study showing strong expression of *Crmp4* mRNA in the olfactory bulb (OB) from postnatal day (PD) 0 to PD7 suggested important roles of CRMP4 in OB development. Here, we aimed to explore phenotypes of *Crmp4*-KO pups by examining morphology and physiology of the OB. In morphological studies on the OB, *Crmp4*-KO pups had longer apical dendrites of mitral cells and thicker external plexiform layer whose main constituents are mitral cells' apical dendrites, compared to those in WTs. With physiological analyses, we found that *Crmp4*-KO pups exhibited impaired olfactory discrimination ability by measuring ultrasonic vocalizations emitted from pups. Activity-dependent c-Fos expression revealed that *Crmp4*-KO pups exhibited hyperactivities in the OB. In addition, mRNA expressions of not GABA receptors but glutamate receptors of AMPA type were increased in *Crmp4*-KO pups than WTs, suggesting that enhanced excitatory circuits contribute to their hyperactivity phenotype. Our data indicate that CRMP4 is involved in the morphological as well as physiological development of the OB.
(COI: No)

P3-046

Immunohistochemical analysis of opsin 5, an ultraviolet-absorbing photopigment, in chicken and mouse neural tissues

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Opsin 5 (Opn5) is one of the recently identified opsin groups that is responsible for nonvisual photoreception in animals. We previously showed that a chicken homolog of mammalian Opn5 (Opn5m) and mouse Opn5m are Gi-coupled ultraviolet sensors. We demonstrated that mouse Opn5m evolved to be a more specialized photosensor by losing one of the characteristics of bistable pigments, direct binding of all-trans-retinal, which is acquired by a single amino acid replacement. Thus, chicken and mouse Opn5m have a different molecular property. To know whether there might be different physiological functions of Opn5m between mouse and chicken, here we analyzed the expression patterns of Opn5m in chicken and mouse neural tissues. We found that, like chicken Opn5m, mouse Opn5m was localized to a small subset of cells in the ganglion cell layer and inner nuclear layer of the retina. On the other hand, the mouse Opn5m was expressed in the preopic area of the hypothalamus, while chicken Opn5m is expressed in the posterior hypothalamus, specifically paraventricular organ, which is known to be photosensitive in invertebrates.
(COI: No)

P3-047

Newly-identified sexually dimorphic gene expressions in the mouse medial preoptic area of the hypothalamus

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The medial preoptic area (MPOA) in the anterior hypothalamus has crucial roles for the sexually dimorphic behaviors and physiological regulations. The steroid hormone receptors are abundantly expressed in the MPOA, and such receptor expression must be involved in the behavioral difference between sexes. It is also well known that the some MPOA subregions show the sexual difference in their volume, the neuron density and/or the fiber density (called sexually dimorphic nucleus, SDN). The regulatory role of SDN for some behaviors or physiology, however, has not been identified because of the poor understandings in their anatomical aspects, especially in their gene expression. In this study, we examined the sexually dimorphic gene expression in the mouse MPOA. Several candidate genes which expressed in specific MPOA subregions were picked up through the database search in the Allen Brain Atlas. The cDNAs of candidate genes were subcloned, and transcribed to synthesize riboprobes. We performed *in situ* hybridization on the adult mouse brain sections to elucidate their sexually dimorphic expression. As a result, at least five genes showed sexually dimorphic expressions in the MPOA, and three of them were newly identified molecular markers for the SDN. We also performed the double-labeling study of c-Fos and mRNA, and will discuss the possible role for the SDN on the sexually dimorphic behaviors.
(COI: No)

P3-048

Relationship between cFos- and nitric oxide synthase-immunoreactivity in neurons in the rat subfornical organ after intraperitoneal injection of hypertonic saline

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It is well known that the subfornical organ (SFO) is an osmosensor and administration of hypertonic saline induces elevation of neuronal activity in the SFO. It is essential to clarify the characteristics of such neurons. There are many nitric oxide synthase (NOS)-immunoreactive neurons in the SFO, thus the present study was designed to reveal the relationship between cFos-immunoreactivity (a marker for elevated neuronal activity) neurons after administration of hypertonic saline and NOS-immunoreactivity in SFO. Male SD rats were intraperitoneally injected 5 ml of hypertonic (5.265%) or isotonic (0.9%, control) saline (n=3, each), anesthetized 5 min later, and perfused with 4% paraformaldehyde. cFos-immunoreactivity was found in 3.8% and 1.1% of NOS-immunoreactive neurons in the SFO of the hypertonic and isotonic saline injected rats, respectively, although the ratio was not significantly different. This finding suggests that a certain population of NOS-immunoreactive neurons in the SFO is responsible for elevation of neuronal activity in the SFO in response to application of hypertonic saline.
(COI: No)

P3-049

The expression of the oxytocin-monomeric red fluorescent protein 1 fusion gene in the hypothalamus and spinal cord of acute nociceptive model rats

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Oxytocin (OXT) is a well-known neurohypophysial hormone that is synthesized in the paraventricular (PVN) and the supraoptic nuclei (SON) of the hypothalums. Several lines of evidence have suggested that OXT plays an important role in pain modulation and analgesia. However, little is known about the neuronal spinal networks responsible for OXT effects. The present study examined the effects of acute nociceptive stress on the expression of the OXT-monomeric red fluorescent protein 1 (mRFP1) fusion gene in the hypothalamus and spinal cord of the transgenic rats. As the acute nociceptive model, OXT-mRFP1 transgenic rats were subcutaneously injected with formalin at the bilateral hindpaws. We observed mRFP1 fluorescence in the PVN, the SON, and the dorsal horn in the spinal cord after formalin injection. The expressions of the mRFP1, and the OXT gene in the hypothalamus were also measured by in situ hybridization histochemistry. We revealed that mRFP1 and OXT mRNA levels in the PVN and the SON and the mRFP1 fluorescence in the dorsal horn were significantly increased at 2 hour after formalin injection compared with controls. We have assessed whether increased hypothalamic OXT may associate with spinal pathway and influence mechanical nociceptive threshold.

(COI: No)

P3-050

The effects of hormonal fluctuation during pregnancy and postpartum on the expression of estrogen receptor α and neuronal morphology in the amygdala

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During pregnancy from the first trimester to a few months after delivery, some of the women suffer from depression. Amygdala is one of the central regions which regulate emotion. We investigate the effect of hormonal fluctuation during pregnancy and postpartum on the expression of estrogen receptor α and neuronal morphology in the amygdala. We used virgin female Wistar rats [gestational day 15 (G15), 20 (G20), 4 days after delivery (P4), and normal estrous (E)] to perform immunohistochemistry and the Rapid Golgi Stain. Rats were perfused with paraformaldehyde. And the brains were sectioned, incubated with anti-ER α and treated with diaminobenzidine. At P4, the number of ER α immunoreactive cells in the central amygdala was decreased compared to G15 and G20 ($p<0.05$). The rat brains were stained by using the Rapid Golgi Stain protocol. We selected pyramidal neurons in the amygdala. And the dendritic spines were counted along the first branch of the apical dendrite and spine density was determined by the number of the spines in 10 μm. At P4, the number of mushroom type spine density in the amygdala was decreased compared to G15, G20 and estrous ($p<0.05$). These results suggest that the perinatal dynamic changes of the estradiol concentrations may affect to the morphological changes in the amygdala through the response with ER α, resulting in emotional instability.

(COI: No)

P3-051

Effects of cutaneous stroking on the responses to immobilized stress of serotonin releasein the central nucleus of the amygdala in rats

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The present study aimed to examine the effects of cutaneous stroking on responses of 5-HT release in the CeA to immobilization in rats. A coaxial microdialysis probe was stereotaxically implanted in the CeA and perfused with modified Ringer's solution at a speed of 1 μl/min. The dialysate output from the probe over consecutive periods of 10 min was manually injected into the HPLC and the amount of 5HT was measured with an electro-chemical detector. Rats were placed for 20 min in a handmade restraint box made of paper clay, and fixed all four limbs to a board using strings and adhesive tape. Stroking stimulation was applied manually to the back during immobilization period. The 5HT release in the CeA significantly increased during 20 min-immobilization period. On the other hand, the 5-HT release showed no significant changes in response to the immobilization when stroking was applied simultaneously. These results demonstrate that cutaneous stroking can eliminate the responses of 5-HT release to immobilization stress. Since 5-HT in the CeA is known to cause fear and anxiety behaviors, present results suggest that tactile stimulation of the skin dampen these emotions induced by immobilization stress.

(COI: No)

P3-052

Responses of serotonin release in the central nucleus of the amygdala to cutaneous stroking of rats are mediated via type1 corticotropin releasing factor receptors in the dorsal raphe nucleus

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We have found that responses of serotonin (5-HT) release in the central nucleus of the amygdala (CeA) to cutaneous stroking were abolished after administration of a non-selective corticotropin releasing factor (CRF) receptor antagonist, α-helical-CRF (9-41), into the dorsal raphe nucleus (dRN) in anesthetized rats. In the present study we examined the contribution of both type 1- and type 2- CRF receptors in the dRN to the responses of 5-HT release. A coaxial microdialysis probe was stereotaxically implanted in the CeA and perfused with modified Ringer's solution at a speed of 1 μl/min in anesthetized rats. Stroking stimulation was applied to the back for 10 min. After vehicle injection into the dRN, stroking stimulation decreased the 5HT release. The responses of 5-HT to stroking were abolished after injection of antalarmin, a type 1 CRF receptor antagonist, while those were not influenced by antisauvagine-30 (ASV-30), a type 2 CRF receptor antagonist. These results suggest that responses of 5HT release in the CeA to cutaneous stroking are mediated via the type 1 CRF receptor in the dRN.

(COI: No)

P3-053

Immunohistochemical analysis and behavioral tests for mdx52 mice, a model for DMD

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Duchenne muscular dystrophy (DMD) is a severe X-linked degenerative disorder of the muscle. This disorder is caused by mutations of dystrophin gene. Besides the muscle, full-lengh dystrophin protein, Dp427, is expressed in the brain. Dystrophin is expressed in neurons of the cerebral cortex, cerebellum, hippocampal CA1-CA3 regions and amygdala basolateral nucleus (BLA). Dp427 is an actin-binding scaffold protein selectively localizes in the postsynaptic membrane of GABAergic synapses. Previous studies suggest that a lack of Dp427 induces reduction of the number of $GABA_A$ receptor α2 subunit clusters and failure of maturation of GABAergic synapse in *mdx* mice, a model for human DMD. Besides Dp427, isoforms from *DMD* gene, such as Dp140 and Dp71, are express in the brain. Recent analysis of mutation in *DMD* gene indicates two deletion 'hot spots', which are the regions exons 2 to 20 and exons 45 to 50. Mutation in the former induces a deficit in the expression of Dp427 only, but that in the latter induces deficits in the expression of both Dp427 and Dp140. Previous clinical studies suggest that the mutation in *DMD* gene that induces deletion of Dp140 in addition to Dp427 correlate with cognitive deficits and psychiatric symptoms. However, other studies do not find significant effects of Dp140 deletion in central symptom. The effects of Dp427-deletion in non-intellectual abnormality are not understood. In this study, we examined immunohistochemical staining of GABAA receptors in the BLA and several behavioral tests for *mdx52* mice, which lack both Dp427 and Dp140 because of deletion in the exon 52 in *DMD* gene.

(COI: No)

P3-054

Altered localization of FoxO1 in neuron by methamphetamine

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The mammalian forkhead box O (FoxO) is a family of transcription factors consisting of FoxO1, FoxO3a, FoxO4 and FoxO6. FoxO1 is expressed in liver, fat, pancreas β cells, brain and so on. It has been reported that FoxO1 is involved in various physiological functions, such as apoptosis, cell division and glucose metabolism. FoxO1 is downstream of PI3K/ Akt in the insulin signaling pathway. FoxO1 shifts into the cytoplasm and its transcriptional activity is decreased by phosphorylation by Akt. FoxO1 is abundantly expressed in the striatum and remains to be incompletely understood about the physiological function in the striatum. The striatum receives the modification by dopamine nerves. Then, we investigated whether FoxO1 in the striatum is regulated by dopamine by administering dopamine-related drugs to ICR mice and conducting immunostaining. FoxO1 translocated to the nuclei from the cytoplasm by the administration of metamphetamine, which increases dopamine concentration in the synapse cleft. Metamphetamine is known to also promote the release of catecholamines such as noradrenaline, but the nuclear translocation of FoxO1 by metamphetamine was inhibited by pre-treatment of each SCH23390, antagonist for D1 dopamine receptors and Haloperidol, antagonist for D2 dopamine receptors. Moreover, co-administration of each A68930, agonist for D1 receptors and Qunipirole, agonist for D2 receptors was required for the nuclear translocation of FoxO1. From the above findings it was suggested that dopamine is involved in the transcriptional regulation of striatal FoxO1.

(COI: No)

P3-055

Ferulic acid decreases serotonin metabolism of the striatum in vivo with no tocxicity to dopaminergic neurons

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Ferulic acid (FA) is a phytochemical compound naturally presents in several plants and foods, having antioxidant effect. It is recently known that FA exerts beneficial action in depressive-like behaviors and it interacts to monoamine neurotransmitter such as dopamine (DA), norepinephrine and serotonin (5-HT). To know FA effects on DAergic neurons, we first investigate the toxicity to cultured DAergic neurons. DAergic neurons were prepared from embryolic mesencephalon of Wistar rat and cultured in Neurobasal A + B27 at a density of 1.0 x 105 cells/cm2 for 5 days, followed by staining to tyrosine hydroxylase (TH). There was no change of TH-positive cell numbers by FA treatment (0-30 ug/ml). Furthermore, FA did not show neuroprotective effect of DAergic neurons against 6-hydroxyDA oxidative stress. We next investigated the effects of FA on monoamine metabolism in the striatum using in vivo microdialysis-HPLC. A tendency of decrease of HVA level as shown just after FA treatment. FA caused in decrease of 5-HT metabolite (5-HT and 5-HIAA), indicating that FA might interact serotonin synthetases (tryptophan hydoxylasearomatic or L-amino acid decarboxylase) in vivo. Data suggest that FA changes monoamine levels without toxicity to DA neurons.

(COI: No)

P3-056

GABAergic neurons are present as a cluster in the A11 region of rat brain

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A11 dopaminergic (DA) neurons are the only DA neurons that innervate the spinal cord and dysfunction of A11 DA system may cause restless legs syndrome. Based on recent findings, the DA neuron-enriched regions such as A8, A9, and A10 regions are composed of not only DA neurons but also GABAergic and glutamatergic neurons. Moreover, these non-DA neurons in the A10 region regulate neuronal activities of the DA neurons in the A10 region. However, little is known about neuronal composition of the A11 region. In this study, to determine whether or not the A11 region contain GABAergic or glutamatergic neurons, we performed DIG in situ hybridization for non-DA neurons and examined the distributions of the GABAergic and the glutamatergic neurons throughout the A11 region. Interestingly, we detected GABAergic neurons as a cluster in the middle of the A11 region and this cluster was located adjacent to a TH cluster, but not overlapped completely with the TH cluster. In contrast to the GABAergic neurons, the glutamatergic neurons were sparsely distributed in this region. These results suggest that 1) A11 region contain not only DA neurons but also GABAergic neurons and glutamatergic neurons as previously reported in the A8-A10 regions. 2) In the middle of the A11 region, GABAergic neurons are present as clusters adjacent to the TH clusters. These GABAergic neurons may regulate the activity of the DA neurons that project into the spinal cord.

(COI: No)

P3-057

Neuronal activations in midbrain regions after peripheral administration of peptide YY in mice: an effect of postingestive consequences

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Peptide YY (PYY), one of anorectic gut hormones, is released from gastrointestinal tract after meal and reduces subsequent food intake. Several studies showed that peripheral administration of PYY activates various brain regions including the nucleus of solitary tract, arcuate nucleus, dorsal striatum, nucleus accumbens (NAc), central nucleus of the amygdala and ventral tegmental area (VTA) in rodents. From these results we can assume that neural activations by PYY in several brain regions are involved in postingestive consequences such as reduction of hunger drive and/or visceral discomfort. To test this assumption, we explored neural activity in extensive midbrain regions using c-fos immunohistochemistry. Seventy to ninety minutes after an intraperitoneal administration of PYY, mice were perfused with 4% paraformaldehyde and brains were removed. Fos expressions were detected with immunohistochemical staining. Fos-immunopositive cells were obviously found in the VTA, rostromedial tegmental nucleus (RMTg) and reticulotegmental pontine nucleus. Furthermore, prominent Fos expression was found in the periaqueductal gray (PAG) and dorsal raphe (DR). These results suggest that neural activations by PYY in the ventral (VTA and RMTg) and dorsal (PAG and DR) midbrain regions contribute to positive and/or negative postingestive consequences after meal.

(COI: No)

P3-058

Moxibustion activates brain reward system of rats

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Moxibustion, one category of the Oriental medicine, originated in ancient China and developed in modern Japan. As a folk remedy, it is believed to be effective for keeping health and for the treatment of various chronic illness. In contrast to acupuncture, for which scientific evidences have accumulated very recently, nothing have been reported on the effect of moxibustion on the body. As the first step of its exploration, its effect on the brain was attempted in order to throw light on the physiological effect of moxibustion.

Rats treated with moxibustion were examined by brain microdialysis to measure secretion of dopamine, by conditioned place preference test (CPP) with unbiased method to know whether moxibustion acted as reward, and by immunohistochemical and quantative-PCR analysis of the brain tissues to measure the expression of c-fos as a marker for the activation of neuron.

Moxibustion treatment induced dopamine secretion in the nucleus accumbens, dorsal striatum and medial prefrontal cortex where the expression of c-fos was detected. Animals showed a preference to moxibustion in CPP.

These results suggested that moxibustion acted as a reward, and reactions of the dopamine reward system supported these physiological phenomena. This could be the first evidence on the effect of moxibustion on the body and be the fundamental basis for further clarifying the mechanism of the oriental medicine and development of its new therapy.

(COI: No)

P3-059

Chronological Changes of Prosaposin in the Dentate Gyrus after Birth

Matsuda, Seiji[1]; Morishita, Midori[1]; Nabeka, Hiroaki[1]; Shimokawa, Tetsuya[1]; Doihara, Takuya[1]; Yamamiya, Kimiko[1]; Kobayashi, Naoto[2]; Hamada, Fumihiko[3] ([1]*Grad. Sch. Med., Ehime Univ., Ehime, Japan;* [2]*Med Education C, Grad. Sch. Med., Ehime Univ., Ehime, Japan;* [3]*Anat, Ohita Med Univ., Ufu, Japan*)

Chronological Changes of Prosaposin in the Dentate Gyrus after Birth. Seiji Matsuda, Midori Morishita, Hiroaki Nabeka, Tetsuya Shimokawa, Takuya Doihara, Kimiko Yamamiya, Naoto Kobayashi*, Fumihiko Hamada**Anat Embryol, Education C*, Ehime Grad Med. Anat, Oita Med Univ**Prosaposin (PS), a highly conserved glycoprotein, is a precursor of saposins A-D. Many reports suggest PS to be a neurotrophic factor that induces differentiation in a variety of neuronal cells. This study investigated changes in PS in the dentate gyrus of young rats using double immunohistochemistry with antibodies to PS, PSA-NCAM, and NeuN. PS immunoreactivity was intense in the dentate gyrus at postnatal day 3 (P3) and P7, but decreased gradually after P14. In the dentate gyrus at P28, immature PSA-NCAM-positive neurons localized exclusively in the subgranular zone where neurons were PS -negative, whereas mature Neu-N-positive neurons were positive for PS. Laser microscopy images at higher magnification were examined for PS immunoreactivity in the nuclei and cytoplasm at P1, P7, P14 and P21. In situ hybridization assays showed that PS in the adult dentate gyrus is dominantly a secreted type of PS (Pro+9). These results imply that PS secreted from mature neurons stimulates proliferation and maturation of immature neurons in the dentate gyrus.

(COI: No)

P3-060

Immunohistochemical study on the substructures of the mouse subiculum

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

Subiculum (Sub) is the major output part of the hippocampal formation. It receives afferents mainly from the CA1 region and sends efferents to many cortical and subcortical areas. Tracer experiments have revealed the topographies of these projections inside the Sub, but the cytoarchitecture of the Sub is not fully understood. So we explored the substructures of the Sub immunohistochemically using antibodies against several different substances. In line with previous studies, calbindin (CB)-immunoreactivity showed the border between the proximal and distal parts of the Sub, with the former being more intensely labelled than the latter. Both the CA1/Sub border and the Sub/presubiculum (PreS) border could also be determined by CB-immunolabelling. The sharp border between the Sub and PreS was further represented by immunolabeling for glutamate receptor 1. The above three borders among CA1/proximal Sub/distal Sub/PreS, which were delineated by CB-immunolabeling, matched the borders identifiable with vesicular glutamate transporter 2 (VGluT2)-immunolabelling. Moreover, a specific subregion in the proximal Sub was newly found by VGluT2-immunolabeling. This subregion was occupied by accumulation of large VGluT2-positive boutons that surrounded somata and proximal dendrites of VGluT2-negative neurons in the intermediate depth of the subicular cell layer. This subregion was also characterized by higher densities of parvalbumin-positive and SMI-32-positive neurons than the surrounding regions and was observable at the ventral part of the hippocampus but disappeared more dorsally.

(COI: No)

P3-061

A novel function of CPEB1 mRNA 3'UTR in dendrites

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The cytoplasmic polyadenylation element-binding protein 1(CPEB1) is a mRNA-specific translational control factor that has two RNA-recognition motifs and two zinc-finger motifs, and inhibits translation of CPE-containing target mRNAs by recruiting specific eIF4E-binding proteins, such as neuroguidin in neurons. Upon glutamatergic stimulation, CPEB1 is phosphorylated by either Aurora kinaseA or CaMk2α, resulting in enhanced mRNA polyadenylation and local translation in post-synaptic regions. In this study, we focused on the mechanism of CPEB1 mRNA regulation, including RNA trafficking and activity-dependent local translation in dendrites. To investigate whether CPEB1 mRNA resides in dendrites, we visualized CPEB1 mRNA dynamics using the MS2-GFP system in living hippocampal neurons and found that the 3'UTR of CPEB1 mRNA was transported to distal dendrites and co-localized with either Staufen or CPEB1 itself. Furthermore, addition of CPEB1 3'UTR to GFP resulted in robust reduction in the GFP expression level compared to GFP alone although there was little down-regulation of its mRNA in hippocampal neurons. The precise mechanisms that regulate the translational repression of CPEB1 in dendrites have not yet been identified, but these results suggest that the CPEB1 might be controlled by other translational repressor(s) or CPEB1 itself, which enables the regulation of CPE-containing mRNAs in response to various neural stimuli.

(COI: No)

P3-062

Visual experience regulates MeCP2 expression in the dLGN in developing mice

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In the dorsal lateral geniculate nucleus (dLGN), retinogeniculate synapses develop through three phases. The first phase is synapse formation (P0-P10), and the 2nd is synaptic elimination and strengthening (P10-P20). These processes do not require visual experience. The 3rd is the visual experience-dependent synaptic maintenance phase (P20-), because visual deprivation for one week after P20 induces remodeling of retinogeniculate fibers. The Methyl CpG binding protein 2 (MeCP2) is reported to be important for visual experience-dependent maintenance of retinogeniculate synapses. However, the MeCP2 expression pattern in the dLGN throughout the three developmental phases has been unclear. MeCP2 immunopositive glutamatergic neurons in the dLGN were very few at P10 (11.1%), and then dramatically increased after P20 (>83.6%). Almost all GABAergic neurons were immunopositive for MeCP2 throughout the developmental phases (>98.3%). Interestingly, dark rearing from P21 for 10 days decreased MeCP2 expression only in glutamatergic neurons in the dLGN. These results raise the possibility that the MeCP2 expression level in glutamatergic neurons in the dLGN is regulated in visual experience-dependent manner during the synapse maintenance phase.

(COI: No)

P3-063

Molecular heterogeneity of perineuronal nets in the thalamic reticular nucleus

Ohgomori, Tomohiro; Jinno, Shozo (*Grad. Sch. Med., Kyushu Univ., Fukuoka, Japan*)

The perineuronal net (PNN) was first described by Camillo Golgi in 1882 as a reticular structure that enwrapped the soma and dendrites of neurons. Seminal histochemical works have shown that PNNs are closely associated with parvalbumin-positive (PV+) GABAergic inhibitory neurons. Although PNNs and PV+ neurons play a critical role in regulation of neural plasticity in the developing visual cortex, the function of PNNs in other brain regions remains largely unclear. To address this issue, here we examined the molecular characteristics and topographic distribution of PNNs in the thalamic reticular nucleus (TRN), the gateway of thalamocortical pathways. The component of PNNs was characterized by using Wisteria floribunda agglutinin (WFA) and antibodies against proteoglycans. Considering the connections with cortical regions, the TRN was divided into three distinct sectors: the rostral sector is related to motor cortex and limbic areas, the intermediate sector is associated with somatosensory cortex, and the caudal sector has connections with visual and auditory cortices. In mature mice, WFA+ PNNs were seen in all sectors of the TRN. Interestingly, the labeling was more intense in the rostral and caudal sectors than in the intermediate sector. Aggrecan+ PNNs were most prominent in the intermediate sector, and they were rarely found in the caudal sector. Versican+ PNNs were seen in the dorsal part of the rostral sector only. PV+ neurons were seen in all sectors of the TRN. Our findings suggest that the molecular heterogeneity of PNNs in the TRN may be involved in regulation of topographically organized thalamocortical pathways.

(COI: No)

P3-064

Pin1 gene deficient mice impaired spatial cognitive function and exhibited frontotemporal lobar atrophy

Ohtaki, Hirokazu[1]; Kiriyama, Keisuke[1]; Watanabe, Jun[1]; Yamamoto, Rena[1]; Matsumoto, Minako[1]; Takahashi, Katsuhiko[2]; Uchida, Takafumi[3]; Shioda, Seiji[1] ([1]*Dept. Anat. Showa. Univ. Sch. Med., Tokyo, Japan;* [2]*Inst. Medicinal Chem., Hoshi Univ., Tokyo, Japan;* [3]*Mol. Enzymol., Dept. Cell Sci., Grad. Sch. Agricultural Sci., Tohoku Univ., Sendai, Japan*)

Pin1 is a ubiquitous peptidyl-prolyl cis/trans isomerase (PPIase) and has been shown to be necessary for cell growth and apoptosis. While pin1 deficit was suggested to contribute to Alzheimer's disease, the relation between behavior and pin1 has not been understood in detail. Pin1-/- and wild-type mice were obtained as a littermate and examined behavior test battery 9 to 24 month old. The test was Y-maze, open-field, object recognition and Morris water maze to examine the recognition, learning and memory function. After the test, the animals were measured T2-weight MRI images in brain and were sacrificed to collect brain samples followed by 4% PFA fixation. The brain was cut into 40-μm serial sections, and were stained by 1% thioflavin-S to detect β-amyloid. Pin1-/- mice showed significant difference with wild-type in object recognition and Morris water maze. Brain weight and volume by MRI did not show any different in both mice. However, the size in coronal plane at bregma -0.5 mm and more anterior showed a decrease in Pin1-/- mice. The mice also increased thioflavin-S reactions consisted with the piriform cortex. These results suggest that Pin1 deficit could impair entorhinal cortex including piriform cortex and result in spatial recognition impairment.

(COI: No)

P3-065

Excitatory and inhibitory inputs to vasoactive intestinal polypeptide-expressing neurons in the mouse primary somatosensory cortex

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GABAergic interneurons in the mouse neocortex have been classified into three subgroups based on gene expression: (1) parvalbumin-containing (PV+) neurons; (2) somatostatin-immunoreactive (SOM+) neurons; (3) 5HT3a receptor-expressing neurons, including vasoactive intestinal polypeptide-expressing (VIP+) neurons and the others. In the present study, we conducted morphological examination on the excitatory and inhibitory inputs to VIP+ neurons in layer 2/3 of the primary somatosensory cortex. The somata and dendrites of VIP+ neurons were visualized by using VIP-Cre knock-in mice and adeno-associated viral vectors (AAV2/1). The injected brain sections were then triple-immunostained for GFP, presynaptic markers and postsynaptic markers. The apposed presynaptic punctae on GFP-positive membrane were considered as putative input sites only when the postsynaptic markers existed on the apposed points. After reconstruction of somata and dendrites of VIP+ neurons in layer 2/3, the excitatory and inhibitory inputs to VIP+ neurons were counted. Both cortico- and thalamocortical excitatory inputs were frequently observed on the distal portions of dendrites of VIP+ neurons. On the other hand, each input of subgroups in GABAergic interneurons varied from proximal to distal; the inputs from PV+ neurons were frequently observed on the cell bodies and proximal dendrites of VIP+ neurons, whereas those of SOM+ neurons and VIP+ ones were mainly found on the distal dendrites.

(COI: No)

P3-066

Freely-moving mice exhibit emotional sweating on their soles in response to stress stimulus and during sleep from onset -A finding by the use of sweating-aided electrocardiogram floor sensor-

Sato, Shinichi; Kanbayashi, Takashi; Shimizu, Tetsuo (*Dept Psychiatr, Grad Sch Med, Akita Univ., Akita, Japan*)

Non-primate mammals have eccrine sweat grands only on their soles of paws. The sweating from the soles is considered to be prerequisite to improve the friction that is needed when they do hunt, fight, escape or climb up a tree. Such sweating is defined as emotional sweating, which is mediated by sympathetic activity enhancement. We have been investigating an electrocardiogram (ECG) recording of freely-moving mice using a floor sensor, which is made of a plate with multiple stripes of gold-plated electrode on the surface and designed to detect ECG when at least two paws touch different electrodes separately. However, actual ECG-detection rate of the sensor was very low because of the high electrode-skin contact impedance due to dried up soles, so that ECG appeared only when mice have sweat on their soles. In fact, ECG appeared immediately after hitting a cage with a stick, suggesting that the stress stimulus caused sweating by sympathetic activation. Interestingly, ECG also appeared during the period of Quiet Sleep in contrast to Quiet Waking state without ECG appearance. The heart rate immediately after the stress stimulus and 2-min after the onset of quiet sleep was 773 ± 21 (n = 8) and 374 ± 70 b/m (n = 5), respectively. The sweating during quiet sleep seemed not due to sympathetic activation because the heart rate was lower than the intrinsic heart rate (478 ± 36 b/m, n = 7). Further study is needed to clarify the unclear mechanism of the sweating during sleep on soles of paws in mice.

(COI: No)

P3-067

Oxidative stress in the lateral/ventrolateral periaqueductal gray does not play a role in evoking abnormal fear bradycardia in rats with heart failure

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We previously reported that 1) bradycardia in response to fear in rats was parasympathetically mediated by activation of lateral/ventrolateral periaqueductal gray (l/vlPAG), and that 2) fear bradycardia was enhanced in rats with heart failure (HF) compared to that in healthy controls. Mechanisms underlying abnormal fear bradycardia in HF have been unknown. In the present study, we attempted to determine if oxidative stress developing in the l/vlPAG of rats with HF contributes to enhancement of fear bradycardia. HF was induced in rats after myocardial infarction by coronary artery ligation. Superoxide generation in the l/vlPAG, as evaluated by dihydroethidium staining, was enhanced in rats with HF [fractional shortening (FS) < 25%, N=5] compared with that in sham-operated healthy rats (FS > 40%, N=5). In another set of conscious HF rats (N=11), five-min exposure of white noise sound (90 dB), which induced freezing behavior (an index of fear), evoked bradycardia response [-55 ± 10 beats per min (bpm) vs. baseline, mean ± SE]. Bilateral microinjection into the l/vlPAG of Tiron (200 mM, 100 nl), which has a superoxide scavenging activity, did not modulate the fear bradycardia (-60 ± 12 bpm). The present data did not support the concept that oxidative stress in the l/vlPAG plays a role in enhancing fear bradycardia in HF.

(COI: No)

P3-068

Expression of hemoglobin in presympathetic neurons of rat hypothalamic paraventricular nucleus with bombesin-induced activation

Tanaka, Kenjiro; Yuri, Kazunari (*Kochi Med Sch, Kochi Univ, Kochi, Japan*)

A hemoglobin a-chain-derived peptide RVD-hemopressin is reported as a ligand for cannabinoid CB1 receptors. We previously showed that RVD-hemopressin inhibited centrally administered bombesin (a stress-related peptide)-induced secretion of adrenal catecholamines (adrenaline and noradrenaline) in the rat, suggesting an inhibitory role of brain cannabinoid system in the sympatho-adrenomedullary outflow. In this study, presympathetic spinally projecting neurons of the rat hypothalamic paraventricular nucleus (PVN), which have been shown to regulate the adrenal response, were labeled with a fluorescent retrograde tracer. Then, we examined the immunoreactivity of hemoglobin a-chain or RVD-hemopressin with neuronal activation marker Fos in the fluorescently labeled presympathetic neurons following the intracerebroventricular administration of bombesin. The bombesin induced Fos immunoreactivity in presympathetic PVN neurons with hemoglobin a-chain immunoreactivity. RVD-hemopressin-like immunoreactivity was also detected in presympathetic neurons of the PVN. These findings implicate the involvement of hemoglobin a-chain and RVD-hemopressin in modulating the sympatho-adrenomedullary outflow.

(COI: No)

P3-069

Hypothalamic mechanisms of autonomic nerve regulation by GLP-1

Tanida, Mamoru; Kuda, Yuhichi; Kurata, Yasutaka; Shibamoto, Toshishige (*Dept Phyiol2. Kanazawa Med Univ, Ishikawa, Japan*)

In the present study, we examined effects of intracerebroventricular (ICV) injection of GLP-1 on autonomic nerve outflows in anesthetized mice, and found that GLP-1 dose-dependently increased sympathetic nerve activity to the kidney, and that activated renal nerve outflow was inhibited by pre-treatment of Exendin-3 (9-39), suggesting that sympathetic regulation by hypothalamic GLP-1 is mediated with GLP-1 receptor. In addition, sympathetic nerve activities to the white adipose tissue and the liver were stimulated by ICV injection of GLP-1. To clear hypothalamic intracellular mechanism, we investigated effects of ICV injection of GLP-1 on MAP kinase, PI3 kinase and PKA signaling in the hypothalamus, and found that GLP increased phosphorylation levels of PKA substrate, not ERK1/2 and Akt in mice. In addition, ICV injection of KT5720, PKA inhibitor, suppressed renal sympathetic response to GLP-1. Using rats, we found that c-Fos induction in the hypothalamic PVN was increased by ICV injection of GLP-1. Thus, these lines of evidence let us suggested that hypothalamic GLP-1 might act to PVN through the receptor-mediated intracellular PKA signaling and regulate autonomic nervous system for homeostasis.

(COI: No)

P3-070

The submandibular salivary secretory responses elicited by the activation of the non-NMDA and NMDA receptors in the superior salivatory nucleus neurons

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The parasympathetic preganglionic cells in the superior salivatory nucleus (SSN) receive inputs from sensory nerves as well as many of CNS nuclei. We investigated whether activation of the non-NMDA and NMDA receptors in the SSN neurons, performed by the microinjection of non-NMDA receptor agonist (AMPA, 0.1mM, 50nl, pH7.4, Sigma) and NMDA receptor agonist (NMDA, 0.1mM, 50nl, pH7.4, Sigma) into SSN region, elicit submandibular salivary secretory pressure responses in urethane-chloralose anesthetized rats. The submandibular salivary secretory pressure responses were elicited by microinjection of AMPA. Mean total volumes of the AMPA-induced saliva was 23.8 mg. The salivary secretory pressure responses was induced at mean latency of 23.6 seconds and lasted for mean time of 535 seconds. The average of the time constant in initial secretory pressure response was 34.4 seconds, and that of initial pressure increasing rates was 7.1 mmHg/s. The submandibular salivary secretory pressure responses were elicited by microinjection of NMDA. Mean total volumes of the NMDA-induced saliva was 12.4 mg. The salivary secretory pressure responses was induced at mean latency of 23.0 seconds and lasted for mean time of 190 seconds. The average of the time constant in initial secretory pressure response was 28.1 seconds, and that of initial pressure increasing rates was 6.8 mmHg/s. In conclusion, activation of the non-NMDA and NMDA receptors in the SSN neurons elicits submandibular salivary secretion.

(COI: No)

P3-071

Expression of c-Fos in the hypothalamus and the cardiovascular response during stress in Parkinson's disease model rats

Mori, Rintaro; Ishihara, Jun; Harasawa, Kazutaka; Ohashi, Hiroki; Takahashi, Tomoyuki; Horiuchi, Jouji (*Dept Biomed Eng, Toyo Univ, Saitama, Japan*)

Parkinson's disease (PD) is a neurodegenerative disease that evoked by lack of the dopamine neurons in the substantia nigra of the midbrain. It is known that PD patients have an autonomic dysfunction, such as orthostatic hypotension. However, the mechanism of the autonomic dysfunction is still unclear. Recently, it has been reported that orexin neurons in the hypothalamus are reduced in the PD patients. In addition, the orexin neurons are localized at the hypothalamus, especially in perifornical area (PeF) and around dorsomedial nucleus (DMN). The PeF and DMN play an important role on the autonomic response to stress. Therefore, we hypothesized that function of the PeF and DMN against stress metamorphoses in the PD model. To verify this hypothesis, we stained c-Fos protein, a marker of neuronal activation, expressed by air-puff stress in the PD rats. The PD rat was developed by injecting 6-hydroxdopamine into the medial forebrain budle. After establishment of the PD model, the air-puff stress was made to the PD rat. In sham-operated rats, the air-puff stress caused pressor and tachycardic responses during the air-puff stress. However, increase in HR during the stress reduced in the PD rat. Expression of c-Fos protein in the DMN was similar level with the sham rat. In contrast, c-Fos positive-neurons in the PeF were significantly suppressed in the PD rat. The results suggest that cardiac dysfunction may occur in the PD model and the dysfunction is related to poorly-reactive neurons in the PeF against the stress.

(COI: No)

P3-072

Arterial blood pressure response to L-homocysteine microinjected in the rat ventrolateral medulla autonomic areas

Takemoto, Yumi (*Basic Life Sci, Institute of BHS, Hiroshima Univ., Hiroshima, Japan*)

Elevated plasma L-homocysteine concentration is related to cardiovascular and neurological diseases. Arterial blood pressure (ABP) is regulated by the brain autonomic nuclei including the rostral ventrolateral medulla (RVLM) and caudal VLM (CVLM). However, the cardiovascular actions of L-homocysteine in those brain nuclei are unknown. ABP and heart rate (HR) are modulated by the homologue L-cysteine stimulation in the RVLM and CVLM of rats, via ionotropic NMDA and non-NMDA glutamate receptors. Therefore, the present study examined 1) if microinjected L-homocysteine influences ABP and HR in the RVLM and CVLM, and 2) if the action is mediated by ionotropic glutamate receptors. Microinjected L-homocysteine increased ABP and HR in the RVLM and decreased in the CVLM, the same as L-glutamate did, in urethane anesthetized paralyzed male Wistar rats. Prior microinjection of MK801 for NMDA receptor blockade, but not CNQX for non-NMDA receptor blockade, abolished responses in both brain nuclei. Results indicate central action of L-homocysteine via NMDA receptors of the autonomic brain nuclei. High concentration of L-homocysteine would stimulate NMDA receptors in the RVLM and CVLM cardiovascular neurons to influence cardiovascular regulation.

(COI: No)

P3-073

Activation of 5-hydroxytryptamine-1A receptors suppresses tachycardia evoked from the dorsomedial hypothalamus

Sato, Fumitaka; Nagaoka, Yuya; Horiuchi, Jouji (*Toyo University, Saitama, Japan*)

The psychological stress such as air-jet stress causes pressor response and tachycardia. The stress-induced response is mediated via the dorsomedial nucleus in the hypothalamus (DMH), so called "defense area". In addition, activation of serotonin 5-hydroxytryptamine-1A (5-HT1A) receptors in the central nervous system suppress the stress-induced autonomic response, though the central pathway of the response is still unclear. In this study, we investigated that effect of microinjection of 5-HT1A receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) into the DMH on the cardiovasular response evoked by disinhibition (activation) of the DMH. Microinjection of bicuculline (BIC), GABAa receptor antagonist, in the DMH caused significant increases in blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RNA) and these increases gradually returned to the pre-BIC injection level (30-40min). At approximately 10 min after the BIC injection, the all parameters plateaued at the peak, and then microinjection of 8-OH-DPAT was made into the same site of the BIC injection. Soon after the 8-OH-DPAT injection, HR increase elicited by the DMH activation started quicker reduction compare to the control BIC response without 8-OH-DPAT injection. In contrast, the 8-OH-DPAT in the DMH did not affect the pressor and tachycardic responses to the DMH activation. The results indicate that activation of 5-HT1A receptors located in the DMH inhibits the tachycardia evoked by stimulation of neurons in the DMH.

(COI: No)

P3-074

This poster presentation was withdrawn.

P3-075

Comparison of skin sympathetic nerve activities evoked by visual discrimination tasks

Kuwahara, Yuko[1]; Tsukahara, Reiko[1,2]; Iwase, Satoshi[1]; Shimizu, Yuuki[1]; Nishimura, Naoki[1]; Sugenoya, Junichi[1]; Sato, Motohiko[1] (*[1]Dept Physiol, Grad Sch Med, Aichi Med Univ, Nagakute, Japan; [2]Inst. Develop. Res., Aichi Human Service Center, Kasugai, Japan*)

We hypothesized that the skin sympathetic nerve activity (SSNA) contained sudomotor activity is evoked by voluntary movement to prevent slipping. We reported that SSNA bursts evoked by go trials of muscle contraction were related to MRCPs (2013). While sudomotor activity (SSNA followed by SSR: sympathetic skin response) is also known to be evoked by cognitive processing. We could not clarify the relationship between SSNA bursts with go trials and cognition. SSNA bursts were evoked by not only go trials but also no go trials. In this study, we compared SSNA bursts evoked by visual go and no go stimuli to investigate the difference of them. We recorded SSNA from the tibial nerve by microneurography, with corresponding sympathetic skin response (SSR). Electromyogram (EMG) was recorded from dorsal interossei muscles. To reveal cortical cognitive processing of visual cue (go or no go stimuli), electroencephalogram (EEG) on the scalp was recorded and averaged. Event related potential (ERP) was classified by the occurrence of SSR. The average amplitude of SSNA bursts of go trials was larger than that with no go trials. The average onset latency of SSNA bursts of go trials was longer than those of no go trials. In no go trials, ERPs classified by SSR showed the different pattern from ERPs classified by no SSR. These results suggest that SSNA bursts of visual no go trials may be related to cognitive information processing.

(COI: No)

P3-076

Tonic influence of corticotropin releasing factor on arterial pressure, heart rate, and serotonin release in the central nucleus of the amygdala in rats

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In addition to the well-known hormonal action of stimulating ACTH and adrenal cortical hormone secretion, corticotrophin-releasing factor (CRF) acts as a neuronal transmitter. For example, CRF neuron evokes fear and anxiety behavior and sympathetic activation such as increases in arterial pressure (AP) and heart rate (HR). However, tonic influence of the CRF neuron on these functions is not known. We therefore investigated the tonic influence of CRF on the AP and HR, and serotonin (5HT) release in the central nucleus of the amygdala (CeA), which causes fear and anxiety behavior, using of α -helical CRF(9-41), a non-selective CRF receptor antagonist. α -helical CRF(9-41) was i.c.v. administered in the anesthetized rats. AP was recorded continuously from the right carotid artery, and HR was calculated from the arterial pulse wave. 5HT release was measured with use of microdialysis technique and HPLC. Administration of α -helical CRF(9-41) significantly decreased AP and 5HT release in the CeA, but not HR. These results indicate that brain CRF contribute to stimulates arterial pressure and 5HT release tonically.

(COI: No)

P3-077

Muscarinic receptor-mediated excitation of rat intracardiac ganglion neurons

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Modulation of membrane excitability of rat parasympathetic intracardiac ganglion neurons by muscarinic receptors was studied using perforated patch recording configuration. Activation of muscarinic receptors by oxotremorine-M (OxoM) depolarized the membrane, accompanied with repetitive action potentials. OxoM evoked an inward current under voltage-clamp conditions at a holding potential of -60 mV. Removal of extracellular Ca^{2+} markedly increased the inward currents. The OxoM-induced current in the absence of extracellular Ca^{2+} was fully inhibited by removal of extracellular Na^{+}, indicating the contribution of non-selective cation channels. The OxoM-induced current was antagonized by muscarinic antagonists with following rank of affinity: 4-DAMP > pirenzepine > methoctramine, suggesting that M_3 receptors have a dominant role. The OxoM-induced current was inhibited by U73122, a phospholipase C (PLC) inhibitor. The membrane-permeable IP_3 receptor blocker xestospongin-C also inhibited the OxoM response. Furthermore, pretreatment with thapsigargin and BAPTA-AM inhibited the OxoM-induced current, while KN-62, a blocker of Ca^{2+}/calmodulin-dependent protein kinase II, had no effect. These results suggest that the activation mechanism involves PLC pathway, release of Ca^{2+} from intracellular Ca^{2+} stores and calmodulin. The cation channels activated by muscarinic receptors may play an important role in neuronal membrane depolarization in rat intracardiac ganglion neurons.

(COI: No)

P3-078

Functional mapping of visceral sympathetic nerve activity and skeletal muscle blood flow in the hypothalamus of the rat

Nagaoka, Yuya; Sato, Humitaka; Horiuchi, Jouji (*Toyo Univ., Saitama, Japan*)

It is known that there are two different types of behavioral response to stress. One is an active stress reaction known by "fight or flight", and another is a passive response known by "freezing behavior". Both reactions are mediated via the hypothalamus and accompany with the autonomic change. These active/passive reactions, which are recognized to "defense reaction", cause increases in blood pressure (BP), heart rate (HR), and skeletal muscle blood flow (SMF), and reduce visceral blood flow as a result of sympathetic activation. In the present study, we made functional mapping in the hypothalamus, especially in the dorsomedial nucleus and the marginal area (DMH), with measuring the SMF in the left hind leg and the renal sympathetic nerve activity (RNA) in the anesthetized rat. To activate neurons in the DMH, 15nl of DL-homocysteic acid (DLH, 50mM) was injected. As previously reported, the DLH microinjection into the DMH caused increases in BP, HR and RNA and the functional localizations of neurons was observed in the DMH. We also found the areas where increased or decreased the SMF at the left hind leg within the DMH. These opposite responses to the SMF were independent upon the responses to BP, HR and RNA. Therefore, we conclude that there are at least two populations of neurons to constrict or dilate skeletal vascular bed in the DMH and that the neuronal populations in the DMH may play important roles to make redistribution of the blood flow sympathetically during the stress condition.

(COI: No)

P3-079

Expression of c-Fos in the midbrain and the cardiovascular reaction during social defeat stress in rats

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Psychological stress caused by an interpersonal problem is involved not only the development of cardiovascular disorders but also depression and panic attack with excessive autonomic reaction. The stress reaction is elicited by neurons in the hypothalamus in mammals. It is still unknown, however, that the midbrain participates in the stress induced-autonomic response in mammals. In the present study, we investigated the distributions of expression of c-Fos (a marker of neuronal activation) in the midbrain during social defeat stress in conscious Wistar rats. The Wistar rat (an intruder) was moved into a home cage of a Long Evans rat (a resident). After the social-defeated relationship was established between the intruder and the resident, the rats were separated with a wire-mesh in the same cage for 60min. In the intruder rat, blood pressure (BP) and heart rate (HR) transiently increased at the period of first 25-30min and then gradually decreased, whereas both BP and HR maintained at higher level than the pre-stress condition during the defeat stress period. Numerous c-Fos expressions were observed at dorsal region (dPAG) and also at ventral region (vPAG) of the midbrain periaqueductal grey (PAG). It is known that the dPAG is associated to "fight or flight" responses, and that the vPAG is associated with immobility response. The immunohistochemical data are consistent with a recently published result. Therefore, it is likely that the mechanism underlying the response evoked by the social defeat stress involves at least two different PAG regions in the midbrain.

(COI: No)

P3-080

Brain-derived neurotrophic factor immunoreactive vagal sensory neurons innervating the gastrointestinal tract of the rat

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We have determined whether brain-derived neurotrophic factor immunoreactive (BDNF-ir) neurons in the vagal ganglia innervate the gastrointestinal tract. Many BDNF-ir neurons were medium in size and located throughout the jugular and nodose ganglia. When Fluorogold was injected into the wall of the cervical esophagus, many retrogradely Fluorogold-labeled neurons were found in both the jugular ganglion and the nodose ganglion. When Fluorogold was injected into the body of the stomach or applied to the cut end of the subdiaphragmatic vagus nerve, numerous Fluorogold-labeled neurons were found mostly in the nodose ganglion. Double-labeling combining immunohistochemistry for BDNF and retrograde tracing with Fluorogold showed that more than 90% of the neurons in the jugular ganglion and the nodose ganglion projecting to the cervical esophagus expressed BDNF-like immunoreactivity. In the cases of both Fluorogold injection into the stomach and Fluorogold application to the subdiaphragmatic vagus nerve, almost all Fluorogold-labeled neurons in the nodose ganglion expressed BDNF-like immunoreactivity. These results indicated that almost all vagal sensory neurons located in either the jugular ganglion or the nodose ganglion that innervate the gastrointestinal tract are BDNF-ir neurons. Thus, BDNF is thought to be a neurochemical marker for vagal afferent neurons projecting to the gastrointestinal tract.

(COI: No)

P3-081

Intrathecal administration of capsaicin enhances the colorectal motility in rats

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It is known that capsaicin-sensitive sensory neurons modulate gastrointestinal motility. However, their functions in the lumbosacral defecation center are unclear. In the present study, we investigated whether capsaicin acts on the lumbosacral defecation center and affects the colorectal motility. For assessing colorectal motility, rats were anesthetized with alpha-chloralose and the distal colon and anus were cannulated to measure the intracolorectal pressure and propelled intraluminal liquid volume. Stable spontaneous contractions of the colorectum appeared within 1 hour after cannulation in most cases. Intrathecal administration (L6-S1 region of the spinal cord) of capsaicin caused large contractions. The enhancement of phasic contractions after intrathecal administration of capsaicin was accompanied by increased fluid output through the anal cannula. The stimulation evoked by intrathecal capsaicin was prevented if the pelvic nerve was severed. These results suggest that intrathecal administration of capsaicin stimulates colorectal motility maybe by acting on capsaicin-sensitive neurons in the lumbo-sacral defecation center.

(COI: No)

P3-082

Effects of thermal cutaneous stimulation on renal sympathetic nerve activity in rats

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We have investigated the effects of thermal cutaneous stimulation on renal sympathetic nerve activity (RSNA) in anesthetized rats, to clear the relationship between somatosensory system and autonomic nervous system. Using intraperitoneal urethane (1.2 g/kg), and the left renal nerve was exposed through a left flank incision and dissected free from surrounding tissue under a dissecting microscope in Sprague-Dawley rats. Bipolar silver electrodes were put under the nerve to record. The nerve and electrodes were covered and stabilized with silicone rubber gel. Thermal cutaneous stimulation with a hot pad (approximately 42 degree Celsius) was applied to the forepaws and hindpaws of rats, and RSNA was recorded with a power lab system. After a brief stabilization period, RSNA was recorded for 15 min under thermal cutaneous stimulation. RSNA during thermal cutaneous stimulation to the limbs decreased from the control level. However, efects of RSNA during thermal cutaneous stimulation fluctuated by different thermal temperature of the hot pad. These results suggest that thermal cutaneous stimulation to the forepaws and hindpaws may affect RSNA to indicate autonomic nervous system, but they have various effects by different temperature of thermal cutaneous stimulation. We should try to detect the relationship between the condition and the effects of thermal cutaneous stimulation to the limbs.

(COI: No)

P3-083

Regulation of energy metabolism by intracellular Ca^{2+} signals

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Obesity is a leading cause of life-threatening diseases such as myocardial infarction; therefore, clarifying its molecular mechanisms is therapeutically important. Recent evidence suggests that intracellular Ca^{2+} signals play a critical role in the regulation of energy metabolism. We have previously reported that mice lacking the Ca^{2+} sensor protein NCS-1 (KO), which is important for excitable cell functions, exhibit significant obesity as they age *(Circ. Res. 2011)*. In the present study, we investigated the molecular mechanisms of this phenomenon. Using metabolic cages, we found that food intake and locomotor activity were similar between WT and KO groups. However, energy metabolism and thermogenesis indicators such as O_2 consumption/ CO_2 emission and rectal temperature were significantly lower in KO than WT mice. Indicators of mitochondrial function and number (respiratory rate and the levels of UCP1, PGC-1 α, and VDAC) were also lower. Lipid droplets in both brown and white adipose tissues (BAT and WAT) were dramatically enlarged in the KO group and interestingly, NCS-1 was expressed in the BAT. Metabolomic analyses demonstrated that in the KO group, the metabolites involved in energy consumption decreased in the BAT, whereas those involved in energy storage increased in the WAT, leading to massive obesity. Taken together, these results suggest that NCS-1 is a novel regulator of energy metabolism in adipocytes, and hence can be an important target for the treatment of metabolic syndrome.

(COI: No)

P3-084

GLP-1 suppresses reflex swallowing via the medial part of nucleus tractus solitarius

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Our previous study demonstrated that microinjection of glucagon-like peptide-1 (GLP-1) into the medial part of dorsal vagal complex (m-DVC) suppressed reflex swallowing. However, it has not been clarified the effective site where GLP-1 acts to suppress reflex swallowing among the m-DVC. In the present study, we examined the effective site of GLP-1 to suppress reflex swallowing among the m-DVC by the selective lesion of the dorsal medulla. Swallowing was induced by the electrical stimulation of the central cut end of the superior laryngeal nerve and was identified by the electromyogram lead penetrated the mylohyoide muscle through bipolar electrodes. Each animal underwent one of three lesions; 1) ablation of the area postrema (AP) by suction; 2) electrical lesion of the commissural nucleus tractus solitarius (NTS); 3) electrical lesion of the medial NTS. GLP-1 was injected into the m-DVC. The electric lesion of the medial NTS abolished the suppression of reflex swallowing induced by injection of GLP-1 into the m-DVC. In contrast, ablation of the AP and electrical lesion of the commissural NTS did not abolish the suppression of reflex swallowing induced by injection of GLP-1 into the m-DVC. These results suggest that GLP-1 suppresses reflex swallowing via GLP-1 receptor situated in the medial NTS. This work was supported by JSPS KAKENHI Grant Number 24500630.

(COI: No)

P3-085

Local application of sympathetic nerve blockers around dorsal root ganglion reduces painful behavior in a lumbar radiculopathy model

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The purpose of the present study is to examine the effects of sympathetic nerve blockers in a lumbar radiculopathy model using behavioral study. We prepared a lumbar radiculopathy model, and placed a catheter on the dorsal root ganglion to administer the sympathetic nerve blockers. We administered phentolamine (non selective α-antagonist), prazosin (α_1-antagonist), silodosin(α_1-antagonist), and yohimbine (α_2-antagonist) for 3 consecutive days after 0th, 4th and 11th post-operative days. The concentration of sympathetic nerve blockers was 10 or 100mM. Control rats received vehicle injections. Behavioral analysis using mechanical and thermal stimulation was performed before the operation until 28th post-operative day. Phentolamine and yohimbine reduced painful behavior for 28days. Pain analgesic effect of yohimbine was stronger than that of phentolamine. Prazosin relieved painful behavior almost all experimental periods, however, the effect was weaker than that of phentolamine and yohimbine. In contrast, silodosin had no pain analgesic effect. Phentolamine administerd at 4th and 11th experimental periods, attenuated pinful behavior once generated. The present study showed that sympathetic nerve blockers attenuated the painful behavior via α_2-adrenoceptor. Sympathetic nerve blockers were effective after generation of painful behavior. So we consider that sympathetic nerve blockade of α_2-adrenoceptors may contribute to pain relief in neuropathic pain.

(COI: No)

P3-086

Prediction error responses in the mouse posterior parietal cortex are dependent on protocadherin-αdiversity

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Higher brain areas are responsible for detecting and reducing prediction errors. Previously, we have reported that prediction errors between whisker and visual inputs were detected in the posterior parietal cortex (PPC) of mice. We recorded neuronal activities in PPC using flavoprotein fluorescence imaging. Visual stimulation or whisker stimulation alone hardly activated PPC. However, anti-phase combination of moving grating patterns and whisker stimulation, which is very unlikely in natural environment for mice, produced prediction error responses in PPC. As expected, in-phase combination failed to produce any clear activity in PPC. We have reported that cortical depression and map shifts were induced by prediction errors between visual and whisker inputs in the primary visual cortex (V1) of young mice that had worn a monocular prism goggle, suggesting that the prediction errors detected in PPC were reduced in V1. Clustered protocadherins (cPcdhs) are neuron-specific cell adhesion molecules with multiple clusters. The prism-induced depression in V1 and the prediction error responses in PPC induced by the anti-phase combination of visual and whisker stimulation were impaired in mice with reduced cPcdh-α diversity. These results strongly suggest that the diversity of cPcdh-α is important for the PPC function to detect prediction errors between visual and whisker inputs.

(COI: No)

P3-087

Higher visual cortices responsible for shape recognition in mice

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Higher visual cortices responsible for shape recognition were not identified in mice. We hypothesized that the cortical area involved in shape recognition might be activated by sound stimuli after mice had acquired sound-shape association memory. We have reported that wild-type mice can learn the sound-shape association memory using an M-shaped maze equipped a screen and a speaker. In the present study, we investigated cortical responses to the associated sound stimuli using flavoprotein fluorescence imaging. In trained mice, the responses to the sound stimuli appeared in the auditory cortex and the higher visual area located dorsally to the auditory cortex. Two-photon calcium imaging revealed the presence of shape-specific neurons in this area, indicating that the activated higher visual area plays an important role in shape recognition. Many genetically-manipulated strains of mice are available. Clustered protocadherins (cPcdhs) are neuron-specific cell adhesion molecules with multiple clusters. Wild type mice have 12 clusters (α1-α12) of cPcdh-α, while, cPcdh-α1, 12 mice have only α1 and α12 clusters. We found that cPcdh-α1, 12 mice had impaired sound-shape association memory using the M-shaped maze test. Furthermore, the higher visual cortical area was not activated by the associated sound stimuli in these mice.

(COI: No)

P3-088

Divisive normalization during multisensory integration by neurons in macaque area MSTd

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Neurophysiological studies of multisensory integration in single neurons have revealed a set of empirical principles that describe a variety of nonlinear interaction between stimuli from multiple sensory modalities. We previously showed that many of these principles could be explained by a single model based on the divisive normalization mechanism operating in brain regions where multisensory integration is taking place. This normalization model of multisensory integration makes a critical prediction which distinguishes the model from other existing models: that a non-preferred sensory input, which is excitatory on its own, can suppress the response to a preferred input of another modality. We tested this prediction by recording from multisensory neurons in macaque area MSTd that play a critical role for perceiving self-motion by integrating visual (optic flow) and vestibular cues. We show that many MSTd neurons show the diagnostic form of cross-modal suppression. The finding provides strong support for divisive normalization acting at the level of multisensory representations in the brain.

(COI: No)

P3-089

Neuronal activity in the monkey orbitofrontal cortex related to reward value processing during decision-making

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When we make a choice from the alternatives, we consider their values and workloads. To understand the neuronal mechanism of such a decision-making process, we developed a decision-making schedule task and recorded single unit activity from monkey orbitofrontal cortex (OFC) which has been reported to be one of the important brain areas for the reward-guided behavior. Two monkeys were trained to perform a reward schedule task which consists of 1, 2 or 4 trials of visual discrimination to earn 1, 2 or 4 drops of liquid reward. After learning this task, the decision-making schedule task in which two kinds of choice target (CT) were sequentially presented was introduced. The CT brightness and length indicated reward amount and required number of trials, respectively. Then, these two CTs were simultaneously reappeared (choice phase). The monkey was required to choose one of them, and then the chosen reward schedule started. We recorded from 246 neurons in the OFC. In the second CT period, 43.1% (106/246) of the recorded neurons showed correlation between the difference in value of the two CTs and the neuronal firing. Some neurons coded only reward amount (24/246) or workload (5/246) information in the first and second CT period. These results suggest that OFC neurons play an important role in the decision-making by reward value information processing.

(COI: No)

P3-090

Synchronization between respiratory cycles and olfactory neural activations -EEG and fMRI study-

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The link between respiration and olfaction is evidenced explicitly by observed synchronization between respiratory cycles and neural activation of the olfactory circuit during odor perception. Taking advantage of the time-locked nature of inspiration and olfactory processing, electroencephalogram dipole modeling (EEG/DT) has previously been used to identify a cascade of inspiration-triggered neural activity moving from primary limbic olfactory regions to frontal cortical areas during odor perception. In this study, we leverage the spatial resolution of functional magnetic resonance imaging (fMRI) alongside the temporal resolution of EEG to replicate and extend these findings. Brain activation identified by both modalities converged within association regions of the orbitofrontal cortex that were activated from approximately 150ms to 300ms after inspiration onset. EEG/DT was additionally sensitive to more transient activity in primary olfactory regions, including the parahippocampal gyrus and amygdala, occurring approximately 50ms post-inspiration. These results provide a partial validation of the spatial profile of the olfactory cascade identified by EEG source modeling, and inform novel future directions in the investigation of human olfaction.

(COI: No)

P3-091

Effects in EEG and the autonomic nervous system when listening to ringing sound of a wind-bell

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In order to investigate physiological effects of listening to ringing of a wind-bell, we measured EEG and heart rate. Subjects were young healthy 2 men and woman. Two kinds of wind-bell were used, i.e. Nanbu-furin made of iron and glass-furin. As a control sound, monotonic sound generator, digital metronome was used. Average amplitudes of θ, α and β band were not changed during listening to ringing sound. However difference of change width was suppressed by each sound, effectively in occipital regions. Ringing of Nanbu-furin induced suppression of the amplitude difference in 3 bands of 3 persons. Suppression was induced by glass-furin in 2 persons less effectively. Metronome weakly induced the same effect on 2 subjects. These results showed that Nanbu-furin most effectively reduced fluctuation of neural activity in the central nervous system. Reduction of θ and α might show awakening activities and reduction of β might be reduced-neural activities. The parasympathetic nervous activity, analyed by fluctuation of heart rat, was activated and the sympathetic nervous activity was reduced at the beginning of 3 sounds. By using psychological test, the General Arousal Check List (GACL), value of general deactivation was effectively augmented by Nanbu-furin compared to other sounds. These data suggested that listening to Nanbu-furin reduced general neural activity of subjects, namely leading to relaxation in mind, and stimulated the parasympathetic nervous system. This is consistent with the deactivation of arousal level measured by subjective psychological feelings.

(COI: No)

P3-092

Attentional neural network activated by a simple task design and the effect of genetic variations in human brain

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Attention is the climax of mental integration and closely related to consciousness. In order to explore attention in humans, we should use a simple behavioral task. We employed ANT (attention-networks test) that has also been applied to fMRI brain imaging (Fan J. et al., 2005). The task was to request participants to report the direction of an arrow projected on a screen. By using ANT we are able to detect activations in brain associated with alerting by presenting a cue, orienting by spatial cues, and conflict resolving by simultaneously presenting disturbing franker arrows heading towards the other end. Several significantly activated areas have been detected in alerting and orienting. These areas are almost in accord with the dorsal frontoparietal network proposed by Corbetta et al. (2008), involving dorsal parietal and dorsal frontal cortices. Visual association areas were also activated in alerting possibly by the top-down signal. No significant activation was detected in conflict resolving at present (14 subjects). Brain activities are "intermediate" phenotypes situated between genes and human behavior. To assess the intermediate phenotypes, brain imaging by using fMRI is most suited. We are investigating several attention-related and unrelated genetic variations (SNPs) to find the effect of genes on the activation pattern detected.

(COI: No)

P3-093

Keratan sulfate in cortico-basal ganglia circuits is involved in acquired vocalization

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The brain system corresponded to skill movements are constructed during the developments. The learned vocalizations including human speech are acquired in postnatal age. They have sensitive or critical period in juvenile stage. Birdsong learning in the zebra finch occurs during a sensitive period, and the involved brain areas are identified as "song system" in cortex and basal ganglia. However neurochemical properties which clearly discriminate song system from the other circuits remained unclear. Here we report that the highly sulfated keratan sulfate is specifically expressed in song system including HVC (the proper name), robust nucleus of the arcopallium (RA), lateral magnocellular nucleus of the anterior nidopallium (LMAN), and Area X. They are distributed in the extracellular matrix especially in supra cell membrane area. Synaptic connections are formed through their defected area. We evaluated the differences of the keratan sulfate intensities among developmental stages and sex. We also examined the molecular properties by the use of Western blotting and chondroitinase. Our results imply that the highly sulfated keratan sulfate are involved in maturation of the learned vocalization. These findings offer new information to facilitate an understanding of the formation of the experience-dependent vocal learning including human language.

(COI: No)

P3-094

Cell-type-specific sustained activity of LIP neurons during covert search but not in overt saccade in a visual search paradigm

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When a target object is embedded in a complex visual scene and less salient, we should carefully search for that target. Model studies suggest that a degree of carefulness in discrimination tasks might be neuronally set by adjusting the criterion level for decision making. However, this assumption is not consistent with the results of neurophysiological studies. To address this issue, we recorded single-neuron activity in the lateral intraparietal area (LIP) when monkeys performed a color-singleton search task involving both target and no-target (catch) trials. Monkeys had to make a saccade to the target in the target trials, whereas they had to maintain fixation throughout the trial in the catch trials in which no target element was presented in a search array. We found that a part of neurons exhibited the stronger activity when the target appeared in the receptive field in the target trials (acceleration-type neurons), whereas another part of neurons exhibit the stronger activity especially when the monkeys successfully maintain the fixation in the catch trials (break-type neurons). The enhanced activity of break-type neurons disappeared when the monkeys simply fixated into the spot stimulus (not fixation cells) and when they erroneously made a fixation break by making saccades in the catch trials. These findings suggest that break-type neurons activated especially when the monkeys make careful covert search during visual search.

(COI: No)

P3-095

The anxiety- and fear-related behavior on the maternal separated mice

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Early life stress is known to induce long-term alterations in emotional and anxiety-related behaviors. Rodent models of neonatal maternal separation (MS) stress have been used to explore the effects of early stress on changes in affective and cognitive behaviors. MS are associated with structural changes in brain regions linked to cognition and mood regulation. Here, we studied the effects of MS on the alteration of neurogenesis in linbic system and anxiety-related behavior on C57Bl/6 mice. The MS was performed daily for 3 hr from P1 to P14 and behavioral test was started at 10 weeks of age. We used a battery of stress and anxiety-related behavioral tests in C57Bl/6 mice. 1. The open field test, which measures the basal anxiety level, showed that MS mice tended to spend shorter in the center area, although total moving distance did not differ. 2. The acoustic startle response induced by the sudden loud tone stimulus was significantly elevated in MS mice. 3. The contextual and cued fear conditioning test provides a measure of memory by assessing a memory for the association between an aversive stimulus and a tone stimulus. Ms mice showed decreased fear conditioning to the context and the tone compared to control. Especially, freezing time during tone stimulus was significant attenuated in MS. Thus in MS mice, fear memory was impaired, although startle response was elevated. 4. Neurogenesis in the limbic system was increase in MS mice. These results suggest that neonatal MS treatment enhances the neurogenesis and alters the anxiety- and fear-related behavior.

(COI: No)

P3-096

The 5-HT3 receptor is essential for exercise-induced hippocampal neurogenesis and antidepressant effects

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Exercise has a variety of beneficial effects on brain structure and function, such as hippocampal neurogenesis, mood and memory. Previous studies have shown that exercise enhances hippocampal neurogenesis, induces antidepressant effects, and improves learning behavior. Brain serotonin (5-hydroxytryptamine, 5-HT) levels increase following exercise, and the 5-HT system has been suggested to play an important role in these exercise-induced neuronal effects. However, the precise mechanism remains unclear. In this study, analysis of the 5-HT type 3A receptor subunit-deficient (htr3a-/-) mice revealed that lack of the 5-HT type 3 (5-HT3) receptor resulted in loss of exercise-induced hippocampal neurogenesis and antidepressant effects, but not of learning enhancement. Furthermore, stimulation of the 5-HT3 receptor promoted neurogenesis. These findings demonstrate that the 5-HT3 receptor is the critical target of 5-HT action in the brain following exercise, and is indispensable for hippocampal neurogenesis and antidepressant effects induced by exercise. This is the first report of a pivotal 5-HT receptor subtype that plays a fundamental role in exercise-induced morphological changes and psychological effects.

(COI: No)

P3-097

Possible mechanisms of glucose-induced facilitation of spatial memory and hippocampal plasticity

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We have previously reported that intrahippocampal injection of 7 mM glucose, which is similar to the glucose concentration of the cerebrospinal fluid during food intake, facilitates spatial learning and memory in rats. The high glucose pre- and postsynaptically enhances basal synaptic response and tetanus-induced long term potentiation in the rat Schaffer collateral/commissural pathway through increases in CAMKII and PKC autophosphorylations. In the present study, we further sought to clarify the cellular mechanisms of the glucose effects using neuronal (N2A) cell lines. When glucose was increased from 3.5 mM to 7 mM, N2A cells showed an increase in expression of brain-derived neurotrophic factor (BDNF) along with the enhanced phosphorylation of AKT (PKB) and CREB. Interestingly, the glucose-induced upregulation of *BDNF* was blocked by the knock down of CREB using lentiviruses encoding short hairpin-RNA against CREB, while high glucose increased CREB recruitment onto the *BDNF* promoter. Furthermore, glucose stimulation reduced histone deacetylase (HDAC) recruitment near the *BDNF* promoters and an HDAC inhibitor, suberanilohydroxamic acid (SAHA) increased *BDNF* expression. These findings, taken together, suggest that glucose enhances spatial learning and memory at least partly through an epigenetic regulation of BDNF gene expression.

(COI: No)

P3-098

Hippocampal plasmalogens regulate memory related gene expressions via modulating the BDNF-TrkB signaling

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Plasmalogens (Pls) are unique glycerophospholipids carrying the vinyl ether linkage at the sn-1 position of the glycerol backbone. Although it has been reported that Pls content is reduced in the brain of Alzheimer s disease (AD) patients, the precise function of Pls is mostly elusive. To understand the impact of the Pls reduction in the brain, we constructed lentiviruses delivering shRNAs against the Pls synthesizing enzyme, glyceronephosphate O-acyltransferase. Intra-hippocampal injection of the shRNAs in mice reduced the Pls content in the hippocampus, impaired Morris water maze task and reduced hippocampal expression of memory related genes such as BDNF, Trk B, Synapsin-1, Synapsin-2, Synaptotagmin-1, PSD-95, CamKII-α and Homer-1. Since BDNF and its receptor TrkB could regulate expression of those genes, we investigated the role of Pls in the BDNF-TrKB signaling. The function of TrkB receptor was known to be dependent on its localization in the lipid rafts, where we confirmed that Pls were also highly present. Interestingly, when Pls were reduced in the hippocampus the TrkB receptor localization was shifted from raft to non-raft fraction. Furthermore we have observed that Pls diet in mice for 6 weeks increased the expression of BDNF and enhanced the memory task, which was blocked by the knock down of BDNF/TrkB. We therefore, propose that hippocampal Pls are necessary for normal memory task through maintaining the BDNF-TrkB signaling in the hippocampus.

(COI: No)

P3-099

Somatomotor integration underlies tactile learning

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Animals actively acquire sensory information via their interactions with the environment. This is especially evident in active touch, where self-generated movements drive tactile inputs. However, due to the lack of an efficient learning task, the mechanisms of tactile learning have yet to be elucidated. We created a tactile-version of an object recognition task, named the object-floor recognition task (OFRT). This task utilizes floor texture as tactile cue to allow mice to discriminate between the same objects placed on different floors. It was observed that after experiencing only one floor on Day 1, on Day 2, the mice preferred the object on the novel floor compared to object on the familiar floor. This learning did not require room light, indicating that the mice could discriminate floors using tactile cues alone. Optogenetic silencing of the primary somatosensory (S1) hindlimb area impaired memory acquisition. Similarly, silencing of the projections from the secondary motor cortex (M2) to S1 or the S1-M2 pathway also impaired memory acquisition. These results suggest that direct mutual somatomotor projections are important for the perceptual learning for discrimination of tactile texture.

(COI: No)

P3-100

Molecular mechanism of the sensitive period of filial imprinting

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The timing and duration of the sensitive period for learning has been believed to be developmentally fixed and unable to be changed. However, there is now reason to believe that the sensitive period can be flexible in terms of the timing and duration. Filial imprinting in birds is the process of forming a social attachment during a sensitive or critical period, restricted to the first few days after hatching. Imprinting is considered to be part of early learning to aid the survival of juveniles by securing maternal care. We showed that the thyroid hormone determines the start of the sensitive period. Imprinting training in chicks (Gallus gallus domesticus) causes rapid inflow of thyroid hormone. The hormone thus initiates and extends the sensitive period to last more than 1 week via non-genomic mechanisms. It can also confer what we term "memory priming (MP)" to prime subsequent learning. Once chicks have achieved MP, it is maintained for long periods. Even in non-imprinted chicks whose sensitive period has ended, exogenous thyroid hormone enables imprinting. It is possible that the sensitive period closes only if MP is not conferred at an appropriate time of development. Under natural conditions, chicks will learn spontaneously with the help of parents and siblings. In a sense, the closing of the sensitive period for learning may not exist under usual physiological conditions probably because the sensitive period does not close as long as MP is acquired. Our study elucidates the critical role of imprinting to subsequent learning as being governed by the acute action of thyroid hormone.

(COI: No)

P3-101

Neuronal activity in the monkey prefrontal cortex during a temporal classification task

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To address the neuronal mechanism for interval timing in the prefrontal cortex (PFC), we examined neuronal activity in the PFC of a monkey during a temporal classification task. In the task, a visual cue was presented on the center of the monitor from 0.8 to 4.8 sec. Following a 1 sec delay period, the subject was required to press the proper key according to the classification of cue duration; the right, center, and left keys for long (3.2-4.8 sec), middle (1.6-2.4 sec), and short (0.8-1.2 sec) categories, respectively. For the spatial control of key selection and movement, the subject also performed a spatially cued delayed response task, in which a visual stimulus spatially cued the proper key. Of 277 PFC neurons we recorded, 124 neurons were related to the temporal classification task. Two types of task-related activities were interesting. The first one was phasic activity during the cue period with constant peak time after the cue onset. The peak times of these activities broadly distributed with a few peaks including 1.2 and 1.8 sec after the cue onset. These phasic cue activities might function to filter current cue duration with the peak time. The second one was phasic activity during the delay period, which changed according to the cue duration categories. The delay activity might represent temporal classification results of cue duration. These results suggest that the PFC contributes to a classification process of visual signal duration.

(COI: No)

P3-102

Effect of mother-infant interaction on the relationships between amygdalar dopamine release and open-field behaviors

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Early-weaned rodents exhibited changes in behavioral and emotional traits (Shimozuru et al, 2007) and myelin formation in the anterior basolateral amygdala (aBLA; Ono et al, 2008), which the prefrontal efferents terminated reportedly. We previously found that early-weaned SD male rats (postnatal day [PND] 16) exhibited lower paired-pulse facilitation than did late-weaned rats (PND 30), in the prefrontal-aBLA pathway of urethane-anesthetized juvenile rats. Rosenkranz & Grace (e.g., 2001) suggested the efferents regulated the BLA inhibitory interneurons to suppress the affective response of the BLA driven by the sensory cortex: Dopamine (DA) attenuated this regulation and synergistically enhanced the sensory cortex-driving response. We found here, by using in vivo microdialysis, the amygdalar DA were statistically 2.5 times higher for the basal release concentration at home cage in the early-weaned group: The relative reactivity tended to be higher at transfer timing from the home cage to open-field and vice versa. On the contrary, locomotor and rearing tended to be lower at either the timing in the early-weaned group. We will show correlations between the DA release and associated open-field behaviors on the mother-infant interaction at the meeting. The study was supported by KAKENHI (M.T).

(COI: No)

P3-103

Optogenetic silencing of serotonin neurons on escalated aggression in male mice

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Aggression is ethologically important behavior for many animals. However, if the level of aggression becomes escalated, that behavior is no more adaptive and it is important to understand neural mechanism of escalated aggression which is relevant to preclinical and clinical concerns. To escalate aggressive behavior of male mice, we used social instigation procedure in which test animal can see the existence of rival in the protected cage but cannot physically attack it. In vivo microdialysis showed that serotonin (5-HT) release was increased by social instigation and during escalated aggressive behavior in the dorsal raphe nucleus (DRN) and the medial prefrontal cortex. On the other hand, there was no change of 5-HT release during basal species-typical aggression. Therefore, an activation of 5-HT system may be involved in escalated aggression induced by social instigation, but not in species-typical aggression. To examine this possibility, we manipulate activity of 5-HT neurons by optogenetics during aggressive behavior of male mice. KENGE-tet transgenic mice that express inhibitory archaerhodopsin (ArchT) specifically on the 5-HT neurons were used. As expected, there was no effect of optogenetic silencing of 5-HT neurons during species-typical aggression. The effect of optogenetic silencing on escalated aggression will also be presented.

(COI: No)

P3-104

Resting-state brain networks related to personality traits

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A number of brain regions have been suggested to be related to personality traits; however, their neural correlates remain largely unknown. In this study, we determined the relationship of personality estimates with a network centrality of the brain as measured by resting-state fMRI. Personality estimates were obtained from Cloninger's Temperament and Character Inventory (TCI). Big-Five scores were estimated by TCI scores. We used a 3-Tesla MRI (PHILIPS) to obtain T2*-weighted functional (5 min x 3) and T1-weighted structural images from healthy right-handed male subjects (N=89, 18-24 years old). Subjects were asked to stay awake with their eyes closed. BOLD signal was preprocessed through SPM8 and in-house software developed on MATLAB (Mathworks). An adjacency matrix was obtained from all gray matter voxels (down sized to 6x6x6 mm) to determine various network centrality measures (such as degree centrality) for each voxel. We examined whether seven TCI scores and Big-Five estimates were significantly related to these centrality measures ($p<0.05$, FWE corrected by factorial design performed on SPM8). We observed in detail the score-specific spatial distribution of significant voxels not only in the cerebral cortex, but also in subcortical structures. Our results suggest that the personality traits are represented in the resting-state brain networks that involve specific cortical and subcortical regions related to each trait.

(COI: No)

P3-105

Postweaning period is critical for the effect of MSG on social behavior in ADHD model rat

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Attention-deficit/hyperactivity disorder (ADHD) is characterized by hyperactivity, impulsivity and inattention. Dysfunction of mesocorticolimbic dopaminergic (DAergic) system such in the amygdala and prefrontal cortex is related to emotional regulation in ADHD. We reported that oral intake of monosodium L-glutamate (MSG), a taste substance for umami, for 5 weeks from postnatal day 25 (P25) to P60 altered social behavior in ADHD model rat (SHR), and dopamine receptor expression (D1R and D2R) was significantly increased in MSG-treated amygdala. In this study we investigated the critical period in MSG effect on social behavior. SHR rats (P25) were housed in an isolated condition (one rat per cage) and treated with 0.6% MSG for various periods until P60: early-treated group (P25-P40), late-treated group (P40-P60), all-period group (P25-P60) and non-treated group. Early-treated group decreased the number of riding (parameter of aggression to unfamiliar rat) compare with control group, which is the same level as all-period group. However, no significant difference was found between late-treated group and non-treated group. Early-treated group also showed faster reduction in the sniffing time (parameter of exploration), indicating that the rats easily accept to unfamiliar rat. Data suggest that MSG intake during early life (P25-P40) may affect mesocorticolimbic DAergic system, relating to social behavioral changes that persist into adulthood.

(COI: No)

P3-106

Effects of aging and idiopathic Parkinson's disease on tactile temporal order judgment

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It is generally accepted that the basal ganglia play an important role in interval timing that requires the measurement of temporal durations. By contrast, it remains controversial whether the basal ganglia play an essential role in temporal order judgment (TOJ) of successive stimuli, a behavior that does not necessarily require the measurement of durations. To address this issue, we compared the effects of idiopathic Parkinson's disease (PD) on the TOJ of two successive taps delivered to each hand, with the arms uncrossed in one condition and crossed in another. In addition to age-matched non-PD participants, we examined young healthy participants. There was no significant difference between PD and non-PD participants in any parameter of TOJ under either arm posture, although reaction time was significantly longer in PD compared with non-PD participants. By contrast, the effect of aging was apparent in both conditions. With their arms uncrossed, the temporal resolution in elderly participants was significantly worse compared with young participants. With their arms crossed, elderly participants made more errors at longer intervals (~1 s) than young participants, although both age groups showed similar judgment reversal at moderately short intervals (~200 ms). These results indicate that the basal ganglia do not play essential roles in tactile TOJ and that the effect of aging on TOJ is mostly independent of the dopaminergic systems.

(COI: No)

P3-107

Cortical mechanisms of top-down control for precise sensory perception

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Little is known about mechanisms how higher cortical areas control sensory processing. Here, we report the identification and characterization of a neural circuit mediating top-down control in the mouse somatosensory system. The circuit comprises a long-range recurrent horizontal projection between primary somatosensory cortex (S1) and secondary motor cortex (M2). Physiological recordings revealed that M2 top-down input provides temporally coincident input to the upper and lower layers of S1, evoking dendritic spikes and large firing of layer 5 neurons. Contrarily, optogenetic inhibition of top-down input in S1 decreased L5 firing and lead to inaccurate perception. These data demonstrate that bottom-up and top-down inputs to S1 are necessary for accurate sensory perception.

(COI: Properly Declared)

P3-108

A new stop-signal task to explore inhibitory function in operant learning to habituation process

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To control our behaviors properly, we need not only a function to execute a behavior but also a function to inhibit it (inhibitory function). It is becoming clear that the execution of behavior is controlled by a network of the cerebral cortex and basal ganglia, which shows dynamic shift in an operant learning to habituation process. On the other hand, little is known about how inhibitory function is controlled in the course of operant learning to habituation process. To elucidate the mechanism of inhibitory function at cellular and network level, it is necessary to record neuronal activity from animals (e.g., rats) working at a behavioral task which requires inhibitory function. Stop-signal task is often used to assess inhibitory function; however, (1) this standard task takes them a very long period for learning/habituation, and (2) it might be hard to record neuronal activity stably and precisely in usual freely-moving animals during their task performance. To address the issues, we constructed a novel stop-signal task for rats in a head-fixed condition. We took advantage of our spout-lever system (Kimura et al. 2012) to shorten the period of learning/habituation effectively. Thereby, we established the stop-signal task that the head-fixed rats learned to perform in three weeks. This novel stop-signal task will enable us to elucidate the mechanism of inhibitory function in the operant learning to habituation process at a cellular and network level.

(COI: No)

P3-109

Salicylate-induced neural changes of the FM function in the primary auditory cortex of guinea pigs observed by optical recording

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The influence of salicylate on neural activities to frequency-modulated (FM) sounds with different FM sweep rates in the primary auditory cortex (AI) of the guinea pig was investigated using optical imaging with a voltage-sensitive dye (RH795). Eight guinea pigs were anesthetized with ketamine (80 mg/kg) and xylazine (40 mg/kg). Activity patterns to the FM sounds (upward and downward linear sweep: 0.5-16.5 kHz in 16-160 ms duration or FM sweep rate 0.1-1 kHz/ms) and tones (0.5, 16 kHz) at 75 dB SPL were recorded from the AI on both sides before (control) and 8 hours after the intraperitoneal injection of 200 mg/kg salicylate. The peak of the response to the 0.5-kHz tone showed the maximum amplitude at the 40- or 160-ms duration and the peak amplitude of the 0.5-kHz frequency-band (FB) was larger than other FBs independent of the duration. The peak of the response to the FM sound showed the maximum amplitude at the 16-ms duration in the 16-kHz FB and the peak decreased when the FM duration increased up to 64 ms after salicylate injection, but under the control condition up to 40-ms duration. The difference of the amplitudes between the 16-kHz and another FB at the 16-ms duration was larger for the upward FM sound than the downward FM sound under both the salicylate and control condition.

(COI: No)

P3-110

Anti-inflammatory protein TSG-6 secreted by MSCs reduces neural damage and improves memory defects after traumatic brain injury in mice

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Traumatic brain injury (TBI) causes multiple long-term defects including a loss of working memory that is frequently incapacitating. Administrations of mesenchymal stem/stromal cells (MSCs) previously produced beneficial effects in models of TBI as well as other disease models. In several models, the beneficial effects were explained by the MSCs being activated to express TSG-6, a multifunctional protein that modulates inflammation. Intravenous human MSCs or TSG-6 decreased neutrophil extravasation, expression of matrix metalloproteinase 9 by endothelial cells and neutrophils, and the subsequent blood brain barrier leakage. Administration of TSG-6 also decreased the lesion size at 2 weeks. Importantly, the acute administration of TSG-6 within 24 hour of TBI was followed 6 to 10 weeks later by improvements in memory and the number of newly born-neurons. The data suggested that acute administration of TSG-6 may be an effective therapy for decreasing some of the long-term consequences of TBI.

(COI: No)

P3-111

Pre-weaning behavioral patterns in prenatal bisphenol A treated rats

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Bisphenol A (BPA) is well known as one of the environmental endocrine disruptors. Past our study showed that pre- and postnatal administrations of low-level BPA impaired the gender differences in open-field behaviors in rats. BPA also induced the depression-like behavior and enhanced the response to the predator odor in adult age. In this study, we focused on the behaviors in the pre-weaning period. We administered low-level BPA (1.5 mg/kg/day) to prenatal rats, examined the behaviors in 8 days and in 20 days old. In this, we designed the methods using the predator odor (fox odor). In 8 days old, a twitching, pivoting, head-moving, crawling and an immobility were examined. There were no significant effects in the gender and in the treatment. In the presence of fox odor, the decreasing of head-moving and the increasing of immobility were revealed in all groups. Number of animals which displayed the crawling was decreased by the fox odor only in BPA rats. In 20 days old, a rearing, locomotor activity, grooming and the immobility were examined. In the control rats, gender difference was revealed in the locomotor activity, then, this difference was not displayed in the BPA rats. Under the fox odor, the rearing and the locomotor activity were decreased, the immobility was increased in all groups. Corticosterone levels after exposure of fox odor, were increased in all groups. It suggested that stress-induced behavioral alterations were observed in both 8 days and 20 days old. The odor-related changes by BPA treatment were revealed in the crawling, but, generally not so remarkable in other parameters.

(COI: No)

P3-112

Effect of a rubber hand on cross-modal dynamic captures

Wada, Makoto; Ide, Masakazu (*Dev Disorder Sect, Dept Rehab Brain Func, Res Inst of NRCD, Tokorozawa, Japan*)

Apparent motion is sometimes affected by other sensory modalities ('cross-modal dynamic capture', CDC); and tactile temporal order judgment (TOJ) is known to be affected by visual apparent motion. Furthermore, hand images enhance temporal integration of visuo-tactile inputs during TOJ and temporal recalibration. Here, we investigated effects of the hand presentation on the CDC effects during tactile TOJ. In front of a participant, a rubber hand was placed at a forward direction (Forward condition); and it was placed at an inverted direction in the other session (Inverted condition). Participants (n = 12) were required to judge temporal orders of the tactile stimuli to an index finger and ring finger of their hidden right hands and to ignore visual stimuli from LEDs that were placed on corresponding fingers of the rubber hand. When incongruent visual stimuli were delivered, participant's judgment was notably reversed at moderately short intervals during Forward condition. In contrast, amounts of the reversals were significantly decreased during Inverted condition ($P < 0.01$). Furthermore, the changes (Inverted / Forward) were negatively correlated with each Autism Spectrum Quotient ($R = -0.64$, $P < 0.05$). Our present results suggest that the rubber hand corresponding to one's own hand facilitates visuo-tactile CDC effect; and present results might indicate relationships between the multisensory processing and social cognitions.

(COI: No)

P3-113

Effects of periodicity of brush stroking on the rubber hand illusion

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Participants experienced illusory ownership sensation of a dummy hand when the dummy hand and participants' hidden hand were synchronously stroked by brushes (Rubber Hand Illusion, RHI). Previous studies have revealed that synchronous stroking to participants' and dummy hands enhances the RHI rather than asynchronous stroking, and acceptable range of synchronicity to occur the illusion is relatively large (300 ms). However, to date, effect of periodicity of stroking has not been investigated yet. In this study, we examined whether the periodicity of stimulation influences occurrence of the RHI with PC controlled brushes. Participants (N = 24) experienced two times three conditions: 1. Cyclic stroking, 2. Non-cyclic stroking, 3. Asynchronous stroking (e.g., 6 sessions in total). We asked them subjective feelings as for ownership sensation of a dummy hand and amounts of proprioceptive drifts induced by the RHI after each sessions. We found that both cyclic and non-cyclic stroking vividly caused the RHI. However, when non-cyclic stroking was delivered, the ownership sensation was significantly decreased in second session compared to the conditions with cyclic stroking ($p < 0.05$). Amounts of the proprioceptive drifts were not different between the two conditions. These findings indicate that the periodicity of the stimulation intervals as well as the synchronicity of stimulation can influence the ownership sensation of the dummy hand.

(COI: No)

P3-114

A rubber hand experiment using an EMG controlled robotic arm

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Feeling ownership of our limbs represents a fundamental aspect of self-consciousness, and in some circumstances, the feeling is extended out of our own body, as in the rubber hand illusion (RHI, Botvinick and Cohen, 1998). In our rubber hand experiment, we used an in-house electromyography (EMG) controlled robotic arm, and evaluated sense of agency (SA) and sense of ownership (SO) (Kalckert & Ehrsson, 2014). A plastic board was placed horizontally in front of participants (n=15), and each participant placed their right hand under the board. EMG signals were recorded from the participants' arm to control the robotic arm, which was placed above the board. The robotic arm and a participant's own hand are synchronously or asynchronously stroked by paintbrushes. After the experiment, SA and SO were evaluated by subjective ratings (-3 to +3). SO was also measured by a proprioceptive drift. Subjective ratings (SA/SO) were significantly greater than 0 under the synchronous condition ($p < 0.05$). When the participants' arm was synchronously stroked, significantly bigger proprioceptive drift to the robotic arm side (SO) was observed. These results suggest that rubber hand illusion was induced by using an EMG controlled robotic arm.

(COI: No)

P3-115

Real-time change of neural activity in the hippocampal CA1 and medial prefrontal cortex before, during, and after the exposure to a specific episode

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The episodes of the first love or sexual relationship remain in memory strongly. To monitor the process of episodic memory, we recorded neuronal activity in the hippocampal CA1 and medial prefrontal cortex in freely moving young male rats before, during, and after the first encounter with young female rats for 10 min in the male rat's home cage. A few minutes after the female rat was placed in the male rat's home cage, CA1 neurons fired with high frequency (~100Hz) for seconds several times. In addition, the ripple-like events and the amplitude of miniature EPSC and IPSC were increased after the female rats was taken out. Furthermore, the ripple-like events came to be observed in the medial prefrontal cortex 30 min after the female rat was taken out, and which were synchronized with ripple-like events in the CA1. The episode of the first encounter with female rats changed neural activity and synaptic plasticity in the CA1 of male rats. The synchronization of ripple-like events between the CA1 and medial prefrontal cortex might have a role in memory consolidation. These observations shows a real-time change in the neural activity in the hippocampal CA1 and medial prefrontal cortex before, during, and after a specific episode, and could be involved in the process of episodic memory formation.

(COI: No)

P3-116

New periodical components in EEG frequency spectrum observed by corresponding electrode near T5/6 of the brain stem

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Human electroencephalogram (EEG) reflects their brain activities, and it has been widely used in clinical diagnoses as well as basic studies for neurosciences. However, the under -lying mechanism of EEG still not yet well understood. Especially, it must be elucidated how are composed of the electrical activities of the brain stem, as well as those of the cortical and subcortical brain. To address this issue, we here search for areas of normal body, near the brain stem where any localized activities may be recorded or not. Their resting state EEG was recorded from the electrodes placed not only at Cz and Oz, but also at the scalp bow-edge behind the auricle near T5 (left side) and T6 (right side of brain), which is defined here as T5'/6', and really succeeded to obtain an interesting waveforms. We have also investigated the wave signals using the FFT spectrum analyzer, and found that both of T5' and T6' showed the same spectral results of sharp line peaks in low frequency range (2-8Hz) separated almost equivalent frequency spacing of 1Hz. Such components were little influenced by eye-opening and changing the posture. We also observed the activity of nebula by attaching an electrode at inion, and found that almost similar frequency line-spectra. On the other hand, the spectra observed by Cz and Oz electrodes showed only typical alpha peak without any such line peak series. These frequency spectra may attributed to electrophysiological activities derived from the mid-brain and the brain stem.

(COI: No)

P3-117

Relation between hippocampal sharp wave ripple events and prefrontal theta activities during delayed reinforcement task

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Hippocampal sharp-wave ripples are component of the local field potential and characterized short oscillatory activity at high frequency (about 100-250Hz) during slow wave sleep. Importantly, the sharp-wave ripple events are well correlated with memory formation. These events are observed during the awake quiescent state immediately before or after the learning task as well. On the other hand, the interaction between the hippocampus and the prefrontal cortex is an important for memory consolidation. However, the interaction of the hippocampal ripple and prefrontal activities during learning stage has not been established. In the present study, we recorded the hippocampal local field potential during the delayed reinforcement task, one of memory retention test, and analyzed the sharp-wave ripple events. After the recording, the ripple events were determined during delay period of the task and were analyzed separately for correct trials and incorrect trials. In the correct trials, the number of the ripple events immediately before reward cue during delay period was increased and the peak of the number of events was correlated with performance of the task. In contrast, the number of ripple events during incorrect trials showed no significant change. In addition, prefrontal theta activities with hippocampal ripple events were increased at term of immediately before reward cue. The ripples before reward cue may affect prefrontal activities for a reward related memory recall or memory consolidation.

(COI: No)

P3-118

Characteristics of Postural Control Disturbance in Rats with Cerebellar Vermis Lesion: a Study using Posturography Technique

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We aimed to reveal characteristics of postural control disturbances in rats with cerebellar vermis lesion using a posturography technique. For this purpose, under pentobarbital anesthesia, the cerebellar vermis of male rats with a weight of about 300g (n = 5) was removed using a suction device. We measured changes of center of pressure (COP) of the animals during floor inclination in four directions, i.e. left-right and antero-posterior directions, at an angle from 0 to 30 degrees with different angle velocities from 1.8 to 15 degree/sec. In 2 of 5 animals, we also made chronic recordings of EMG activities of extensor muscles of fore- and hind-limbs together with measurements of COP changes. All measurements were made in 2 days before lesion and 2, 7, 14 days after lesion. In 2 days after lesion, most of the animals showed remarkably increases of COP changes during floor inclinations, especially in antero-posterior directions, thus indicating disturbances of postural control. In addition, they also exhibited uncoordinated EMG activities during postural control. These tendencies became much remarkable in 7 and 14 days after lesion. These results suggest that the cerebellar vermis is closely related to the postural control in antero-posterior direction in rats.

(COI: No)

P3-119

Aerobic treadmill training prevents the obesity caused by gene disruption of Ca^{2+}-sensor protein in mice

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Regular exercise with appropriate nutrition can help reduce body fat as well as protect against chronic failure like cardiovascular diseases. Genetic disruptions sometimes induce obesity via unknown mechanism in mice. The neuronal Ca^{2+}-sensor 1 (NCS-1) is one of such genes. Gene knockout (KO) of NCS-1 results in obesity, probably via reduced basal metabolism. NCS-1 is expressed in excitable cells like neurons and hearts, and regulates many physiological functions. In this study, we examined whether proper exercise can prevent obesity in NCS-1 KO mice. A group of age-matched wild-type (WT) and KO mice were subjected to 8-weeks (5 days/week) aerobic treadmill running (slope 10 degree, 15-20 m/min, 60 min) and others were placed to sedentary. In both WT and KO mice, compared to sedentary, training groups exhibited outstanding exercise effects; high running ability at lactate threshold level and enlargement of tibialis anterior muscle fibers. However, echocardiography showed no apparent sign of athletic heart. In both WT and KO mice, training much decreased the body weight and the epididymal fat weight. Training was very effective to reduce the size of lipid droplets in brown and white adipose tissues within interscapular fat, indicating increased fatty acid consumption by training. These findings suggest that aerobic training is very effective to prevent obesity even in genetically inherited obese constitution.

(COI: No)

P3-120

Meso-limbic outflow toward descending motor pathways in monkeys

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Most people had experiences that led to better motor performance when they had higher motivation. In addition, the result of better performance is thought to boost one's motivation. Recently, we found that the meso-limbic system involved in processing of motivation increased the activity in association with that of the primary motor cortex during recovery course from the spinal cord injury in monkeys. Motivation might be a key issue for motor performance and functional recovery. However, it is unclear how the activity of the meso-limbic system affects that of motor-related areas and motor outputs. To clarify this question, evoked-electrocorticogram and upper limb muscle responses induced by electrical stimulation of the meso-limbic system were obtained from two sedated monkeys. Electrical stimulation was applied to the ventral tegmental area (VTA) or the nucleus accumbens (NAc), which are included in the meso-limbic system. The result showed that VTA stimulation induced stimulus-dependent responses not only in the orbitofrontal cortex (OFC) but also in the primary motor cortex (M1). In contrast, NAc stimulation induced responses not in M1 but in OFC. In addition, repetitive stimulation of VTA induced excitatory responses in upper limb muscles. These results suggest that the meso-limbic system can modulate the M1 activity and motor outputs and demonstrated neural substrate for emotional control of motor outputs.

(COI: No)

P3-121

Contribution of the activity of frontal eye field fixation neurons to the suppression of saccades and smooth pursuit eye movements in the monkey

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Focal electrical stimulation in the frontal eye field (FEF) suppresses the generation of saccadic and smooth pursuit eye movements at an intensity lower than the threshold for eliciting electrically evoked saccades. We previously found a localized area of the FEF in the caudal part of the arcuate gyrus facing the inferior arcuate sulcus in which stimulation suppressed the generation of saccades and pursuit in bilateral directions and also where fixation neurons discharging tonically during fixation were concentrated. Fixation neurons usually showed a reduction in activity during saccades. The present study analyzed the activity of fixation neurons in the FEF during pursuit in trained monkeys. Fixation neurons showed a variety of discharge patterns during pursuit, ranging from a decrease in activity to an increase in activity. Of these, more than two thirds of fixation neurons were found to show a reduction in activity during pursuit toward ipsilateral or bilateral directions. When catch-up saccades during the initiation of pursuit were eliminated by step-ramp target routine, the reduction in activity of fixation neurons survived. The present results suggest that fixation neurons in the FEF may contribute to the generation of pursuit suppression. These findings support the idea that this type of fixation neuron assembly as a whole in the FEF may be part of a more generalized visual fixation system through which suppressive control is exerted on pursuit as well as saccades.

(COI: No)

P3-122

Single-unit activity in supplementary motor area of Japanese monkeys walking on a treadmill

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To further understand cortical mechanisms for controlling bipedal (Bp) gait in humans, we recorded single-unit activity from trunk/hind-limb regions of supplementary motor area (SMA) of an unrestrained monkey walking either quadrupedally or bipedally on a treadmill. EMG activity was simultaneously recorded from up to 16 trunk and limb muscles. We found that majority of SMA neurons analyzed (44/51) discharged phasically and/or tonically during the performance of at least one of the two locomotor tasks. Of these locomotor-related cells, more than a half displayed such activity component(s) differently for quadrupedal (Qp) and Bp locomotion, thus the activity of SMA neurons was task-dependent. Interestingly, for Bp locomotion, some cells correlated their activity highly with one of the trunk/hind-limb EMGs (n=7), or peaked during the mid-stance phase (n=15) in a manner similar to hind-limb extensor muscles. Contrary, one third of the locomotor-related cells modulated their activity bi-phasically, which was broadly tuned to the whole step cycle. In addition, small, but significant proportion of the other cells disclosed brief burst activity at the transition of locomotor modes from Qp to Bp during on-going locomotion. Such activity patterns were remote from the recorded EMG activity. Considering input-output organization of SMA, our results suggest that the monkey SMA significantly contributes to the control of locomotion, so as to possibly coordinate movements of trunk and limbs along the rostro-caudal axis as well as movements on the left and right sides of the body.

(COI: No)

P3-123

Physical pain and muscle tonus during hospital bed rest

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Long duration of hospital bed rest induces physical pain in patients. It is believed that pain is correlated with increase in muscle tonus. But data supporting this hypothesis is sparse. The aim of this study is to observe muscle tonus during bed rest using EMG and to evaluate correlations with physical pain. The subjects were 32 healthy adults (M/F = 16/16, mean age 20 ± 1 y/o). EMG were recorded from bilateral eight muscles including M. trapezius, M. erector spinae, M. gluteus medius, M. Obliquus internus abdominis, M. quadriceps femoris, M. biceps femoris, M. tibialis anterior and M. soleus using Neuropack® X1 (NIHON KODEN, Tokyo, Japan). The subjects were instructed to lie in a four-segment adjustable hospital bed for two hours with the head segment elevated to 0° (control), 30°, 45° or 60° (ANGLE). Subjective pain was scored by visual analogue scale (VAS). Vertical and horizontal deviations of the trunk from the original position were recorded. LF/HF ratio of heart rate variability was monitored as indices of sympathovagal balance. Out of 16 muscles observed, seven showed no difference in tonus with time. In nine muscles, EMG activities significantly decreased with time ($p < 0.001$). VAS, LF/HF and deviation of trunk increased with time ($p < 0.001$). ANGLE had significant effect only on vertical deviation of trunk. EMG activity had no correlation with VAS. It is suggested that increase in physical pain due to bed rest does not correlated with increase in muscle tonus.

(COI: No)

P3-124

Axon regeneration and motor function improvement with scaffold-free BMSC sheet transplantation to completely transected spinal cord rat

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In regenerative medicine, the usefulness of cell sheet has been attracting attention. Many studies showed that mesenchymal stem cells (MSC) promote the axonal regeneration of central nervous system, but there is no report of spinal cord injury treatment with sheeted MSC. In this study, we produced bone marrow derived mesenchymal stromal cell sheet (BMSCs) to verify usefulness of the scaffold-free BMSCs transplantation for the completely transected spinal cord model rats. Bone marrow cells were obtained from 7-week-old female F344 rats. BMSCs was made by culture in the standard medium added ascorbic acid. T8 spinal cords of F344 rats were completely transected for 2mm gaps. At 2 and 8 weeks after transplantation, between the two groups: 1)BMSCs transplantation group and 2)control group (gelatin sponge), histological and motor functional evaluation (BBB score) were performed. BBB score of BMSCs group in 8 weeks after injury (mean: 6.75) was higher than the control group. A lot of GAP-43 and Tuj-1 positive axons were observed in the site of sheet transplantation. GFAP strongly positive reactive astrocytes were less than control in the spinal cord stump. By scaffold-free BMSCs transplantation for completely transected spinal cord model rats, axons were regenerated, glial scar formations were inhibited, and motor functions were improved. It was suggested that neurotrophic factor and vascular endothelial growth factor that are secreted by BMSC cause axonal regeneration and glial scar inhibition.

(COI: No)

P3-125

Effect of repeated crush injuries at different intervals on functional recovery of the sciatic nerve

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After a single sciatic nerve crush injury, nerve fibers regenerate and functionalrecovery occurs within 4 weeks in the nerve-crushed adult rats. However, we reported previously that the motor function in the rats with the triple nerve crush injuries every week did not recover to normal range up to 8 weeks because of delay of the reinnervation. In this study, we investigated the effects of repeated nerve crush injuries at different intervals on functional recovery. Double and triple nerve crush injuries of the sciatic nerve were inflicted on adult rats at 1, 2, 3, or 4 weeks intervals. Motor functions were estimated every week until 8 weeks after the last crush injury by the sciatic static index (SSI), a conventional footprint analysis in animal models. SSI is measured by two parameters of 1-5 toe spread and 2-4 toe spread lengths on both sides and widely used for the evaluation of motor function in sciatic nerve-injured rats. At the point of 8 weeks after the last crush injury, reinnervation of the tibialis anterior muscles was estimated by the ratio of β III-tubulin-positive presynaptic nerve terminals for α-bungarotoxin-positive neuroreceptors in the postsynaptic membrane. We report the effects of repeated sciatic nerve crush injuries at different intervals on functional recovery in adult rats.

(COI: No)

P3-126

Architecture of whisker movement related neurons in rat primary motor cortex

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The purposeful movement of biological sensors, such as the motion of the eyes or hands, is an essential part of perception. We don't know what algorithms incorporate movement as part of perception at the level of cortex. Rats move their whiskers to locate and identify objects in their environment. The whiskers provide important tactile information to rats, and therefore have an extended representation in somatosensory and motor cortex. Vibrissa area in primary motor cortex (vM1) controls whisking movements directly via Facial Nucleus (FN) and indirectly via the whisking central pattern generator (CPG) in the brainstem and so on. The facial motor neurons drive the muscles involved in whisking movement. However, what kinds of information from vM1 are sent and where they are sent have not been understood completely. To characterize the motor cortex representation of whisking movements, here we recorded single neuronal activity of vM1 in head-fixed rats performing a behavioral task with juxtacellulary recording method. After recording, we labeled the recorded neuron by injecting plasmid encoding palmitoylation green fluorescent protein (palGFP). We reconstructed and quantitatively analyzed the differences of dendrites and axonal arborization of single vM1 neurons among their firing properties related to whisking movements.

(COI: No)

P3-127

Repetitive masseter muscle activities during NREM sleep in guinea pigs

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Objectives: Rhythmic masticatory muscle activity is observed during NREM sleep in humans. We aimed to physiologically characterize repetitive/rhythmic masseter activities (RMAs) during NREM sleep in guinea pigs in association with cortical and cardiac activities.
Methods: Polygraphic recordings simultaneously with electromyographic (EMG) activity from masseter muscle were made for three hours in the freely-moving guinea pigs. The episodes of the RMAs were visually scored. The electromyographic patterns of RMAs were analyzed quantitatively and compared with those during chewing. Time-course changes of cortical and cardiac activities in relation to the onset of RMAs were also assessed.
Results: RMAs occurred during NREM sleep with a large inter-individual variability in the frequency. Compared to chewing, RMAs was characterized by lower burst activity ($p < 0.001$), longer burst duration ($p < 0.001$) and longer burst intervals ($p < 0.05$). The onset of RMAs was preceded by a decrease in RR-intervals. After the onset of RMAs, the delta power of the electroencephalographic activity and RR interval were transiently changed.
Conclusion: Repetitive/rhythimic patterns of masseter contractions occationally occurred during NREM sleep in the guinea pigs and the occurrence of these episodes were associated with physiological signs of transient arousals.

(COI: No)

P3-128

Tail proprioceptive representation area in the cerebellum identified by using Aldoc-venus knock-in mice

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Aldolase C (Aldoc, also known as zebrin II), a brain type isozyme of the glycolysis pathway, is expressed heterogeneously in subpopulations of cerebellar Purkinje cells that are arranged longitudinally in a complex striped pattern in the cerebellar cortex. Aldoc expression is visualized by expression of a fluorescent protein in Aldoc-Venus knock-in mice. Although Aldoc gene was knocked out in these mice, anatomical features of the brain (size, shape or striped pattern in the cerebellum) and motor coordination (rotarod test) were not different among wild type, heterozygotes or homozygotes. These mice (heterozygotes in particular) enabled in vivo experiments of identified Aldoc stripes in the cerebellum. Although functional localization in vermal lobule VIII has been unclear, we found that Aldoc stripes 1+ and 1- in the caudal apex of this lobuleis intensely innervated by spinocerebellar mossy fiber axons originating from the sacral spinal cord. Flection of the tail produced dynamic spike response in the granular layer in this area, presumably representing mossy fiber activity, in these areas in Aldoc-venus mice. Localized cortical lesioning in this area produced significant decrease in motor coordination in the rotarod test. The results suggest that this area is involved in control of body posture and locomotion by utilizing proprioception of the tail. Kakenhi 25430032.

(COI: No)

P3-129

Glutamatergic plasticity at layers II/III synapses is dependent on the stage of motor learning in rat primary motor cortex

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Synaptic plasticity via AMPA glutamate receptors is associated with memory and learning. To investigate the neuronal mechanism of motor cortical plasticity, we performed a rotor rod test and analyzed layers II/III neurons in the primary motor cortex (M1) using patch clamp method. Motor skill consistently improved within 2 days of training in all animals. In current clamp analysis, 1-day trained rats showed lower, but 2-days trained rats showed the higher resting membrane potential than untrained rats, resulting in a significant increase of firing rate. In voltage clamp analysis, 1-day trained rats exhibited significantly higher AMPA/NMDA ratios and miniature EPSC (mEPSC) amplitude than untrained rats, suggesting an increase in postsynaptic AMPA receptors in the early phase of motor learning. Western blot analysis further indicated a specific phosphorylation of GluA1 subunit of AMPA receptors in 1-day trained rats. On the 2nd day of training, the AMPA/NMDA ratio decreased to the levels in untrained rats. In addition, 2-days trained rats showed the significantly higher mEPSC amplitude and frequency than untrained rats. Moreover, paired-pulse response of EPSC significantly decreased, suggesting the increase in presynaptic glutamate release at the late phase of learning. These results suggest that dynamic changes in the property and glutamatergic plasticity depending on the phase of motor learning in layers II/III neurons in the M1.

(COI: No)

P3-130

Facilitation from the flexor digitorum superficialis to the extensor carpi radialis in humans: a study using a post-stimulus time-histogram method

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Effects of low threshold afferents from musculus (m.) flexor digitorum superficialis (FDS) to m. extensor carpi radialis (ECR) in humans were examined using a post-stimulus time-histogram method with electrical (ES) and mechanical conditioning stimuli (MS) in 4 healthy human subjects. ES to FDS with the intensity below the motor threshold induced excitatory effects (facilitation) in 14/31 ECR motor units in every subject. The remaining motor units received no excitatory or inhibitory effects by ES. The central delay of the facilitation was almost equivalent to that of the homonymous facilitation of ECR. MS to FDS with the intensity below the threshold of the tendon-reflex induced excitatory effects (facilitation) in 31/31 ECR motor units in every subject. The difference between latencies of the facilitation by ES and MS was almost equivalent to that of the homonymous facilitation of FDS by ES and MS. These findings suggest that facilitation from FDS to ECR exists in humans. Group Ia afferents should mediate the facilitation through a monosynaptic path.

(COI: No)

P3-131

Morphological substrates of tectal commissural inhibition and excitation in relation to saccade coordinates and "Listing's law"

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Our previous electrophysiological study showed that excitatory and inhibitory commissural connections existed between the two superior colliculi (SCs). To obtain morphological substrates for these electrophysiological findings, we examined tectal distributions of excitatory and inhibitory commissural neurons (CNs) in the cat SC by injecting tracers into various parts of the SC and double-labelling CNs with GABA and gold particle-conjugated WGA -HRP. Lateral SC injections labeled small GABA-positive CNs in the medial SC and medium-sized GABA-negative CNs in the lateral SC, whereas medial SC injections labeled small GABA-positive CNs in the lateral SC and medium-sized GABA-negative CNs in the medial SC. These morphological results support our electrophysiological findings that mirror-symmetric excitatory pathways link medial to medial upward saccade areas and lateral to lateral downward saccade areas of the SCs, whereas the medial upward saccade area in one SC is inhibited by the lateral downward saccade area in the other SC and vice versa. This pattern of commissural inhibition between two SCs is comparable to that between bilateral vestibular nuclei for the vestibuloocular reflex, indicating that the saccade system uses the semicircular canal coordinates. The excitatory tectotectal commissural connections in bilateral symmetric sites of the rostral SCs might seem to minimize torsional eye movements and contribute to Listing's law.

(COI: No)

P3-132

Plasticity of indirect cortico-motoneuronal excitations in relaxed hand muscles in humans

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We reported that repetitive combined stimulation (RCS) of pyramidal tract and peripheral nerve could induce long-term potentiation (LTP) in indirect cortico-motoneuronal excitations in biceps brachii (BB) of human subjects, which are mediated by cervical propriospinal neurons (PNs). However, the LTP could be induced only when the target muscle was voluntarily contracted, which limits possible clinical use. In animal studies, PNs are known to project various forelimb motoneurons. Because RCS could induce plastic changes in synapses from pyramidal tract to PNs, we hypothesized that the LTP of C-M excitations could also be induced in non-target muscles in upper limb, which are relaxed during RCS. RCS intervention (0.2 Hz, 10 min) was the same as in the previous study. With BB EMG recording under weak contraction, transcranial magnetic stimulation (TMS) to the arm area of left motor cortex (M1) was delivered with right ulnar nerve stimulation. Inter-stimulus interval for the combined stimulation was set at 10 ms, where inputs by both stimuli to reach PNs simultaneously. As previously reported, motor evoked potentials (MEPs) in BB induced by TMS were potentiated after RCS, which lasted for ~65 min. Furthermore, the potentiation could be observed in hand muscles, which showed similar time course to that in BB. These results show that LTP could be induced in muscles without contraction, if RCS induces LTP in another muscle under weak contraction.

(COI: No)

P3-133

Disturbance in information flow through the cortico-basal ganglia pathways in parkinsonian monkeys

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Parkinson's disease (PD) characterized by motor symptoms, such as bradykinesia, rigidity and tremor, is caused by loss of dopaminergic neurons. To elucidate the mechanism causing such symptoms, we examined neuronal activity in the internal pallidum (GPi), the output nucleus of the basal ganglia (BG), of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP, dopaminergic neurotoxin)-treated PD monkeys. The monkeys exhibited moderate to severe motor symptoms. Motor cortical stimulation induced triphasic responses composed of early excitation (Ex) followed by inhibition (Inh) and late Ex in the GPi of normal monkeys. After MPTP treatment, however, cortically evoked Inh was mostly lost with little changes in spontaneous firing rates. Intravenous L-dopa injection alleviated motor symptoms and restored cortically evoked Inh in the GPi. Blockade of subthalamic nucleus (STN) by muscimol injection improved bradykinesia, suppressed cortically evoked Ex, and restored cortically evoked Inh in the GPi. These results suggest that disturbance in information flow through the cortico-BG pathways is responsible for PD symptoms. Under normal conditions, signals through the cortico-striato-GPi *direct* pathway induce Inh in the GPi and properly release motor actions by disinhibiting the thalamus. On the other hand, in PD, signals through the *direct* pathway induce diminished Inh in the GPi and fail to disinhibit the thalamus, resulting in bradykinesia.

(COI: No)

P3-134

Synaptic inputs underlying motoneuronal firing during locomotion in the α-chimaerin knockout mouse spinal cord in vitro

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For the formation of the motor circuit in the developing mammalian spinal cord, interaction between tyrosine kinase receptor EphA4 and its ligand ephrinB3 is crucial, regulating the projection of a subpopulation of ipsilaterally projecting neurons. The interaction of these two molecules functions as a barrier preventing ipsilaterally projecting EphA4+ axons from crossing the midline. Deletion of the EphA4 or its downstream-signaling molecule alpha-chimaerin causes aberrant midline-crossing of these ipsilateral-projecting axons in the spinal cord and leads to a hopping gait in mice. In this study we examined how lumbar motoneurons (MNs) are modulated by excitatory and inhibitory synaptic inputs during locomotor-like rhythmic activity in the isolated spinal cord preparation taken from alpha-chimaerin knockout (Chn1-KO) neonates. Similar to wildtype MNs, firing of an individual Chn1-KO L2-flexor-related-MN was time-locked with the ipsilateral flexor activity but not with that of the contralateral side indicating that it is unlikely that the firing pattern of Chn1-KO MNs is shaped by direct synaptic inputs from the contralateral network. Furthermore, we estimated the instantaneous frequency of excitatory and inhibitory synaptic inputs during the rhythmic activity from the membrane voltage trace based on the Ornstein-Uhlenbeck model. Preliminary results suggest that during locomotion, Chn1-KO MNs are synaptically modulated in a similar way to wildtype MNs.

(COI: No)

P3-135

The effect of cyclic illusory movement on corticospinal excitability of muscles in the contralateral limb

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When human move their wrist periodically, the neural activities in the contralateral forearm muscles are modulated so as to correspond to the phase of the wrist movement. It is considered that such neural modulation in remote limb enhances synchronization in bimanual movements. The detailed neural mechanisms are still hardly known. In order to investigate the effect of sensory process on neural modulation, we tested whether just illusion of rhythmic movement of one hand modulates corticospinal activity in the contralateral forearm muscle depending on the phase of illusory movement. The experimental tasks were 1) voluntary movement of right hand, 2) kinesthetic illusion induced by tendon vibration (80Hz), 3) no illusion with vibration, 4) passive movement. During these tasks, transcranial magnetic stimulation of the left motor cortex was delivered at random timing, and then motor evoked potentials (MEPs) were recorded from extensor and flexor of resting right wrist. Kinesthetic illusion of the left wrist without overt movement or muscle activity modulated the MEP amplitude in the contralateral forearm muscles depending on illusory movement phase just as observed during voluntary movement. These results indicate that only perception of kinesthetic movement can produce the MEP modulation of resting limb depending on movement phase of contralateral limb. Thus, sensory process would be important in the functional connectivity between bimanual hands.

(COI: No)

P3-136

Convergence of multi-pathway signals in single cerebellar granules cells in vivo

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Somatosensory signals from the facial area are conveyed to the cerebellar cortex directly via trigeminocerebellar pathway as well as indirectly via cortico-ponto-cerebellar pathway. Both of these pathways, forming mossy fibres, project to the granule cells in the cerebellar cortex. It was not known whether these pathways project to different populations of granule cells or converge onto the same granule cells. To address this issue, we made whole-cell patch-clamp recordings from single granule cells in the crus II area of anaesthetized mice. In these experiments, knock-in mice expressing Venus fluorescent protein by aldolase C promoter were used to visually identify the sagittal zones. When the upper lip was stimulated by air puff, excitatory postsynaptic currents (EPSCs) were evoked in granule cells in the aldolase C compartments 5+ and 5-. A majority (4/6) of the responding cells had two components of EPSCs. The latency (time from the onset of stimulation to the peak) of fast and slow components were 6.3 ms and 25.9 ms (n = 4), respectively, while the field potentials simultaneously recorded from the cerebral sensory cortex had a single component with intermediate latency (19.6 ms), suggesting that the fast and slow components in granule cells were the direct trigeminal input and the indirect pontine input, respectively. These results suggest that a substantial population of paravermal granules cells receive convergent inputs from direct and indirect pathways.

(COI: No)

P3-137

Anatomically structured burst spiking in the thalamic reticular nucleus: implications for a novel functional organization of thalamocortical loop circuitries along the rostrocaudal neural axis

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Thalamic reticular nucleus (TRN), a cluster of GABAergic cells projecting to thalamic nuclei, occupies a highly strategic position to regulate information processing in the thalamocortical loop. TRN cell activity is characterized by burst spiking that imposes significant influences on thalamic and thereby cortical cell activities. In view of the previous findings that the intensities of burst spiking in spontaneous activities of visual and auditory cells in the caudal TRN are graded in a similar manner along the rostrocaudal neural axis, the present study was carried out to address the question of whether the gradient of burst spiking extends in the whole rostrocaudal range of the TRN. Recordings of spontaneous activity were obtained from 49 cells in four anesthetized rats. Cells were labeled with neurobiotin to determine locations. The number and averaged inter-spike interval of spikes in a burst became larger and shorter as cells were located more rostrally in the putative domain that contains visual, auditory and somatosensory cells. This gradient, however, appeared to be reversed in the rostral end of the TRN that operates for motor and limbic functions. The results suggest that burst spiking is anatomically structured along the rostocaudal neural axis in the whole sensory domain of the TRN. There could be a novel functional organization that allows the TRN to impose graded influences on sensory processing in the thalamocortical loop across sensory modalities.

(COI: No)

P3-138

Visual short-term memory dependent on the diversity of protocadherin-α cluster in mice

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The clustered protocadherins (Pcdh) are assumed to play important roles for the formation of sophisticated neural networks. Of the 12 clusters in cPcdh-α, cPcdh-α 1, 12 mice have only cPcdh-α 1 and α 12, so that cPcdh-α diversity is largely reduced. To investigate the roles of clustered structure of cPcdh-α in higher brain function, we investigated visual short-term memory. First, we tested short-term memory of spatial information using a T-shaped maze task. The performance of cPcdh-α 1, 12 mice was significantly worse than that of wild-type mice. We further tested visual short-term memory of shape information. We developed an M-shaped maze equipped with a display. In a control task, a cue shape was presented at center of the display, and the two choices including the original cue were presented at both branches with overlapped timing. Mice must select the cue, or the other in other experiments, to get reward. In the short-term memory task, the delay period was set at 20 s between the presentation of the cue and two choices. The performance in cPcdh-α 1, 12 mice was significantly worse than that in wild-type mice. After this visual short-term memory sessions were finished, we confirmed that mice could choose a pair of alphabets, which they had never seen, based on the short-term memory. These results indicate that mice can memorize shapes as complex as alphabets, and the diversity of Pcdh-α is required for the visual short-term memory.

(COI: No)

P3-139

Different members of face-responsive neurons in monkey area TE contribute to global categorization of faces and to upright-face versus inverted-face categorization

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We have reported that face-responsive neurons in monkey area TE represent information about a global category, i.e. human vs. monkey vs. shapes earlier than information about more detailed categories, e.g. facial expression. To examine whether neurons respond to inverted faces showing similar characteristics to upright faces on a face-by-face basis, we analyzed activities of 119 face-responsive neurons in area TE of two rhesus monkeys (Macaca mulatta), performing a fixation task. Test stimuli were colored pictures of monkey faces (4 models with 4 expressions), human faces (3 models with 4 expressions), geometric shapes, and inverted pictures of the faces. Population vectors consisting of responses for each stimulus were computed in a window 115-165 ms after a stimulus onset. Sparse logistic regression was applied individually to the vectors for the upright monkey vs. human faces (GL), for the upright vs. inverted human faces (HUI), and for the upright vs. inverted monkey faces (MUI). The number of neurons contributed to GL, HUI, and MUI was 2, 4, and 5, respectively. Three neurons were found in common for HUI and MUI. No neuron and one neuron was found in common for GL and HUI and for GL and MUI, respectively. The results suggest that different members of the neuronal population contribute to GL and to HUI or MUI, but that HUI and MUI have contributing members in common. Supported by Grants-in-Aid for Scientific Research on Innovative Areas "Sparse modeling" (26120535).

(COI: No)

P3-140

Representation of binocular depth in macaque visual area MT proved with varied temporal frequencies of visual stimuli

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Binocular disparity is a cue for binocular stereopsis. The visual system has correlation-based and match-based representations for binocular disparity. These representations are characterized by disparity tuning functions to anti-correlated RDSs (aRDSs). In aRDSs, the luminance contrasts of dots are reversed in one eye. The correlation-based representation should have inverted tuning functions for aRDSs relative to those for normal, correlated RDSs; the match-based representation should lose disparity selectivity for aRDSs. Human psychophysical experiments suggest that stimulus temporal frequency changes the relative contributions of the two representations to stereopsis. Here, we hypothesized that the temporal frequency alters the neural representation of binocular disparity in the visual cortex. To test this hypothesis, we recorded responses from 41 disparity-selective neurons in macaque middle temporal area (MT). The disparity-tuning curves of these neurons were tested both with normal and anti-correlated RDSs and with slow and fast pattern refresh rates of the RDSs. We fitted Gabor functions to the tuning curves to estimate the tuning-curve shape. For both slow and fast refresh rates, the tuning curves inverted the shapes when binocular anti-correlation was applied to RDSs. Our results indicate that temporal frequency does not alter the correlation-based representation of disparity in area MT.

(COI: No)

P3-141

Superior colliculular neurons are involved in detection of face-like patterns in monkeys

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Facial recognition plays an important role in social communication in primates. Previous neurophysiological and imaging studies suggest that the superior colliculus (SC) is implicated in social behaviors and facial information processing. However, specificity of SC neuronal responses to facial stimuli remains unclear. In this study, we recorded monkey SC neuronal activities during discrimination of various monochrome face-like and non-face-like patterns in a delayed non-matching to sample (DNMS) task. Each pattern consisted of one of 4 face contours (rice scoop, star, circle, square) and 5 facial features (2 eyes, 2 eyebrows, 1 mouth). Each non-face-like pattern consisted of the same facial contours, but the facial features were random positioned in the facial contours, or included no facial features. Of 405 SC neurons recorded, 138 neurons responded to visual stimuli. Of these, 116 neurons were tested with the all stimuli. The results showed that SC neurons responded stronger and faster to upright and inverted face-like patterns than to non-face-like patterns. Furthermore, response latencies were shorter in SC neurons with upper receptive fields than those with lower receptive fields. Mean response latencies were also shorter in SC neurons in the superficial layers than in the deep layers. Furthermore, response magnitudes to original images were significantly correlated to those to white/black reverse images in about 50% of SC neurons. These results provide evidence for SC involvement in detection of face-like patterns.

(COI: No)

P3-142

The similarity of receptive field properties in connections between the retina, the lateral geniculate nucleus and the primary visual cortex of the cat

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In the early visual system, receptive field (RF) properties, such as orientation selectivity and spatial frequency (SF) selectivity, are key features of visual information processing. Although many studies have investigated retinal, geniculate, and cortical functions, relatively few studies have been conducted with a particular focus on the similarity/difference of the functional properties among the regions. In this study, we simultaneously recorded multi-unit activity from the retino-geniculate and geniculo-cortical neural populations from anesthetized cats. Then, we compared the RF properties obtained using the reverse correlation method with noise or flashing grating stimuli between the populations with or without the significant functional connections. We found that, regardless of the connections, preferred SF became low to high through the pathway, which might reflect the converging retino-geniculate projections and the narrowly tuned geniculo-cortical projections. Also we found that the populations with the functional connections sharing the similar preferred orientations, suggesting that there were the connections depending on the preferred orientations through retina to V1.

(COI: No)

P3-143

Interaction between neuronal responses to multi-site microstimulation in the mouse visual cortices in vivo

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It has long been suggested that cortical microstimulation is feasible for artificially restoring a certain level of auditory, tactile, or visual sensation, by directly exciting the target neurons in the cerebrum. In such a cortical neuronal prosthesis, it is required to deliver electrical stimuli to multiple sites in accordance with the topological map, regardless of their sensory modalities. However, little has been known about interactions of the neuronal responses induced with multiple electrodes. In the present, we examined the spatio-temporal neuronal responses to multi-site microstimulations in the mouse visual cortices in vivo using the voltage-sensitive dye imaging technique. When electrical pulse stimuli were delivered simultaneously from spatially separated two electrodes, depolarizing responses were evoked in the corresponding regions in the primary, and then secondary visual cortical areas (V1 and V2). These responses were smaller in amplitude as well as in spatial extents in both V1 and V2 compared with a linear summation of two responses that are evoked independently with each of the electrodes. Such a suppressive interaction became less significant as the stimulation interval between the two electrodes was increased from 10 to 100 msec. Similar results were obtained when the stimuli were delivered from three electrodes. The nonlinear interaction among responses induced with multi-site stimulations should be taken into account for designing effective stimuli in cortical neural prostheses.

(COI: No)

P3-144

Postnatal developmental observations of abnormal retinal terminal aggregation in the cPcdh-α KO mice

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Clustered protocadherins (cPcdhs) belong to the cadherin superfamily and are playing important roles on the cellular diversity and interaction at neuronal surface in the mammalian central nervous system (CNS). cPcdhs consist of three families, cPcdhs-α, -β and -γ, and each family shows distinct spatial and temporal distribution in the CNS. Their roles in the establishment of nervous system, however, are still unclear. To investigate the functional features of cPcdhs-α during postnatal development, we produced cPcdhs-α deleted mice. They could grow up without gross abnormality, but we found aberrant terminal distribution of retinal axons in the dorsal lateral geniculate nucleus (LGd), the primary relay center of the visual system. There were many strange huge aggregations of retinal terminals, as large as neuronal soma size, within the LGd. In the postnatal developmental study, we found that the huge aggregations firstly appeared during P10－P14, just before eye opening. The retinal terminals of the earlier stages of postnatal development were not apparently different from those of the wild mice. This suggests that cPcdhs-α might play important roles in the refinement stage which starts around the eye opening, following the completion of eye-specific segregation stage of retinal terminals in the LGd.

(COI: No)

P3-145

Transporter-independent choline uptake in the mouse retina

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Choline, an essential precursor for acetylcholine synthesis, is transported into synaptic terminals through high affinity choline transporter (hCT) in cholinergic neurons. We found that the immunoreactivity for hCT of the ON-cholinergic amacrine cells (ON-CACs) was significantly stronger than the immunoreactivity for hCT of the OFF-CACs in the mouse retina. We have previously reported that P2X2-purinoceptors, which are permeable to large cations, are specifically located in OFF-CACs. In this study, therefore, we examined whether the less accumulation of hCT in the OFF-CACs is compensated by choline uptake through P2X2-purinoceptors. When ATP was applied to P2X2-purinoceptor expressing HEK293 cells, inward current was detected even when the extracellular Na^+ were replaced with equimolar $choline^+$. The permeability to $choline^+$ was also found in the OFF-CACs in the mouse retina. Choline current was activated by an application of ATP-γ-S but not by α, β-methylene ATP or benzoyl-benzoyl-ATP. In the presence of pyridoxalphosphate-6-azophenyl-2', 4', -sulfonic acid, an application of ATP did not induce any choline current. Furthermore, cholinergic current was increased when extracellular Ca^{2+} concentration was reduced. These physiological and pharmacological characteristics support the notion that P2X2-purinoceptors permeate choline, and that cholinergic transport mechanism is different between ON-CACs and OFF-CACs. The P2X2-purinoceptors might work as an alternative pathway of choline transport especially in the OFF-CACs of the mouse retina.

(COI: No)

P3-146

The dry eye enhances cold cell sensitivity to capsaicin

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Previous studies have found that cold cells innervating the cornea are sensitive to the ocular fluid status of the corneal surface and may be responsible for the regulation of basal tearing. In addition, we have shown that an experimental dry eye condition modifies the thermal and menthol responses in these corneal primary afferent neurons. In the present study, we examined the effect of dry eye on the sensitivity of cold cells to the TRPV1 agonist capsaicin. Unilateral dry eye condition was created by excision of the left exorbital and infraorbital lacrimal glands. Extracellular, single-unit recordings were performed in anesthetized animals 1 week after gland excision and in age matched controls. Electrodes positioned in the trigeminal ganglion were used to isolate and characterize cold-sensitive neurons. Responses to thermal stimulation were examined 5 min after the application of capsaicin (3 nM- 3 uM). At low concentrations (<300 nM), capsaicin did not affect the rate of ongoing and cold-evoked activity in both groups of animals. High concentrations of capsaicin (>= 300 nM) suppressed the ongoing and cold-evoked activity in both groups of animals, overall, capsaicin induced greater suppression in dry eye animals. We applied TRPV1 antagonist, capsazepine 30 min before capsaicin application. Capsazepine blocked the capsaicin-induced suppression of cold cell activity. These results indicate that dry eye sensitizes cold cells to capsaicin mediated by TRPV1 channels.

(COI: No)

P3-147

Phagocytic ability of Müller glia in the damaged retina

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It is well established that microglia, the resident phagocytes in the central nervous system, play a major role in the clearance of dead cells after neuronal damage. Microglia are also present in the retina and have been considered as the principal phagocytes in the retina that are recruited to the site of damage and remove dead neurons. However, we have recently observed that, in the rat model of photoreceptor damage, dead photoreceptors were removed from the outer nuclear layer immediately after induction of photoreceptor death and replaced by proliferating Müller glia. This suggests that Müller glia may repair damage by removing dead photoreceptors by phagocytosis. To test this possibility, we examined the phagocytic ability of Müller glia in the rat retina after methyl nitrosourea-induced photoreceptor damage. Double immunofluorescence for glutamine synthetase, the Müller glia marker, and rhodopsin, the rod photoreceptor marker, revealed rhodopsin-positive photoreceptor debris within the cytoplasm of Müller glia, consistent with the possibility that Müller glia are able to phagocytose dead photoreceptors. On the other hand, few microglia were detected in the ONL during the stage of photoreceptor removal. These results indicate that Müller glia have a previously unrecognized role as phagocytes that remove dead neurons after retinal damage.

(COI: No)

P3-148

Differentiation of gap-junctionally connected amacrine cells and modulation of channel opening of their electrical synapses

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Electrical synapses are present in retinal neurons expressing channel subunit, connexins (J Neurosci, 2004; Brain Res, 2012). Electrical current spread through connections of cells is expected to modulate chemical synapses. Our studies revealed channel opening of gap junctions between several types of retinal amacrine cells is regulated by intracellular cyclic AMP as well as intracellular Ca^{2+} concentration (Brain Res, 2012). Individual amacrine cells show specific coupling patterns (J Intgra Neurosci, 2005). Based on coupling patterns and dendritic morphology, six different classes of homotypic lateral connections between amacrine cells of the same subtype were identified. Two types of cell-specific dendritic contacts, tip-contacts and cross-contacts, were found. Electrical synapses between tip-contact cells are regulated by intracellular cyclic AMP. I investigated synaptic contacts of these amacrine cells by electron microscopy as well as laser scanning confocal microscopy. Tip-contact cells make output synapses only onto axon terminals of retinal bipolar cells. Whereas cross-contact cells make conventional synapses onto dendrites of retinal ganglion cells. I investigated channel opening of electrical synapses between cross-contact cells under dual whole-cell patch clamp recordings. Gap junctions between cross-contact cells closed under intracellular high Ca^{2+} concentration (>300nM), but did not close under intracellular application of cyclic AMP. These results suggest that cell-specific electrical synapses play important roles in differential synapses from retinal amacrine cells.

(COI: No)

P3-149

Mechanisms of the retinotopic map reorganization induced by monocular enucleation

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Visual input is received by retinal ganglion cells and their axons project mainly to the lateral geniculate nucleus (LGN) of the thalamus. Then neurons in the LGN send their axons to the primary visual cortex (V1). In this visual pathway, the retinogeniculate and geniculocortical connections are organized to form an accurate retinotopic map during the developing stage. Both molecular guidance and neural activity are thought to play an important role in the formation of the topographical arrangement, although the precise mechanism is still unknown.

A previous electrophysiological study using hamsters reported that monocular enucleation in early postnatal days induces a disarrangement of the retinotopic map in V1, resulting in the duplication of the central visual field. We used an optical imaging technique to investigate the change of the retinotopic map more precisely in the monocularly enucleated (ME) animals. We observed the duplication of the retinotopic map clearly across V1 which is ipsilateral to the intact eye. The responding region to the ipsilateral eye in V1 of the ME animals was larger compared with that of normal animals. This functional change may reflect a reorganization of the neural connections. We demonstrate possible anatomical correlates of the map duplication and the influence on the expression of guidance molecules which affect axon growth.

(COI: No)

P3-150

Relationship between barometric hypersensitivity and autism - morphological analysis of the autism model rat

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Autism spectrum disorder (ASD) is one of the developmental disorders according to three core features of social deficits, communication impairments, and repetitive or stereotyped behaviors. In addition to these features, hypersensitivity is often appended to the symptom in ASD. It has been reported that the receptor organ of barometric pressure locates in inner ear, but its mechanisms has not been known. Previously, we reported the autistic rat model through prenatal thalidomide exposure and its abnormal development of serotonergic neuron in the brain with morphological and behavior analyses. In this study, we examined morphological analyses of inner ear in the autistic rat model. Pregnant Wistar rat were exposed to thalidomide on embryonic day 9. Each offspring was perfused with paraformaldehyde and inner ears were dissected out. Immunohistochemical analyses of frozen sections in the inner ear and surface preparation of organ of Corti were performed. Histological differences between thalidomide-exposed rat and control could not be found about inner and outer hair cells in the organ of Corti and these synapse formation. These results suggest that the inner ear in the autistic model rat is morphologically normal.

(COI: No)

P3-151

Local Na^+ current forms the unique electrical property of the epithelial tissue essential for the endocochlear potential in the inner ear

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Cochlear endolymph exhibits a potential of +80 mV relative to an ordinary extracellular solution, perilymph. This called endochclear potential (EP) is essential for hearing. The EP is maintained by the lateral cochlear wall that comprises outer and inner epithelial layers. The basolateral surface of the former is exposed to the perilymph, whereas the apical surface of the latter face the endolymph. Between the two layers lies the extracellular compartment that shows a highly positive potential. This interlayer potential (ILP) is a source of the EP and governed by a K^+-diffusion potential that depends on a large K^+-gradient across the apical surface of the outer layer. Our electrophysiological experiments and computational model revealed that the gradient is controlled by the unidirectional K^+-transport across the lateral wall. We previously found that the outer layer is continuously depolarized of +7 mV relative to the perilymph. Although this unique property is indispensable for the positive ILP, how it is formed remains uncertain. The theoretical approaches based on our former model predicted that Na^+ current depolarizes the basolateral surface of the outer layer. In support of this, perilymphatic perfusion of low [Na^+] solution hyperpolarized the outer layer and reduced the ILP and EP. Thus, the Na^+ current is a critical regulator for the EP.

(COI: No)

P3-152

Proteomic analysis of the epithelial tissue that drives membrane transport systems in the cochlea of the inner ear

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Cochlear endolymph in the inner ear exhibits 150 mM [K^+] and a positive potential of +80 mV. These properties are essential for hearing and maintained by the K+-transport across the epithelial tissue, stria vascularis. Majority of the proteins involved in the K^+-transport have been identified. The stria vascularis also drives a variety of other membrane transports to function the cochlea. To clarify the proteins underlying these transport systems, we analyzed the membrane fractions of the stria by a mass spectrometry. We identified 1,664 membrane proteins, which contained 25 ion channels and 79 transporters. 16 of the former and 65 of the latter have been for the first time detected in the stria. Network analysis suggested that Ca^{2+} signaling would play pivotal roles in the strial transport. Of interest, we identified 20 candidates for uncloned deafness genes. Our protein library is useful to elucidate not only molecular architecture of the membrane transport systems in the stria but also pathological processes of hearing disorders.

(COI: No)

P3-153

Evidences of the K^+-circulation current that controls the electrochemical properties in the cochlea of the inner ear

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Cochlear endolymph exhibits a high [K^+] of 150 mM and a highly positive potential of +80 mV. We previously revealed by electrophysiological assays and a computational model that the lateral cochlear wall maintains these unique properties with the K^+-circulation current which flows between the perilymph and the endolymph. Its dysfunction causes deafness. The lateral wall consists of two epithelial layers; the inner and the outer layers. The latter expresses Na^+, K^+-ATPases on its perilymphatic surface. However, it remains uncertain whether they contribute to the circulation current. An inhibition of these ATPases decreased the intracellular [K^+] of the outer layer and consequently impaired the endolymphatic potential. Based on this experimental data, in this study we renewed the computational model, where the K^+-circulation current set to flow through the ATPase in the outer layer. The model predicted that the block of the ATPase reduced the circulation current, which leaded to a decrease of the extracellular [K^+] between the two layers. Indeed, this alternation could be observed by *in vivo* electrophysiological experiments. These results support the concept of our model that the K^+-circulation current occurs across the lateral wall and establishes the endolymphatic properties.

(COI: No)

P3-154

5-HT3 receptor expression in the mouse vestibular ganglion

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Introduction: 5-HT3 receptor is a ligand-gated ion channel. Previous studies have shown 5-HT3 receptor expression in various neural cells of the central and peripheral nervous systems. Although the function and distribution of the 5-HT3 receptor has been well established, no study has yet determined its localization and function in the peripheral vestibular nervous system. To address this question, we investigate here the localization of the 5-HT3 receptor in the mouse peripheral vestibular nervous system.
Methods: C57BL/6J wild-type (WT) and 5-HT3 receptor knock-out (KO) mice were used in this study. We performed RT-PCR and in situ hybridization to examined 5-HT3 receptor mRNA localization in the inner ear. Moreover, we studied the physiological effects of a selective 5-HT3 receptor agonist (SR57227A) on freshly isolated VG neural cells from adult mouse using the measurement of intracellular calcium ion concentration (Ca2+ imaging).
Results: We found that both 5-HT3A and 5-HT3B receptor mRNA is expressed in VG neurons and also that 5-HT3 receptor mRNA is localized in the VG of the inner ear. 5-HT3A receptor mRNA is expressed in approximately 30% of VG neurons, while 5-HT3B receptor mRNA in VG neurons is expressed with a much lower signal. SR57227A induced increases in intracellular calcium in several VG cells from WT mice. However, SR57227A caused no change in VG neurons from 5-HT3A receptor KO mice.
Conclusions: These findings suggest that functional 5-HT3 receptors are synthesized in VG neurons and might modulate the peripheral vestibular nervous system.

(COI: No)

P3-155

Brain responses induced by odor and odorless air stimulation in human: 7 Tesla fMRI study

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It has been demonstrated that olfactory sensory neurons respond to both odor and mechanical stimuli. However, processing mechanism of different sensory information received by olfactory sensory neurons remains unclear. To clarify the brain area related to odor and mechanical sensory processing, we investigated brain responses induced by odor and mechanical stimuli application to nostrils with fMRI. BOLD-signal changes evoked by odor stimuli are known to comparatively small. Thus, we used ultra high field (7 Tesla) MRI to increase BOLD signal. Odorant stimulation (isovaleric acid, peppermint and coffee odor) and odorless air (mechanical) stimulation were applied to nose by air pressure. Activation by odor and mechanical stimuli were detected in piriform cortex, amygdalae, hippocampus, thalamus, cingulated cortex, insula, orbitofrontal cortex and somatosensory cortex. In the piriform cortex, odor stimuli induced activation at anterior and mechanical stimuli induced activation at posterior. Mechanical stimuli induced strong activation in thalamus. In the insula, olfactory stimuli evoked activation in the anteroventral portion and mechanical stimuli evoked activation in the posterodorsal portion. These results suggested that odor and mechanical information from olfactory epithelial cells project different brain area.

(COI: No)

P3-156

Responses of mature and immature rats to P-mix derived from wolf urine

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Urine excreted from common grey wolf (Canis lupus) has been used as a repellent for various kinds of mammals. Previous our studies showed that pyrazine analogues contained in wolf urine (P-mix) induced avoidance and fear behaviours in mice (Osada et al., 2013) and Hokkaido deer (Osada et al., 2013). Mice and Hokkaido deer have not any experiences that they have threated by wolves during living periods because wolves died before 100 years ago in Japan. This suggests that P-mix induces innate fear in these animals. In the present study, first, we addressed question that P-mix induces a fear-related behaviour and avoidance in mature rats. Then, we examined effects of P-mix on immature rats to explore whether P-mix induces innate fear responses in immature rats. P-mix also induce avoidance in immature rats. Previous study also showed that P-mix induced excitation of neurons at the accessory olfactory bulb (AOB), which receives pheromonal information. Exposure of mature and immature rats to P-mix induces expression of Fos-ir cells, which are excited neurons, at the AOB of mature and immature rats. The present results showed that P-mix induces fear-related responses in immature and mature rats via the AOB.

(COI: No)

P3-157

Properties of olfaction in patients with panic disorder

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Panic disorder (PD) is classified in V. Anxiety disorder of DSM-5, which is characterized by sudden bursts of panic attacks accompanied by heart palpitations, sweating, dyspnea, and abnormal sense. Patients often suffer from severe terror and discomfort reaching the peak in a few minutes. Although anti-depressants (SSRI) or cognitive behavior therapy are applied, it is very difficult to recover completely. It is reported that olfactory function of PD patients is normal, using UPSIT, as well as olfactory-triggered panic attacks experienced in 45 % of patients. Taken together, we examined olfactory function of PD patients by T&T olfactometry and Open Essence, after answering to the questionnaire on self-evaluation. Eight patients of PD participated in this study. All of them have anti-depressant or antianxiety drugs administered. Unfortunately they still suffer from panic attacks at frequency of once per one to 6 months. PD patients has hypersensitive olfactory function, although most of them are unaware of it. These results suggest that approach to olfaction of PD patients or odor administration to them may be much useful in diagnoses or treatment of PD. This study was approved by Kochi Medical School Clinical Research Center (ERB-000397), and we obtained informed consent from all subjects before study participation.

(COI: No)

P3-158

Expression of G proteins in the olfactory organs of *Pelodiscus sinensis*

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Many vertebrates have two olfactory organs, the olfactory epithelium (OE) and the vomeronasal organ (VNO). Since fish and aquatic mammals do not have the VNO, presence of the VNO is thought to be closely related to the adaptation for terrestrial life. In this study, the olfactory organs of a semi-aquatic turtle, *Pelodiscus sinensis*, were investigated to examine their adaptation for the aquatic and terrestrial environment. The nasal cavity was largely divided into the upper and lower chambers. They were lined by sensory epithelia, which can be regarded as the OE and the VNO, respectively. The upper chamber epithelium was supposed to detect odorants in the air and the lower chamber epithelium in the water, since associated glands were found only in the upper chamber epithelium. Immunoreactivity for Golf, a member of G proteins coupled to the odorant receptors, were detected in the cilia on the free border of epithelia lining both upper and lower chambers. In addition, the Golf-positive cilia in the upper chamber epithelium were longer and more intensely labeled than those in the lower chamber epithelium. These results suggest, although differences in the cell shape are exist, that receptor cells positive for Golf are distributed both in the OE and the VNO of *P. sinensis*, and involved in the detection for odorants both in the water and air. It is interesting to note that the same characteristics are found in other semi-aquatic turtles, *Geoclemys reevesii* and *Trachemys scripta elegans*, both of which belongs to the other family than the *P. sinensis*, considering their adaptation for the aquatic and terrestrial environment.

(COI: No)

P3-159

Effects of external Cl^- on the electroolfactogram recorded from the goldfish olfactory epithelium submerged in saline solutions

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Most olfactory receptor neurons (ORNs) of vertebrates bear cilia or microvilli at their dendrite tips. The ciliated ORNs (cORNs) have a signal transduction using adenylate cyclase. The Ca^{2+} influx through cAMP-gated channels opens Ca^{2+}-activated Cl^- channels, which amplify the response. While the microvillus ORNs (mORNs) of many terrestrial vertebrates are mostly located in the vomeronasal system, teleosts have both of cORNs and mORNs in the same olfactory epithelium, allowing us to compare them in the same epithelium. Although mORNs appear to utilize Phospholipase C to induce the olfactory response, the role of C^-l in the fish mORNs are not clear. We tested the effect of the external Cl^- on the electroolfactogram (EOG) of goldfish. The nose tissue was isolated from the body and submerged in the Ringer solution for EOG. The $[Cl^-]$ of the solution was reduced from 126.5 to 4.5 mM just after the end of 1-s application of 250 μM IBMX that raises [cAMP] in cORNs. In this condition, the IBMX response was enhanced by 21 ± 14% (SD, n=4) compared to the control response recorded without the Cl^- reduction, suggesting the contribution of the Cl^- efflux in the IBMX response. When the olfactory epithelium was stimulated by 1 mM serine (0.5 s), the Cl^- removal did not induce the detectable increase of the EOG response; 4 ± 3%, n=8. Serine response may occur mainly in mORNs, and the Cl^- efflux may not produce a detectable contribution to the response of goldfish mORNs in our recording condition.

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P3-160

Whole-cell recording from goldfish olfactory receptor cells in the slice preparation of the olfactory epithelium

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The olfactory epithelium (OE) of teleost fish has different types of olfactory receptor neurons (ORNs), including ciliated ORNs (cORNs) and microvillus ORNs (mORNs), which have been expected to have different signal transduction cascades involving adenylate cyclase and phospholipase C, respectively. Teleosts have no vomeronasal organ (VNO). Both of the cORNs and the mORNs of teleosts are distributed in the same OE, whereas cORNs and mORNs of many terrestrial vertebrates almost exclusively localize in main OE and VNO, respectively. Thus, the teleost OE is a good platform to compare the cell properties of cORNs and mORNs in a same preparation of OE. In the present study, we developed a slice preparation of goldfish olfactory epithelium to record olfactory responses by using whole-cell patch clamp technique. The olfactory organ (rosette) was dissected from the head of goldfish in a 0.5 mM-$Ca2^+$ saline solution. Several lamellae of the olfactory rosette were separated from the tissue, and laid flat on a piece of a nitrocellulose filter membrane (3×8 mm) coated with a cyanoacrylate adhesive. The flat-mount lamellae with the filter membrane were cut into 250 μm slices by a razor-blade tissue chopper slicer. The current responses of goldfish ORNs to IBMX, a bile acid and/or amino acids were successfully recorded with the obtained sliced tissue of goldfish OE. This work was supported in part by KAKENHI (22500351).

(COI: No)

P3-161

5T4 oncofetal trophoblast glycoprotein regulates the sensory experience-dependent dendritic development in newborn olfactory bulb interneurons

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Sensory input regulates the development of various brain structures, including the retina, cortex and olfactory bulb (OB). Little is known about how sensory experience regulates the dendritic development of OB interneurons, such as granule cells (GCs). Recently, we identified, with DNA microarray and *in situ* hybridization screenings, an oncofetal trophoblast glycoprotein *5T4* gene, whose expression in the OB interneurons is dependent on sensory experience (Yoshihara *et al*, J Neurosci 32, 2217, 2012). In this study, we characterized 5T4-knockout mice to know its physiological role in the dendritic development of OB GCs. *5T4*-knockout mice resulted in a significant reduction in the dendritic branching of OB GCs, while *5T4* overexpression could rescue the reduction of the dendritic arborization in its knockout GCs. Then, we conducted behavior tests for *5T4*-knockout mice. Interestingly, *5T4*-knockout mice were less sensitive in odor detection than the wild-type mice, and impaired the acquisition of the two-related-odor discrimination task, although they possessed the olfactory detection ability in the food seeking task. Taking account of electrophysiological data for external tufted cells from *5T4*-knockout mice, these results demonstrate that 5T4 contributes to regulate the sensory experience-dependent dendritic development of interneurons and the formation of functional neural circuitry in the OB.

(COI: No)

P3-162

ER stress induced in the OB inhibits olfactory learning

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It is well known that the endoplasmic reticulum (ER) stress links to neuronal death in various neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. It is also reported that most patients complain impairment of olfactory recognition. Therefore we examined if ER stress provoked by tunicamycin (TM) infusion has an inhibitory effect on synaptic plasticity in the olfactory bulb (OB) underlying aversive olfactory learning. Without vision, young rats can learn their mother's odor and approach her. They do this in part by learning their mother's odor as a conditioned stimulus that is paired with an unconditioned somatosensory stimulus given by maternal care. To establish aversive olfactory learning, an artificial odor can be paired with foot shock during training. TM infused into the bilateral OBs during odor-shock training on postnatal day 11 dose-dependently impaired aversive olfactory learning tested on the next day without affecting memory retention 1 hour after the training. Behavioral pharmacology shows that TM-induced ER stress causes the selective impairment of the long-term memory process of aversive olfactory learning. Electrophysiological experiments using OB slices show that TM administration has an inhibitory effect on the late phase of long-term potentiation induced at the mitral-to-granule cell synapses without affecting the early phase of long-term potentiation. These results are consistent each other to suggest that ER stress impaired aversive olfactory learning by inhibiting synaptic plasticity in the OB.

(COI: No)

P3-163

Two mechanisms underlying maintenance of long-term potentiation at synapses in the mouse accessory olfactory bulb

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Microcircuits in the accessory olfactory bulb (AOB) include the prominent reciprocal dendrodendritic synapse between mitral cells, a single class of projection neurons, and granule cell interneurons. Long-term potentiation (LTP) at the AOB synapse is expected to underlie the pheromonal memory that avoids pregnancy block in mice. We have previously shown that the late-phase LTP at the AOB synapse depends on new protein synthesis. Here we examined whether the late-phase LTP also requires actin polymerization, which is suggested to play a crucial role for LTP maintenance in the hippocampus and the neocortex. Using AOB slice preparations, we measured field potentials (fEPSP) derived from granule cells to examine the effects of an actin polymerization inhibitor or inducer on the late-phase LTP. Tetanic stimulation, consisting of a 100 Hz, 100 pulse train applied four times at 3 min intervals, induced LTP lasting for 180 min. Under bath application of an inhibitor of actin polymerization, cytochalasin D (1 μM), the tetanic stimulation failed to induce the late-phase LTP. The late-phase LTP was induced when subthreshold stimulation (a 100 Hz, 100 pulse train applied twice at 3 min intervals) that only produced short-term potentiation was paired with an inducer of actin polymerization, jasplakinolide (0.2 μM). The results support the hypothesis that both new protein synthesis and actin polymerization underlie LTP maintenance at the AOB synapse.

(COI: No)

P3-164

Modulation of reciprocal synaptic transmission between mitral cells and granule cells in the mouse accessory olfactory bulb through vasopressin V_1 receptors

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Central vasopressin facilitates social recognition and modulates numerous complex social behaviors in mammals. Recent analysis of transgenic rats engineered with an enhanced green fluorescent protein reporter for vasopressin synthesis identified new population of vasopressin neurons in the accessory olfactory bulb (AOB). The AOB has been demonstrated to be a critical site for mating-induced mate recognition (olfactory memory) in female mice. The effect of vasopressin, however, on the synaptic transmission between dendrites in the AOB of female mice is largely unknown.

To address this issue, evoked synaptic currents were measured from mitral cells in slice preparations prepared from 23- to 36-day-old Balb/c mice. To evoke dendrodendritic inhibition, a depolarizing voltage step from -70 mV was applied to a mitral cell under the whole-cell configuration. We have demonstrated that vasopressin significantly reduced the IPSCs in Mg^{2+}-free solution.

In the present study, to determine the contribution of different vosopressin receptor subtypes (V_{1a} and V_{1b}) to reduce the IPSCs, effects of antagonists for V_1Rs on the IPSCs were tested. The suppressive effect of vassopressin on the IPSCs was diminished by an antagonist for V_{1a} receptors, Manning compound, while an antagonist for V_{1b} receptors, SSR149415 unaffected the effects of it. The present results suggest that vasopressin modulates reciprocal transmission between mitral cells and granule cells through vasopressin V_{1a} receptors.

(COI: No)

P3-165

The blockade of $GABA_A$ receptors in the bed nucleus of the stria terminalis further suppresses the intake of conditioned aversive taste solution

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The bed nucleus of the stria terminalis (BNST) receives projections from the taste-related brain regions. However, the involvement of the BNST in conditioned taste aversion has still been unclear. The BNST contains dense GABAergic interneurons. Therefore, we tested the effects of microinjections of $GABA_A$ receptors antagonist bicuculline into the BNST on the intake of conditioned aversive taste solution on the retrieval of CTA. The rats implanted with guide cannulae into the BNST were trained to drink water during a 20-min session. On the conditioning day, all rats received a pairing of a 5 mM saccharin solution with an i.p. injection of 0.15 M lithium chloride. On the retrieval tests (Test 1-3), the rats were presented with the saccharin CS for 20 min. On Test 3, the rats were divided into two groups. One group was microinjected with saline, the other with bicuculline (100 ng/0.25 μl), 30 min before the CS presentation. All rats acquired robust aversion to the CS, because their CS intakes on Test 1 and 2 were significantly lower than those on the conditioning. On Test 3, the bicuculline-injected rats showed significantly lower intake of the saccharin CS than the saline-injected rats. These results indicate that the blockade of $GABA_A$ receptors in the BNST enhance the suppression of the consumption of the CS solution on the retrieval of CTA. Therefore, it is suggested that the GABAergic transmission in the BNST are involved in some taste memory process. This work was supported by JSPS KAKENHI (24500973).

(COI: No)

P3-166

Behavioral and neural mechanisms to ingest vitamin C in vitamin C-deficient rats

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The behavioral and neural mechanisms to ingest sufficient vitamin C (VC) has not been clear. In the present study, we performed the behavioral and the electrophysiological experiments to investigate the mechanisms to ingest sufficient VC. As experimental animals, ODS/Shi Jcl-od/od (od) rats, which cannot synthesize VC in their own metabolism, were used. In the behavioral experiment, the preferences ratio for various concentrations of VC solutions were measured before and after deprivation of VC solution in od rats by using two-bottle preference test. In the electrophysiological experiment, we compared the integrated chorda tympani (CT) nerve response in the VC deficient rats with that in the non-deficient rats. The results were as follows: In the behavioral experiment, the od rats avoided the high concentration of VC solutions on the sufficient VC situation, but this aversive behavior was reduced by the deficiency of VC. On the other hand, preference ratios for low concentration of VC were not changed by the deficiency of VC. In the electrophysiological experiment, the magnitudes of whole CT nerve responses to VC, HCl, NaCl and quinine hydrochloride in od rats with sufficient VC were significantly smaller than those in rats with deficiency of VC. On the other hand, those responses to sucrose and monopotassium glutamate in the VC deficient od rats were as same as those in the normal od rats. These data suggest that rats with sufficient VC can avoid the high concentration of VC solutions by receipting VC as a tastant, but this avoidance is reduced by the deficiency of VC.

(COI: No)

P3-167

Neural pathway contributing to control mechanism of sucrose preference by body weight in mice

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Mice consumed high-caloric sweetener sucrose (Suc) rather than non-caloric sodium saccharin (Sac), when their body weight (BW) reduced to 75% of the original BW by chronic food-restriction. However, Suc consumption abruptly dropped to Sac consumption levels (Saltatory Suppression of Suc Preference, SSSP) immediately after the regain their original BW in 50% mice tested. The SSSP tended to be suppressed by lesions of somatosensory area for the limbs (S1FL/HL). In the present experiment, we examined the expression of a neuronal activity marker c-Fos in S1FL/HL and amygdala to clarify the relationship between those brain regions. The number of c-Fos positive cells in S1FL/HL of pre-SSSP group was significantly lower than that of post-SSSP group. In contrast, the number of c-Fos positive cells in amygdala of pre-SSSP group was higher than that of post-SSSP group. These results suggest that the sensory activation of S1FL/HL encoding increment of BW decreases amygdala function, resulting in suppression of Suc preference. We attempt to examine neural pathway from S1FL/HL to amygdala using anterograde and retrograde tracing.

(COI: No)

P3-168

Glucagon like peptide-1 (GLP-1) underlies sweet taste transmission

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Recent studies demonstrated that taste bud cells express several gut peptides, such as GLP-1 (glucagon like peptide-1), NPY (neuropeptide Y), and glucagon, and secrete these peptides in response to various taste stimuli. Interestingly, the secretion patterns of peptides are correlated with taste qualities, suggesting the possibility that these gut peptides would contribute to taste quality coding. In this study, we report the expression of GLP-1 in some taste cells which possess sweet taste receptor subunit T1R3, and GLP-1 receptor is expressed in gustatory nerve neurons in wild type mice. Mice genetically lacking of GLP-1 receptor showed reduced sweet taste responses in chorda tympani (CT) nerve recordings. GLP-1 is secreted from a subset of sweet responsive cells by sweet taste stimulation in a concentration dependent manner. Furthermore, i.v. injection of GLP-1 produced transient increase of neural activities in a subset of sweet specific single nerve fibers without affecting those of other taste fibers. This activation by injected GLP-1 was observed also in CT nerve of ATP receptor knockout (P2X2/3 KO) mice. All these findings suggest that GLP-1 may be involved in normal sweet taste signal transmission in mice.

(COI: No)

P3-169

Paclitaxel alters sweet-preference in rats

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The alterations in the sense of taste (Dysgeusia) are often overlooked side effects in cancer patients undergoing chemotherapy. Dysgeusia affects the daily quality of life (QOL) of these patients and causes malnutrition in a majority of the patients. However, the research is lacking. The aim of the present experiments was to investigate the effects of paclitaxel (Taxol) on voluntary sucrose-solution intake using a two bottle choice. For this purpose, rats were presented with both a sucrose solution (0.3 M) and water and the consumption of each was measured daily. Usually rats prefer sucrose solution. Intraperitoneal administration of paclitaxel caused a decrease in sucrose-solution intake within a few days after being administered. However, the preference for sucrose solution returned in several days after we stopped. Outcomes indicate paclitaxel attenuates sweet-taste preference in two bottle choice (sucrose vs. water). Since molecular weight of paclitaxel is approximately 850, it cannot pass through the blood brain barrier. These results suggest that paclitaxel affects peripheral taste system or circumventricular organs involved in the sweet taste transduction.

(COI: No)

P3-170

Effects of estrogen on Mu and Kappa opioid inhibition of TMJ-responsive neurons in superficial laminae at the spinomedullary junction in ovariectomized female rats

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Chronic painful temporomandibular joint disorders (TMD) occur more often in women than men and are difficult to manage. There is evidence for significant sex difference in the potency of opioid analgesics in human. The influence of analgesic agents on neurons activated by stimulation of temporomandibular joint (TMJ) region is not well defined. The spinomedullary junction (trigeminal subnucleus caudalis (Vc/C1-2) is major site of termination for TMJ sensory afferents. To determine whether estrogen status influences opioid-induced modulation of TMJ-responsive neurons, mu or kappa opioid agonists (morphine or U50488) was given to ovariectomized (OvX) rats treated for 2days with low-dose (LE2) or high-dose (HE2) estrogen. Under isoflurane anesthesia TMJ units were activated by ATP (1 mM, 20 μl) injected into the joint space before and during cumulative doses of morphine or U50, 488H (0.03- 3mg/kg, iv) given at 20 min intervals. Morphine inhibited evoked activity in units from LE2 rats in a dose-related and naloxone-reversible manner, whereas from HE2 rats were not inhibited. By contrast U50, 488 inhibited evoked activity in units from HE2 rats in a dose-related and nBNI-reversible manner, whereas from LE2 rats were not inhibited. These data indicated that estrogen status differentially affected mu or kappa opioid modulation of TMJ unit activity.

(COI: No)

P3-171

Angiotensin II modulates sweet taste sensitivity via endocannabinoid receptor

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Angiotensin II (AngII) suppresses the chorda tympani (CT) nerve responses to NaCl and enhances those to sweeteners via AngII typeI receptor (AT1) expressed in taste cells. The effect of AngII on sweet taste responses but not on salt taste responses is not observed in cannabinoid receptor (CB1) knock out mice. It is also reported that CB1 is trans-activated by AT1, which induces generation of 2-arachidonoyl glycerol (2-AG), a major endocannabinoid. We, therefore, investigated whether the sweet enhancing effect of AngII is mediated by both CB1 and AT1 using their pharmacological blockers. Intraperitoneal injection of CB1 blocker AM251 specifically inhibited sweet enhancing effect of AngII on the CT nerve responses. Interestingly, we found that repeated taste stimulation with sweeteners but not with other tastants gradually increased individual responses. AngII enhanced the effects of repeated taste stimulation with sweeteners. AM251 or AT1 blocker CV11974 inhibited the AngII's effects. Taken together, we presumed that 2-AG would be produced via sweet taste receptor and/or AT1 activation, leading to enhancement of sweet taste responses via CB1.

(COI: No)

P3-172

The functional analysis of Mash1 in mouse taste bud cell differentiation using Cre-loxP system

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The gustatory cells in taste buds have been identified as paraneuron, they possess characteristics of both neuronal and epithelial cells. Like neurons, they form synapses, store and release transmitters, and are capable of generating an action potential. Like epithelial cells, taste cells have a limited life span and are regularly replaced throughout life. However, little is known about the molecular mechanisms that regulate taste cell genesis and differentiation. In the present study, to begin to understand the mechanisms that regulate taste bud cell differentiation, we have investigated the role of Mash1 in regulating taste bud cell differentiation in Mash1 conditional knockout mice (CKO) using Cre-loxP system. We found that amino acid decarboxylase-immunoreactive (AADC-IR) cells and carbonic anhydrase 4-immunoreactive (CA4-IR) cells were significantly reduced in the circumvallate papilla epithelia of Mash1 CKO mice. In Mash1 CKO/GAD67-GFP mice, GFP-positive (GAD67 expression type III cell) cells were also reduced in the taste papilla epithelia. However gustducin, a marker of type II taste bud cells, was expressed in taste buds in the soft palates of Mash1 CKO mice. These results suggest that Mash1 could play an important role of differentiation of the type III cells in the taste bud.

(COI: No)

P3-173

Leptin's effect on sweet responses is mediated by leptin receptor Ob-Rb and metabolic sensor K_{ATP} channel

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Leptin selectively suppresses neural and behavioral responses to sweet tastants. However, the molecular and cellular basis for the specific link between leptin and sweet taste still has not been elucidated. Here, we report that sweet suppressive effect of leptin is mediated by functional leptin receptor (Ob-Rb) and ATP gated K^+ (K_{ATP}) channel expressed in sweet sensitive taste cells. Ob-Rb was much more abundantly expressed in taste cells expressing T1R3 (a sweet receptor component) than in those expressing GLAST (a Type I cell marker) or GAD67 (a Type III cell marker). Administration of leptin suppressed sweet but not bitter and sour responses of identified taste cells. This effect was inhibited by leptin antagonist and was not observed in leptin receptor deficient *db/db* mice and also in diet induced obese (DIO) mice. K_{ATP} channel subunit SUR1 was well coexpressed with Ob-Rb in T1R3 expressing taste cells and sweet suppressive effect of leptin was inhibited by addition of K_{ATP} channel blocker glibenclamide in dose dependent manner. In addition, K_{ATP} channel activator diazoxide mimicked the sweet suppressive effect of leptin. These results indicate that leptin suppresses taste responses of sweet sensitive taste cells via activation of Ob-Rb and K_{ATP} channel.

(COI: No)

P3-174

Glucuronosyl group of gymnemic acids mainly interacts with the transmembrane domain of human T1R3 in sweet-suppressing effect

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Gymnemic acids (GAs) are triterpene glycosides isolated from the plant *Gymnema sylvestre*. GAs are known to selectively suppress taste responses to various sweet substances in humans, but not in mice. This effect of GAs is diminished by rinsing the tongue with γ-cyclodextrin (γ-CD). Here we focus on the molecular mechanisms for sweet-suppressing effect of GAs. We examined the interaction between GAs and sweet receptor by utilizing a sweet receptor assay based on changes in intracellular calcium activity in HEK293 cells expressing T1R2 + T1R3. Similar to previous studies in humans and mice, GAs suppressed the $[Ca^{2+}]_i$ responses to sweet compounds in HEK293 cells expressing human but not mouse sweet receptor. This effect of GAs rapidly disappeared after rinsing the cells with γ-CD. Using full length or chimera receptors in human and/or mouse, we determined that the transmembrane domain of hT1R3 was mainly required for the effect of GAs. Glucuronic acid, common structure of GAs, also showed sweet-suppressing effect. In our molecular models, GAs were predicted to dock to a binding pocket within the transmembrane domain of hT1R3.

(COI: No)

P3-175

Morphology of P2X3-immunoreactive nerve endings around laryngeal chemosensory cells in rat

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Taste bud-like chemosensory cell clusters and solitary chemosensory cells (SCC) are distributed in the laryngeal mucosa. It has been reported that the laryngeal chemosensory cells were immunoreactive to molecules for taste transduction such as α-gustducin and PLC β2. In the present study, morphology of P2X3-immunoreactive nerve endings around laryngeal chemosensory cells was examined using multilabeling immunofluorescence with confocal laser microscopy. In the taste bud-like chemosensory clusters, P2X3-immunoreactive nerve endings were intruded from basal part and ramified in the cluster. Terminal regions of the P2X3-immunoreactive nerve endings were flattened in shape, and attached with spindle cells immunoreactive to α-gustducin or IP3R3, which are markers for type II gustatory cell, and with the slender cells with immunoreactivity for SNAP25 or syntaxin-1, which are markers for type III cell. On the other hand, solitary chemosensory cells were classified into two types, i.e., spindle cells with α-gustducin and IP3R3 immunoreactivities and flask-shaped cells with SNAP25 and syntaxin-1 immunoreactivities. Axon terminals from branched P2X3-immunoreactive nerve fibers were terminated in both types of the solitary chemosensory cells. P2X3-immunoreactive nerve endings were also immunoreactive to vGLUT1 and vGLUT2, but not immunoreactive to CGRP. These results suggest laryngeal chemosensory cells are similar to type II and type III cells in the lingual taste buds, and P2X3-immunoreactive nerve endings have an important role on signal transmission from laryngeal chemosensory cells.

(COI: No)

P3-176

Movement-specific employment of sensory signals as a basis for rapid task switching

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Much of our flexible behavior is dependent on responding efficiently to relevant information, while discarding irrelevant information. In perceptual decision making, a popular hypothesis for utilizing relevant information is that sensory signals are gated, such that only relevant sensory signals reach decision making circuits. Little is known, however, about how neural pathways governing sensory-motor associations can rapidly switch to accomplish such flexibility. Here, we investigated how the outputs of sensory neurons change in a context in which task demand switches rapidly. We addressed this question by electrically microstimulating middle temporal (MT) neurons selective for both motion direction and binocular disparity in monkeys switching between direction and depth discrimination tasks. We frequently found that the observed psychophysical bias precipitated by delivering microstimulation to neurons whose preferred direction and depth were related to opposite choices in the two tasks (incongruent sites) was substantially shifted toward a specific movement. Furthermore, the degree of movement-specific employment was correlated with how well the monkey ignored the irrelevant information. Our findings suggest that the outputs of sensory signals are movement-specific, and that irrelevant sensory-motor pathways are filtered out depending on task demand, to accomplish rapid attentional switching.

(COI: No)

P3-177

Experience-Dependent Clustering of Sensory Synaptic Inputs in the somatosensory cortex

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Experience-dependent clustered synaptic plasticity is thought to underlie the formation of highly structured synaptic input patterns. However, direct experimental evidence for this hypothesis is lacking. To directly test the effect of sensory experience on synaptic input patterns, we analyzed individual synaptic inputs by using in vivo two-photon calcium imaging and whole-cell recordings from layer 2/3 neurons in the mouse barrel cortex. We found that both sensory-evoked synaptic inputs in local dendrites were sparse but locally clustered, and the majority of sensory inputs were localized to a small subset of spines. Importantly, whisker deprivation during the critical period significantly enhanced co-activation of distant spines. These results suggest that sensory information is represented by highly heterogeneous and structured synaptic inputs that are shaped by sensory experience during the critical period.

(COI: No)

P3-178

Effects of forelimb stimulation on the propagating excitatory wave evoked by hindlimb stimulation in the rat sensorimotor cortex recorded with optical recording system

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We have developed an optical recording system using a voltage-sensitive dye. Using this system, we have reported that the neural excitation induced by a somatic stimulation initiates from the somatotopically corresponding site and spreads outward in the sensorimotor cortex like the wave produced when a stone is dropped into still water. In this study, we examined the influence of forelimb stimulation on the hindlimb stimulation-induced response. The sensorimotor cortex was exposed and stained with a voltage sensitive dye (RH-414). An electrical stimulation (1 mA, 0.5 ms) was given first to the hindlimb then to the forelimb. The interstimulus interval (ISI) was 0, 5, 10, 15 or 20 ms. We compared the amplitude, full width at half maximum (FWHM) and slope of the rising phase of the hindlimb-response signal between with and without forelimb stimulation, at the collision position of the two propagation waves. The collision position was estimated from the isochrone maps based on the latency difference of each pixel. The FWHM was significantly shorter for all ISIs. The slope of rising phase was significantly steeper but only for the ISI of 0 ms. No significant difference was found in the amplitude for any ISIs. In addition, the latency of the hindlimb-response at its initiation site was shorter than the control, for the ISIs of 0 and 5 ms. Thus, the effects of subsequent stimulation were not of simple summation but of complicated phenomena depending on the ISI.

(COI: No)

P3-179

Discrimination of optogenetic whisker pattern in channelrhodopsin-2 transgenic rat

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The rodent whisker-barrel cortical system has been a model to study somatosensory representation in the cortex. Optogenetics would facilitate this with highspatiotemporal resolutions. Recently, we have identified the expression of channelrhodopusin-2(ChR2) in the mechanoreceptive neurons in the trigeminal ganglion in one of Thy1.2-ChR2-Venus transgenic rat lines, W-TChR2V4. Each whisker follicles were thus innervated by ChR2-positive nerve endings. Here, we studied if this rat can discriminate the irradiation patterns on their whiskers. A W-TChR2V4 rat was fixed its in head in awake and irradiated blue LED light on each whisker as a signal of operant conditioning of either Go or No-go task. The Go task was designed so as the rat is allowed to get a reward, when it licked the nozzle within 5s after whisker irradiation. The No-go task was designed so as, the rat have to withhold licking least 5s to get a reward. After training with blue light the W-TChR2V4 rat performed Go task, with success rate of over 80%. However, the success rate was less than 50% with red LED light or the wild type rat that did not express ChR2. When switched from Go to No-go task, once decreased success rate increased again with the repeat of training sessions. One of two points on the same side was irradiated as a Go signal and another as a No-go signal. It learned to discriminate these patterns successively with sessions and even with days. It is suggested that the optogenetic approach would facilitate to study how the spatiotempolal pattern of the mechanoreception would be interpreted in the cortex.

(COI: No)

P3-180

Somatosensory Cortical Responses after Crossing Nerve Transfer in Mice

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To understand the therapeutic effects of crossing nerve transfer for brachial plexus injuries in human patients, we investigated cortical changes after crossing nerve transfer of brachial plexus using flavoprotein fluorescence imaging in mice. The distal cut ends of the left median and ulnar nerves were connected to the central cut ends of the right median and ulnar nerves with a sciatic nerve graft at 8 weeks old. After eight weeks, responses in the primary somatosensory cortex (S1) elicited by vibratory stimulation applied to the left forepaw were visualized. In control mice, direct responses (DRs) mediated via thalamic input was observed in the contralateral S1. Weak indirect responses (IRs) were also observed in the ipsilateral S1. In nerve crossing mice, DRs were observed in the ipsilateral S1. At the same time, clear IRs, which were not observed in control mice, were found in the contralateral S1. In our previous study, it was expected that DRs were initiated by thalamic inputs to layer 4, while IRs were secondarily initiated by callosal inputs from the ipsilateral S1 to the layer 2/3. We next perform the experiment using macroconfocal microscope to analyze the latencices of activity in each layer. After analyzing every layer about DRs of the control mice and IRs of the experiment mice, layer 4 was dominant with control mice, and layer 2/3 was dominant with the nerve crossing mice.

(COI: No)

P3-181

Opt-fMRI study of whisker-barrel cortical responses using channelrhodopsin-2 expressing rat

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The rodent whisker-barrel cortical system has been a model to reveal somatosensory representation in the brain. Optogenetics would facilitate this with high spatiotemporal resolutions. Recently, we have identified the expression of ChR2 in the mechanoreceptive neurons in the trigeminal ganglion (TG) in one of thy1.2-channelrhodopsin 2 (ChR2)-Venus transgenic rat lines, W-TChR2V4 (Honjoh et al., 2014). Each whisker follicles were also richly innervated by the ChR2-positive nerve endings. The whiskers of a ChR2-expressing rat were all trimmed and 16 follicles were attached with plastic optic fibers in array of 4x4. Each endings of optic fiber was connected to blue LED, which turned on and off independently by a pulse generated by a computer. Therefore, the whiskers could be stimulated with various spatiotemporal patterns (optogenetic tactile pattern, OTP). The functional magnetic resonance imaging (fMRI) responses of barrel cortex were investigated using OTP under 7T-MRI system. The whisker irradiation induced a change of blood oxygenation level-dependent (BOLD) responses in the barrel field of contralateral somatosensory cortex in a manner dependent on time. The response to a single whisker irradiation was more regional than the simultaneous 16-whiskers irradiation. It is suggested that the OTP was accompanied with specific spatiotemporal changes of BOLD response. Our OTP, in combination with fMRI, would facilitate to study how the spatiotemporal pattern of the whisker mechanoreception would be represented in the cortex.

(COI: No)

P3-182

Peripheral nerve injury changes neuronal firing patterns in the somatosensory thalamus of unanesthetized mice

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Peripheral nerve injury induces massive remodeling in the central nervous system. Recently, we have demonstrated that transection of the peripheral sensory nerve increases excitatory afferent fiber innervations onto a relay neuron in the somatosensory thalamus (VPm neuron) [Takeuchi Y. *et al.* (2012) J Neurosci 32: 6917]. On the other hand, the transection also increases tonic inhibitory membrane conductance in a VPm neuron (Nagumo Y. *et al*., unpublished data). However, it is currently unknown about the net effects of such changes on VPm neuronal firing in a physiological condition. To address this issue, we here developed extracellular recordings of VPm neuronal firing from unanesthetized young adult mice using a U-frame head holder [Chiken S. *et al.* (2008) J Neurosci 28: 13967]. One week after the transection, spontaneous tonic (non-burst) spike frequency significantly decreased (6.5 ± 5.8 Hz vs. 3.8 ± 4.3 Hz, mean ± s.d., 30 and 41 neurons for control and transection groups, respectively; $^{*}P < 0.05$, Mann-Whitney *U*-test) whereas burst spike frequency did not (2.6 ± 3.6 Hz vs. 1.3 ± 1.3 Hz; $P = 0.34$). Consequently, the proportion of burst spikes to total spikes significantly increased (0.22 ± 0.16 vs. 0.31 ± 0.18; $^{**}P < 0.01$). These results suggest that VPm neuronal firing is globally inhibited by increased tonic inhibitory conductance and shifted from tonic to burst firing mode after the transection.

(COI: No)

P3-183

Parvalbumin-expressing lamina II interneurons are an origin of presynaptic inhibitory inputs on to central terminals of myelinated low-threshold mechano-receptors in the rodent spinal cord

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Axo-axonic synapses have been described on the central terminals of tactile afferents and certain classes of nociceptive fibers, however the origin of these inputs have yet to be identified. A recent report has shown that a significant proportion of inhibitory inputs on to the central terminals of myelinated afferents terminating in lamina II inner (IIi) of the mouse spinal dorsal horn express parvalbumin (PV) and that these inhibitory boutons are likely to be derived from PV-expressing cells in laminae IIi and III. In this study, we aim to confirm the origin of these PV-expressing axo-axonic inputs. We have used in vitro whole-cell patch-clamping techniques to record from and label PV-expressing cells in lamina IIi-III. We have observed that most of the axon terminals from all of the PV-expressing cells recovered form inhibitory presynaptic inputs on to the central terminals of myelinated afferents. Our findings confirm that PV-expressing cells in laminae IIi and III are a source of inhibitory presynaptic inputs on to the central terminals of myelinated afferents. These cells might play an important role in the development of tactile allodynia.

(COI: No)

P3-184

Repeated forced swim stress enhances CFA-evoked mechanical hypersensitivity and affects the expressions of pCREB and delta-FosB and the acetylation of histone H3 in the insular cortex

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Exposure to stressors causes substantial effects on the perception and response to pain. In several animal models, chronic stress produces lasting hyperalgesia. The insular (IC) and anterior cingulate cortices (ACC) are the regions exhibiting most reliable pain-related activity. And the IC and ACC play an important role in pain modulation via descending pain modulatory system. In the present study we examined the expressions of pCREB and delta-FosB and the acetylation of histone H3 in the IC and ACC after forced swim stress and CFA injection to clarify changes in the cerebral cortices that affect the activity of descending pain modulatory system in the rats with stress-induced hyperalgesia. Forced swim stress (day 1, 10min; days 2-3, 20min) or CFA injection into the hindpaw induced a significant increase in the expressions of pCREB and delta-FosB in the IC. However the forced swim stress prior to CFA injection showed significant enhancement of CFA-evoked mechanical hypersensitivity and attenuation of the increase in the expressions of pCREB and delta-FosB in in the IC. Cells of the IC also displayed evidence of chromatin remodeling. The forced swim stress prior to CFA injection attenuated the CFA-evoked increase in acetylation of histone H3 in the IC. These findings suggest neuroplastic and epigenetic changes in the IC after forced swim stress, which may be involved in the enhancement of CFA-induced mechanical hypersensitivity through dysfunction of descending pain modulatory system.

(COI: No)

P3-185

Opposing role of NMDA receptor GluN2B and GluN2D in somatosensory development and maturation

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NMDA receptors are essential for activity-dependent synapse refinement, and thus required for correct somatosensory map formation. However, distinct role of each NMDA receptor subunit remains largely unknown. Here we investigated functional roles of GluN2B (GluR ε 2 or NR2B) and GluN2D (GluR ε 4 or NR2D) in development of whisker-related patterning at trigeminal relay stations. Compared to control littermates, both the appearance of whisker-related patterning and the termination of the critical period plasticity were delayed by nearly a day in each of the trigeminal relay station of GluN2B+/- mice, while advanced by nearly a day in GluN2D-/- mice. Importantly, these temporal shifts were not accompanied by changes in the magnitude of lesion-induced critical period plasticity. Thus, GluN2B and GluN2D play counteractive roles in temporal development and maturation of somatosensory maps. Further analysis revealed that GluN2B was predominantly expressed in non-GABAergic neurons, while GluN2D was selective to GABAergic neurons in each of the trigeminal relay station. Taken together, our findings suggest that GluN2B expressed in ascending projection pathway and GluN2D expressed in inhibitory circuit accelerates and decelerates somatosensory map development, respectively.

(COI: No)

P3-186

The immunohistochemical characterization of the new heat-sensitive primary sensory neuron in mouse dorsal root ganglia

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Transient receptor potential vanilloid 1 and 2 (TRPV1 and V2) has been proposed as the heat sensors in nociceptors. However, several reports did not support major contribution of TRPV2 in heat nociception. We previously reported a new class of the heat-sensitive neurons in mouse dorsal root ganglia (DRG) using immunohistochemical detection of heat-induced phosphorylation of extracellular signal-regulating kinase (pERK). To characterize these neurons, here, we examined them by double-staining immunohistochemistry with antibodies against the marker molecules for the nociceptive neurons. In wild-type mice, ~20% of the neurons were pERK-positive after heat stimuli. The number of the heat-induced pERK-positive neurons was reduced but significantly observed in TRPV1-deficient mice. The most of the pERK-positive DRG neurons were TRPV2-negative, but positive to other molecular markers for the nociceptive neurons, e.g. CGRP (calcitonin-gene related peptide). The results suggest that the new heat-sensitive neurons share the features of nociceptive neurons, but their heat-sensitivities are independent of TRPV1/V2.

(COI: No)

P3-187

Primary nociceptive modulation via pigmentation-dependent dopaminergic signaling in skin

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In human and mice, pigmentation level is highly correlated to heat pain sensitivity in the skin. Tyrosinase does not only control melanin production, but also produces L-dopa that is converted into dopamine. To examine whether the peripheral dopamine mediates pain sensitivity, we investigated effects of local dopamine injection on nociceptive responses and nociceptive receptor expressions on primary sensory neurons in black C57BL/6 (B6) and albino tyrosinase-mutated B6 (B6(Cg)-*Tyr*$^{c-2J}$) mice. In the hind paw and whisker pad, B6 showed significantly lower mechanical and higher thermal sensitivities than albino B6. Subcutaneous injection of dopamine produced sustained hyposensitivity to mechanical stimulation and hypersensitivity to thermal stimulation. The same sustained changes in mechanical and thermal sensitivity were seen after injection of L-dopa or D_1 agonist SKF38393, but not injection of catecholamines or other dopamine receptor subtype agonists. The tyrosinase inhibitor kojic acid and the D_1 antagonist SCH23390 showed opposite effects on mechanical and thermal sensitivities. Injection of dopamine and SKF38393 into the whisker pad also downregulated mRNA expression of the mechano-sensitive receptor Piezo2, and upregulated mRNA expression of the heat-sensitive receptor TRPV1, in the associated trigeminal ganglia. These results suggest that tyrosinase-dependent dopamine production mediates expression levels of nociceptive receptors in sensory neurons via D_1 activation.

(COI: No)

P3-188

Three dimensional reconstruction of trigeminal ganglion cell processes labeled by intracellular injection: Emphasis on club-like endings

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Primary somatosensory neurons are pseudo-unipolar cells. Both the peripheral and central endings of the neurons, as well as their firing characteristics, were identified and characterized, using intracellular labeling and recording in the rat trigeminal ganglion in vivo. Eleven of 35 labeled neurons terminated as club-like endings (Clubs) in the whisker follicles. All the neurons responded to deflection of their corresponding whisker. Eight of the Club neurons never ramified in the peripheral branch. They indicated significantly shorter duration at base time of action potential (1.7 ± 0.6ms) in contrast to the other endings (n=27; 2.5 ± 1.0ms). Analyses of serial semi-thin sections showed 52 Clubs to be arranged side by side along a cylindrically-shaped narrow belt zone around the follicle at connecting level of the ringwulst (Rw). Our findings indicate that approximately 50 neurons innervate a single small zone of the Rw. Rw is a sausage like protrusion into the ring sinus. Clubs were connected with collagen fibers extending from the Rw. We propose that the Clubs are sensitive to momentum changes of the Rw during whisker protraction or retraction.

(COI: No)

P3-189

Three-dimensional distribution of lamellar corpuscles in a human toe

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We investigated three-dimensional distribution of lamellar corpuscles and their afferents in a human infant toe. A distal part of a toe excised from a polydactyl patient (1year-old; informed consented by parents and obtained the permission of Hospital Ethics Committee of Kyoto Prefect. Univ. Med.). The toe was fixed, decalcified and cut into 80 μm-thick serial sections and immunohistochemically stained using primary antibodies against protein gene product 9.5 and myelin basic protein. Pacinian corpuscles (PCs) showed relatively simple and straight axon terminals surrounded by lamellae and Golgi-Mazzoni corpuscles (GMCs) displayed ramified and tangled ones. All PCs and GMCs were mapped on a 3D image. Totally 51 corpuscles (47 PCs and 4 GMCs) existed. Diameters (transverse / longitudinal) of these PCs and GMCs were 0.1-0.7 / 0.2-1.6 and 0.1-0.3 / 0.2-0.5 mm, respectively. 42 PCs were distributed in ventral, the rest 5 PCs were dorsal and all the 4 GMCs were in the lateral side of the toe. All corpuscles were located in the subcutis or deeper but not in the dermis. 33 PCs existed close to the distal phalange but little was beneath it. 14 PCs and 4 GMCs existed in between the tendons and the middle phalange. 10 corpuscles were solitarily scattered, but 41 distributed in 12 groups as 2-7 corpuscles. These observations may contribute to consider three-dimensional architecture of sensing mechanisms in the human finger.

(COI: No)

P3-190

In vivo analysis of visceral sensory inputs to the sacral spinal cord

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Sacral spinal dorsal horn receives synaptic inputs not only from somatic afferent but also from pelvic afferent fibers, and has important roles in integrating the sensory information and controlling functions of pelvic organs including the lower urinary tract functions. Sensory information including pain from the pelvic organs is known to be conveyed to the sacral dorsal horn by small myelinated A δ - and unmyelinated C-fibers. However, little is known about how the sacral dorsal horn receives pelvic sensory information *in vivo*. In the present study, we examined sacral spinal sensory responses from the lower urinary tracts by using *in vivo* patch-clamp and extracellular recording techniques. Sacral dorsal horn (SDH) neurons *in vivo* elicited action potentials in response to bladder filling and voiding. During the micturition cycle, the SDH neurons were classified into two types based on their responsiveness: neurons showing bursts of action potentials that correlated to the rise in intravesical pressure during voiding, and neurons which elicited firings at the peak pressure. Both groups of SDH neurons received inputs from slow conducting afferent fibers and the latter groups of neurons elicited firing by electrical urethra stimulation. These results suggest that sensory information from the bladder and urethra was separately conveyed to the sacral dorsal horn, and the precise pattern of spike timing in each group of SDH neurons may be needed to implement appropriate micturition.

(COI: No)

P3-191

Ultrastructural analysis of itch-related neural network in the spinal cord

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Gastrin-releasing peptide (GRP) has been reported as an itch mediator in the somatosensory system. We demonstrated that GRP was expressed in the small-sized primary afferent neurons, and central axons terminated the superficial layers of spinal dorsal horn and spinal trigeminal nucleus caudalis in rats. Furthermore, ultrastructure of GRP containing axon terminals in the spinal dorsal horn was analyzed by the following electron immunecytochemistry: high-voltage electron microscopy showed GRP containing axon terminals of a series of the varicosities; transmission electron microscopy displayed GRP expressing presynaptic terminals containing excitatory neurotransmitters; 3D-scanning electron microscopy showed GRP containing varicosity surrounded by tens of postsynapses. These results suggested that itch transmission is elaborately controlled by many synapses in the spinal dorsal horn.

(COI: No)

P3-192

Effects of neurotoxic destruction of noradrenergic fibers of the central nucleus of amygdala on rat inflammatory orofacial pain

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The amygdala is a kernel site for chronic pain-induced emotional complications. It receives non-thalamocortical direct inputs through spino- (parabrachio-) amygdaloid pathway, which exhibits robust synaptic potentiation in a variety of chronic pain models. Although it is well documented that exogenous noradrenaline potently modulates neuronal activity and synaptic transmission in the amygdala, the role of endogenous noradrenaline in the amygdala in the various symptoms observed during pain chronification has been only poorly addressed. We injected saporin conjugated with anti-dopamine-beta-hydroxylase (DBH) into the central nucleus of amygdala (CeA) and compared the various behavioral consequences in the inflammatory pain model. To allow evaluation of voluntary choice of floors of different temperatures (thermal preference test, TP) by rats with inflammatory pain, we injected formalin into orofacial regions, which resulted in manifest nocifensive behaviors lasting for < 60 min, and evaluated changes in paw withdrawal threshold (PWT) and TP index for >24 h after formalin injection. Saporin injection into the CeC resulted in elimination of DBH-immunopositive fibers in the CeC and also a partial reduction of DBH-immunpositive neurons in the locus coeruleus. On the basis of distinct effects of saporin treatment on PWT and TP, noradrenaline system might play differential roles in the regulation of nocifensive spinal reflex and voluntary choice of sub-aversive environments.

(COI: No)

P3-193

Functional characterization of pruriceptive dorsal root ganglion neurons in rats

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Itch sensation is different from painful one. However, it has been known that subsets of nociceptive sensory neurons mediate itch sensation. The fundamental question is how the peripheral neuronal activities specificially mediate itch sensation. Here, we used an original method for *in vivo* patch-clamp recording to allow integrated analysis of the diverse properties of dorsal root ganglion (DRG) neurons in rats and investigated the characteristics of chloroqunie (CQ; one of itch-induced chemical agents)-sensitive DRG neurons. Small- and medium-sized DRG neurons that innervate the skin were screened according to axonal conduction velocity (C-type and A δ-type), action potential duration, current expression profiles (Ih, IA, and T-Ca) and could be classified into 5 classes (Class I-V). Intradermal injection of CQ to the receptive field evoked discharges in some DRG neurons that belonged to Class I characterized by a long action potential and small Ih or to Class II characterized by a shorter action potential and IA. The Class I neurons were high-threshold mechanosensitive and also responded to heat (42-55℃) or warm (32-40℃) stimulation to the receptive field, whereas the Class II ones were moderate-threshold mechanosensitive and heat sensitive. All of the somata responded to application of capsaicin. Comparing with the characteristics of CQ-insensitive and nociceptive DRG neurons, we will discuss encoding mechanisms of itch sensation evoked by CQ.

(COI: No)

P3-194

Neural mechanisms of nociception in an animal model of fibromyalgia

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Chronic widespread pain is a serious medical problem, yet the neural mechanisms remain to be elucidated. Using a reserpine-induced animal model for fibromyalgia, this study was undertaken to examine: 1) the expression of pain-related ion channels in the dorsal root ganglion (DRG); 2) activities of peripheral nociceptors; and 3) alterations in spinal microglial cells. Acid-sensing ion channel (ASIC)-3 mRNA was significantly upregulated in the DRG, and a selective blockade of this channel was significantly reversed the behavioral mechanical hyperalgesia. Facilitated mechanical responses of mechano-responsive C-fibers both in the skin and muscle were observed. Spinal microglia labeled with Iba1-immunoreactivity was obviously activated, especially in the laminae I-II. The activated microglia and behavioral hyperalgesia were significantly prevented by a minocycline application. These results suggest that the increase in ASIC3 channels in the DRG, facilitated mechanical response of C-nociceptors, and activated spinal microglia may direct to intensify pain in this model. Pain may be further amplified by reserpine-mediated monoamine depletion in the descending pain inhibitory system (Nagakura et al. Pain 2009).

(COI: No)

P3-195

Psychosocial stress by partner-loss enhanced pain behaviors in monogamous animal, prairie voles

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Nociception is modulated by social environmental factors and stressful events. In particular, social stressors may have adverse effects on psychological and nociceptive functioning. Although "stress-induced hyperalgesia" are common clinical symptoms in chronic pain disorders, the mechanisms have been obscure because of a lack of appropriate animal models. The prairie vole is a socially monogamous rodent that exhibits a partner preference after the formation of the pair bonding. Here we analyzed the effect of partner-loss on anxiety behaviors, sensory thresholds, and pain behaviors of the prairie voles. Adult male voles were paired with strange females for 7 days then tested in a 2-h partner preference test at Day 7. Males that displayed a partner preference were divided into paired or partner-loss group. After that, an array of behavioral testing was conducted on males from both groups. Partner-loss males showed much anxiety behaviors in open field test (Day 11), low threshold of mechanical and thermal stimulus in plantar test and von Frey test (Day 12), and much pain behaviors in formalin test (Day 13) as compared to paired males. Spinal dorsal horn and descending inhibitory pathways from the brainstem play crucial roles in nociceptive processing, therefore, we are proceeding to estimate their activity by cFos immunoreactivity and will report the results on the day.

(COI: No)

P3-196

Joint immobilization by cast modulates not only pain threshold but also itch sensation

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Immobilization of joint by cast is commonly used for resting the injured joint. However the reduction of pain threshold for mechanical stimuli is often induced by cast immobilization of the hind limb. The cast treatment also elicits the itch sensation that we have experienced much stress and affect patient's quality of life. In this study using rats, we examined whether the cast treatment modulates the pain threshold to mechanical stimulation and the itch sensation induced by intradermal injection of serotonin. To examine the effects of cast immobilization on pain and itching behaviors in rats, one hind limb was immobilized for 2 weeks with a cast and observation of behavior was conducted after cast removal for 3 weeks. A wire mesh cast was wrapped around the one hind limb to keep the ankle joint almost straight. The pain threshold was measured by using calibrated von Frey filament test before and after cast immobilization. The joint immobilization elicited the reduction of pain threshold which continued almost over 10 day period after a cast removal. The itch sensation was assessed in rats with cast treatment by using the intraplantar injection of serotonin. The serotonin-induced itch sensation as licking and biting behavior was escalated in the rats with cast treatment than that of sham treatment rats. These results suggest that the joint immobilization by the cast not only reduce the pain threshold but also facilitates the itch sensation induced by intradermal injection of serotonin.

(COI: No)

P3-197

Noradrenaline enhances visual detectability via β adrenergic receptor in freely moving rat

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Noradrenaline (NA) is thought to modulate various brain functions such as sensory information processing. Previous electrophysiological studies revealed that iontophoretically-administered NA enhanced the signal-to-noise ratio in the primary visual cortex. It suggests that NA released into the visual cortex improves the visual detectability. However, this point has not been investigated in freely moving rat. In this study, to evaluate the effect of the endogenous NA by adrenergic receptor types, we measured the contrast sensitivity (CS) of Long-Evans rat in two-alternative forced-choice (2AFC) visual detection task combined with staircase method with or without adrenergic blockade. We applied α_2 receptor antagonist, idazoxan (IDA), and β receptor antagonist, propranolol (PRP), intraperitoneally 30 min before the test. We found that IDA increased the CS, whereas PRP decreased one. However, both of these receptors must work coordinately in the natural state, so it was unclear whether endogenous NA increased or decreased the CS. To answer this question, we administered the cocktail of both drugs resulting in the reduction of CS as with β antagonist. Our results demonstrated that endogenously released NA enhanced the contrast detectability, and suggested that the β receptor might work dominantly in freely moving rat.

(COI: No)

P3-198

Effects of neonatal dopamine depletion on itch and pain related responses in the adult rats

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Previous studies have shown that dopamine (DA) system in not only involved in motor control but also modulation of somatosensory information. Rats with DA depletion during adulthood and neonatal period exhibited akinetic motor activity and spontaneous motor hyperactivity, respectively, indicating that behavioral effects of DA depletion depend on the period of lesion development. Although itch and pain have some similarities, they are different sensation, and roles of DA system in development of the systems of their sensations are still unclear. To clarify effects of neonatal DA depletion on response to pruritic and noxious stimuli during adulthood, we analyzed the behavioral response and c-Fos immunoreactivity (Fos-ir) of spinal dorsal neurons, to injection of serotonin (5-HT) or formalin into the hindpaw in adult rats with neonatal 6-hydroxydopamine treatment. Rats with neonatal DA depletion showed significant increases in the numbers of flinch evoked by 5-HT or formalin injections, and decreases in the number and starting time of biting evoked by 5-HT injection. The numbers of Fos-ir spinal neurons evoked by 5-HT or formalin injections were not affected by neonatal DA depletion, while localization of Fos-ir neurons in the spinal cord evoked by 5-HT injection were different from that evoked by formalin injection. These results suggest that a role of DA system in development of somatosensory system depend on the modality, and the spinal neural circuit for pruritic transmission is not always consistent with nociceptive transmission.

(COI: No)

P3-199

Effects of motor cortex stimuli on rostral ventromedial medulla (RVM) cell activity in spared nerve injury (SNI) rats

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Motor cortex stimuli provides anti-nociceptive effects in chronic pain model rats. However the precise mechanisms of anti-nociception remains to be unknown. In the previous study we have shown the possible involvements of rostral ventromedial medulla (RVM)-on and off cells in cortex stimuli-induced pain relief in chronic constriction injury (CCI)rats. In the present study, using spared nerve injury (SNI) rats, another model of chronic pain made by section of common peroneal and tibial nerve but sparing sural nerve, we re-examined the RVM involvement in this motor cortex stimuli-induced anti nociceptive effects. Single unit activity of the RVM cells were recorded with tungsten/stainless steel microelectrodes under pentobarbital anesthesia. Prior to cortical stimuli, the RVM cells were classified into three groups, on-, off-, and neutral cells, based on their responses to nociceptive pinch stimuli applied at the hind paw. Cortical stimulus current intensity was ranged 30-110 μA. We found that spontaneous activity of off cells facilitated whereas on cells inhibited by cortex stimuli in SNI rats for at least 30 minutes after the stimuli. Taken together with previous results of CCI rats, these results suggest RVM is involved in cortex stimuli induced anti-nociceptive effects in chronic pain.

(COI: No)

P3-200

Degenerative histological alteration is not required for the induction of muscular mechanical hyperalgesia after lengthening contraction in rats

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Mechanical hyperalgesia after lengthening contraction (LC) severely restricts physical activities in daily life. The current study aimed to test a hypothesis that degenerative alteration in the exercised muscle is necessary for LC-induced mechanical hyperalgesia. Under isoflurane anesthesia, the tibialis anterior (TA) muscle of rats was loaded repetitive LC of different stretch range of motion (ROM) and angular velocity (VEL). The degree of mechanical hyperalgesia was quantified by measuring the mechanical withdrawal threshold of the exercised muscle before, 3 hours, 1-5 days after LC. Mechanical hyperalgesia appeared after LC at the ROM of 60, 90, and 120° in a ROM-dependent manner while that of 30° did not, and after LC at the VEL of 100, 200, and 400°/s in a VEL-dependent manner while that of 50°/s did not. Degenerative histological change in the TA muscle was observed only in some cases. However, median cross sectional area occupied with degenerated fibers in the TA muscle was 0~0.27 mm2 in all LC protocols used in this study. The area corresponds to 0~0.8% in the TA. These results suggest that mechanical hyperalgesia was induced by LC in a ROM- and VEL-dependent manner, and that massive degenerative change is not required for the induction of mechanical hyperalgesia after LC.

(COI: No)

P3-201

Involvement of Fractalkine (FKN) in ectopic orofacial pain induced by trapezius inflammation

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The mechanism of the ectopic orofacial pain accompanied with the chronic neck pain remains unclear. We investigated the role of FKN in the ectopic orofacial pain following trapezius inflammation. CFA was injected into the trapezius in rats. We measured the head withdrawal threshold (HWT) to mechanical stimulation of facial skin by von Frey filaments. Moreover, changes in HWTs were examined after i.c.m. administration of neutralizing fractalkine receptor antibody (anti-CX3CR1). The expressions of the Iba1 which is maker of microglia or fractalkine receptor (CX3CR1) positive cells were examined in trigeminal subnucleus caudalis (Vc) and upper cervical cord (C1-C2), immunohistochemically. We studied the effect of fractalkine on nocifensive behavior. HWT was significantly decreased, and the density of Iba1-positive cells was significantly increased by CFA injection into the trapezius. After CFA injection, i.c.m. administration of anti-CX3CR1 produced a complete recovery of reducing HWT, and the density of Iba1-positive cells was also significantly reduced. HWT was decreased and the density of Iba1-positive cells was increased with i.c.m. administration of FKN. CX3CR1 co-expressed with Iba1 in trapezius-inflamed rats. The protein expression of FKN, but not CX3CR1 significantly increased in Vc to C1-C2. The results suggest that up-regulation of FKN following trapezius inflammation regulates microglial activation in Vc and C1-C2, and the activated microglia is involved in mechanical allodynia.

(COI: No)

P3-202

Involvement of LPA receptors and phospholipases in LPA-evoked peripheral itch sensation of mice

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Lysophosphatidic acid (LPA) is a phospholipid which is well corelated to itch intensity of cholestatic patients with itch, while it was also reported to induce acute pain. We reported that LPA caused itch-related behaviors rather than pain-related behaviors by using a cheek injection model. We also showed the involvement of transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) channels in LPA-induced itch *in vivo* and *in vitro*. In this study, we further examined the LPA-induced signaling using calcium imaging methods with mouse dorsal root ganglion (DRG) neurons by focusing on LPA receptors and phospholipases. We used inhibitors of LPA_1, LPA_3, and LPA_5 which were reported to be expressed in mouse DRG neurons. We found that inhibitors of the all 3 receptors and LPA_5 alone were effective to reduce the LPA-responding DRG neurons, suggesting that LPA_5 is involved in the LPA-induced signaling. The downstream signaling of LPA receptors is so complicated that we pharmacologically examined the involvement of phospholipases A_2, C and D. Since lipid production or some membrane lipid depletion by these lipases can activate TRPA1 and TRPV1. An inhibitor of phospholipase D (PLD) decreased the LPA-responding DRG neurons, suggesting that PLD activity is involved in the LPA-induced signaling. Taken together, we concluded that LPA_5 receptor activation and PLD activity were necessary for the LPA-induced itch signaling in DRG neurons in the upstream of the activation of TRPA1 and TRPV1.

(COI: No)

P3-203

Involvement of ASICs in isoproterenol induced ischemic cardiac pain rat model

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Aim: Isoproterenol (Iso) induces myocardial ischemia, however, the cardiac pain associated with Iso-induced myocardial ischemia has not been reported. We characterized Iso-induced myocardial ischemia in rat to clarify the involvement of acid-sensing ion channel (ASIC) 3 in ischemic cardiac pain.
Methods: Male SD rats (200-400 g) were injected with 50 mg/kg of Iso subcutaneously. The control group were administrated with saline. Heart tissues were stained with hematoxylin and eosin. The behavioral changes and c-Fos expression in intra-spinal cord neurons after Iso-injection were analyzed. Furthermore, effects of morphine (Mor, 1 mg/kg) and ASICs antagonist amiloride (Ami, 30 mg/kg) on behavioral responses and c-fos expression were examined.
Results: The heart tissues was infiltrated with inflammatory cells into the subendocardial tissues 24h after Iso-injection. Rats showed characteristic behaviors most frequently from 15 to 30 min after Iso-injection. The animals lay on their side or back with their head extended. Iso group expressed more c-Fos in intra-spinal cord neurons compared to control group significantly. Pre-treatment with Mor significantly reduced the frequency of characteristic behaviors and the number of c-Fos expressed neurons. Furthermore, Ami decreased the characteristic behaviors and the c-Fos expression significantly.
Conclusion: These results suggest that ASICs were involved in Iso-induced ischemic cardiac pain.

(COI: No)

P3-204

Serotonin-mediated modulation on the chemosensory activity of rat carotid body

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We previously reported that immunoreactivities for the serotonin (5-HT) biosynthetic enzyme, tryptophan hydroxylase 1, and 5-HT plasma membrane transporter, were localized in chemoreceptor glomus cells and perivascular sympathetic nerve fibers in the rat carotid body (CB). In the present study, we examined 5-HT-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) responses in glomus cells, as well as smooth muscle cells and pericytes in isolated CB blood vessels. In specimen of glomus cells, 5-HT did not change $[Ca^{2+}]_i$ in glomus cells during normoxia, whereas induced repetitive $[Ca^{2+}]_i$ increases during hypoxia. The frequency of hypoxia-induced $[Ca^{2+}]_i$ changes was enhanced in the presence of 5-HT. These results suggest that 5-HT from glomus cells may increase its own hypoxic chemosensitivity by autocrine-paracrine mechanism. In arteriole specimen, 5-HT did not change $[Ca^{2+}]_i$ in smooth muscle cells. However, 5-HT induced $[Ca^{2+}]_i$ increases in pericytes in capillary specimen, and this response was inhibited by the 5-HT2 receptor antagonist, ketanserin. These results suggests that 5-HT from sympathetic nerve fibers may reduce capillary blood flow in the CB in order to increase chemosensitivity. In conclusion, 5-HT may modulate the chemosensory activity of the CB via two different modulatory pathways.

(COI: No)

P3-205

Enhanced tonic GABA currents after peripheral nerve injury contribute to inhibition of neuronal activity and subsequent remodeling of medial lemniscal fibers in the somatosensory thalamus

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We previously reported that cut of the infraorbital nerve (IONC) recruited additional excitatory medial lemniscal fibers onto somatosensory thalamic neurons (VPM neurons) around 5 days after the operation. On the other hand, it is also reported that peripheral nerve injury induces remodeling in the central nervous system through changes of inhibitory GABA system. However, little is known about details of the postoperative change in inhibitory GABA currents onto VPM neurons after the IONC. Here we report that the IONC significantly inhibited the neuronal activity of VPM neurons in unanesthetized mice and markedly enhanced the amplitude of tonic GABA currents onto VPM neurons. Interestingly, potentiation of tonic GABA currents after the IONC occurred much earlier than the remodeling of lemniscal fibers onto VPM neurons and was selectively observed in VPM neurons that had multiple lemniscal fibers by the IONC. Moreover, we found that chronic infusion of tonic GABA agonist into the VPM of normal mice recruited additional lemniscal fibers onto VPM neurons, whereas lack of tonic GABA currents prevented the remodeling of lemniscal fibers onto VPM neurons after the IONC. These results provide the possibility that the reduction of the VPM neuronal activity through enhanced tonic GABA inhibition by the IONC drives the remodeling of lemniscal fibers on a VPM neuron.

(COI: No)

P3-206

Morphogenesis of the lateral line in the primitive fish *Polypterus*

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A basal actinopterygian fish *Polypterus* possesses ganoid/enamel scales on the surface of the body, which reminds us of a primitive fish. There are a wide variety of shapes in lateral line scale and its neuromast known and we thus investigate into the morphogenesis of the neuromast of the lateral line scale in *Polypterus* as a representative of primitive fish. In a 25mm long larva, the primitive scales mineralized from a rear part toward a front part in a row, where primitive papillae on the vascular bone were formed. SEM images of the surface of a 115mm long juvenile revealed that the lateral line scale has a few pores from which sense hairs on top of the sensory cells project. These pores were surrounded by the epidermises in a concentric fashion and they looked just like the neuromast in other actinopterygians. The neuromasts in *Polypterus*, however, were macroscopically seen where pigment cells gathered, which is different from zebrafish. In a 125mm long juvenile, the isopedin was accumulated and the ganoin on the surface seemed to get formed. Axons from the neuromasts innervated into the vascular cavity of the lateral line scale and they thus went through it to join the lateral line, which was located at the junction of the transverse and the horizontal septa. The behavior of the axons was also immunohistologically confirmed in a 14mm larva. Therefore, the neuromasts started to be observed in the epidermis on the surface of the lateral line scale in the young larva before mineralization occurred, and their axons penetrated the scale and they eventually connected with the lateral line in the juvenile.

(COI: No)

P3-207

The early elevation of hippocampal BDNF by exercise after stroke protects neurodegeneration

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Exercise in the early stage of poststroke has been shown to facilitate the recovery from cognitive dysfunction. We have showed that the recovery of spatial memory function was depend on the hippocampal level of brain-derived neurotrophic factor (BDNF). However, time dependent changes of BDNF and its neuroprotective effects were unknown. In the present study, we investigated chronological changes of BDNF by exercise and apoptotic cell damage after brain ischemia using multifocal cerebral ischemia model rat induced by microsphere (MS) injection. Treadmill exercise was started at 24 h (Early group) or 8 days (Late group) after MS injection for 7 days. BDNF concentration in transected hippocampus were measured at 6hrs., 1, 2, 4, 8 and 15 days after MS injection by ELISA. BDNF concentration was gradually elevated by exercise (Early group: 6 hrs: 2.9 ± 0.21, 8 days: 4.8 ± 0.97; Late group: 8 days: 2.6 ± 0.67, 15 days: 4.9 ± 0.72 pg/mg-protein) and decreased after the completion of exercise in both groups. In addition, neurons immunostained for activated caspase-3 were observed in the hippocampus, especially in dentate gyrus, and tended to decrease in Early group. Taken together with our previous study, these results suggest that the transient elevation of hippocampal BDNF by exercise in early stage might play a major role in neuroprotection after onset of cerebral multiple microemboli.

(COI: No)

P3-208

Spatiotemporal analysis of motor map reorganization after stroke

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Previous studies have shown that motor map reorganization after stroke plays important role for functional recovery. Despite many factors affecting the motor map, little is known as to how or when motor map reorganization occurs and rehabilitative therapy modifies it. In the present study, the spatiotemporal changes of motor map were investigated after stroke. Rats were assigned to either rehabilitative or non-rehabilitative groups. After 3 weeks training of reach test, stroke were induced by photothrombosis in caudal forelimb area (CFA). Rehabilitative therapy was carried out by continuing reach training for 4weeks. To investigate the spatiotemporal changes of motor cortex, intracortical microstimulation (ICMS) were performed chronologically. In ICMS study, almost complete destruction of CFA was confirmed. Rostral forelimb area (RFA) was significantly reduced as well as vibrissa and jaw area, although RFA was located away from the infarction. RFA size increased slowly and its recovery was accelerated by rehabilitation. On the other hand, jaw and vibrissa areas were recovered quickly compared with RFA and not affected by rehabilitation. Interestingly, RFA size was also decreased after muscimol (specific agonist of the GABAA receptor) injection into CFA in normal rats. These results indicate that RFA size depends on the neural activity of CFA, suggesting rehabilitation therapy may modulate neural input into RFA.

(COI: No)

P3-209

Proteomic analysis of the molecular basis of the ischemic tolerance in the rat hippocampus

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Ischemic tolerance (IT) is a phenomenon whereby pretreatment with a non-lethal period of ischemia protects neurons against a normally lethal ischemic event. The cellular mechanisms underlying IT have not been fully elucidated. In the present study, we studied the altered expression of proteins in the rat hippocampus subjected to non-lethal ischemia by proteomic approach. Male S.D. rats were subjected to 3 min of global ischemia or sham-operation induced by four-vessel occlusion method. At 3 days after 3 min of ischemia, the hippocampal CA1 region was dissected and divided into four subcellular fractions. Proteins contained in each four subcellular fractions were separated by 2-DE. The protein spots were detected by CBB or silver staining. Mass spectrometry (MS) analysis was performed on LCMS-IT-TOF (Shimadzu). MS/MS spectra were searched against the NCBI database using MASCOT search program. Two-DE and MS/MS analysis revealed that the expression level of 11 proteins including VCP, aconitase2, transketolase, tubulin, adenylate cyclase-associated protein, protein-L-isoaspartate O-methyltransferase, adenylate kinase 1, PBP1, cytochrome b-c1 complex subunit 2, ACAT, voltage dependent anion channel (VDAC) was different between rats subjected to 3 min of ischemia and sham-operation. Western blot analysis revealed that the expression level of mitochondrial aconitase and VDAC was decreased in the hippocampus of rats subjected to 3 min of ischemia compared to sham-operated rats. The present results suggest that 3 min of ischemia may cause alteration in mitochondrial function.

(COI: No)

P3-210

A novel nucleoside analogue (COA-Cl) exerts neuroprotective effects against stroke events

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2Cl-C.OXT-A (COA-Cl) is a novel synthesized adenosine analogue exerting angiogenetic activity through ERK1/2 activation. As a well-known kinase, ERK1/2 is also involved in the over-activated apoptotic process of neurological disorders, such as stroke. Therefore, we investigate the neuroprotective effects of COA-Cl on stroke events in this study. Both rat transient focal cerebral ischemia and autologous blood infusion models were used for this study. COA-Cl was intracerebroventricularly administrated either immediately after model making or delayed continuously. In ischemic stroke, COA-Cl reduced the infarct volume, decreased the number of TUNEL positive cells and improved neurological deficits. The level of pERK increased by the administration of COA-Cl in vitro, indicating that the neuroprotective effects of COA-Cl may be via ERK1/2 activation. In intracerebral hemorrhage (ICH) models, the rats in the COA-Cl group attenuated the sensorimotor deficits and reduced ICH-induced edema. Furthermore, both TUNEL positive cells and 8-OHdG positive cells were fewer around the hematoma of COA-Cl treated rats compared with ICH ones. In conclusion, COA-Cl exerts neuroprotection against both ischemic and hemorrhagic stroke via anti-apoptotic and anti-oxidative effects.

(COI: No)

P3-211

Effects of running exercise on motor function and dendritic plasticity after unilateral striatal hemorrhage in rats

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We investigated the effect of running exercise on motor functions, dendritic plasticity and growth promoting or inhibiting factors after intracerebral hemorrhage (ICH) in rats. Male Wistar rats (B.W.: 240-270 g) were injected with collagenase into the left striatum to induce ICH. Sham operated animals were injected with saline instead of collagenase. They were randomly assigned to sham-control (SC), sham-exericise (SE), ICH-control (IC), and ICH-exercise (IE). Exercise groups were forced to run on a treadmill at a speed of 9 m/min for 30 min/day between 4 and 14 days after surgery. Behavioral assessments were performed by using motor deficit score, beam walking test and cylinder test. At 15 days after surgery, rats were sacrificed and their brain were removed. Dendritic morphologies in the motor cortex (layer V) were analyzed by Golgi-Cox staining. Expression levels of TrkB, Nogo-A and ROCK in the motor cortex were analyzed by Western blotting. Motor functions of IE improved significantly compared with that of IC. IE had more branches and higer length of dendrite compared with IC. TrkB expression levels of IE incresaed compared with IC. Nogo-A and ROCK expression levels of IE decreased compared with IC. These results suggest that improvement of motor function by treadmill running after ICH may relate to dendritic plasticity by the action of both growth promoting factors (TrkB) and growth inhibiting factors (Nogo-A, ROCK).

(COI: No)

P3-212

Effects of motor skills training on sensorimotor function and AMPA receptor subunits after intracerebral hemorrhage in rats

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The purpose of this study was to investigate the effects of motor skills training on sensorimotor function and AMPA receptor subunits after intracerebral hemorrhage (ICH) in rats. Rats were induced ICH by injection of collagenase into the left striatum. They were randomly assigned to acrobatic training group (ICH+AT) and no training group (ICH). ICH+AT group trained acrobatic tasks for 28 days from 4 days after ICH. Sensorimotor function was assessed using limb placing and postural instability test. The mRNA expression of AMPA receptor subunits, GluR1, GluR2, GluR3 and GluR4, in bilateral sensorimotor cortex was analyzed using real-time PCR at 29 days after ICH. This work was supported by a Grant-in-Aid for Scientific Research from the Niigata University of Health and Welfare, a Japan Society for the Promotion of Science and Nagoya Gakuin University. ICH+AT group significantly improved sensorimotor function compared with ICH group. The GluR1-4 mRNA expression of ICH+AT group significantly increased than those of ICH group in the sensorimotor cortex ipsilateral to the ICH. These results suggest that motor skills training following ICH may promote sensorimotor functional recovery by upregulation of AMPA receptor subunits mRNA expression.

(COI: No)

P3-213

Effects of coffee aroma on the stress: analysis of behavior and gene expression in the mice

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Recently, it was demonstrated that gene expression in the brain was altered by coffee aroma. However, effects of the aroma on brain regions are not yet clear. In the present study, we analyzed changes in behavior and gene expression in the mouse midbrain. After water emersion stress for 24 h, mice were received coffee aroma for 90 min. In elevated plus-maze test, time spent on open arms in the group of coffee aroma after stress was significantly longer than that of stress, suggesting that the aroma has antidepressive effects. Gene expression, measured by RT-PCR, of nerve growth factor receptor (*NGFR*) and activity regulated cytoskeletal-associated protein (*Arc*) in the group of coffee aroma after stress showed tendency of increment and no change was found in brain-derived neurotrophic factor (*BDNF*) and FBJ murine osteosarcoma viral oncogene homolog (*c-fos*). NGFR is known as a receptor of nerve growth factor which has anti-oxidative activity. Arc has been suggested to be involved in cytoskeletal organization and inhibit apoptosis. The present results suggest that anti-oxidative effects by an increase of NGFR in the midbrain may protect neurons from oxidative stress. Moreover, a possible inhibition of apoptosis by Arc may result in protection of neurons. Coffee aroma may have anti-anxiety and anti-stress effects. There are no conflicts of interest to declare. Supported by a grant (#24650506) from the Ministry of Education, Culture, Sports, Science and Technology, Japan to Masuo Y.

(COI: No)

P3-214

Examination of the change of IDO2 expression in the mouse under inflammatory condition

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It has been reported that the depression is related to inflammation. By the way, the serotonin is produced from tryptophan, while indolamine 2, 3 dioxygenase (IDO) is the enzyme which converts tryptophan into kynurenine. Although the local existence in the normal brain of IDO is still discussed, it has been reported that expression of IDO rises to several times in brain when inflammation happens. When IDO increase under the inflammatory condition, tryptophan would be converted into kynurenine, and production of serotonin would be decreased. Therefore, we supposed that depression would happen as a result of serotonin decrease. However, it is still unclear that which tissue express IDO1 and IDO2, a novel isoform indolamine 2, 3 dioxygenase, under normal condition, because that is different according to reports. We think that it is necessary to examine expression of IDO1 and IDO2 in normal tissue in detail at first. Next, we examine the expression of IDO1 and IDO2 in the mouse tissue under inflammatory condition. Now, we investigate the expression of IDO1 and IDO2, a novel isoform indolamine 2, 3 dioxygenase, in the mouse tissue which given lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (poly I:C, a toll-like receptor-3 agonist), and caused inflammation, using in situ hybridization and northern blot analysis. We cannot yet say clearly, but the expression of IDO2 also may change by inflammation. In this conference, we show the results and discuss the change of IDO expression in the mouse tissue under inflammatory condition.

(COI: No)

P3-215

Changing in sociality, learning ability, and neural activity induced by the juvenile isolation in mice

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Social isolation for the period from weaning to sexual maturity interferes with the development of the medial prefrontal cortex (mPFC)-dependent behaviors in mice. Also, psychological stress leads to activation of microglia in mPFC. Based on these findings, it is thought that the neural activity in mPFC of mice, which are isolated for two weeks immediately after weaning, is abnormal as a result of microglial activation caused by psychological stress. However, no direct evidence has been reported. In this study, therefore, we made the mice which are isolated for two weeks after weaning, and compared the social interaction and learning ability with those of control mice. Social interaction and learning ability of isolated mice were found to be lower than that of control mice. In control mice, the blockade of microglial activity did not change the neural activity in mPFC. In contrast, the blockade of microglial activity increased the neural activity in mPFC of isolated mice. And, after blocking ionotropic glutamate receptors, the blockade of microglial activity increased the neural activity in mPFC of isolated mice.

(COI: No)

P3-216

Analysis of neurotransmitters in the brain and behavioral abnormalities of offsprings from stressed mother in mice

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Previous studies have shown that giving to stress in pregnant mice increases autism and attention deficit hyperactivity disorder (ADHD)-like behaviors in offsprings. However, the mechanisms involved are still unknown. In this study, we examined whether or not the molecular expression in the brain and behavior of the offspring are altered by giving stress to the pregnant mice. All experiments were performed using male offspring that had been prenatally exposed to stress, and male offspring whose parents had been exposed to prenatal stress. We examined sociality and anxiety-like behavior of the offsprings by behavioral experiments. In addition, we have examined the molecular expression in the brain by immunostaining method. Anxiety-like behavior was not observed in the first and second generation of offsprings. However, the reduction of sociality and anxiety associated with obsessive-compulsive disorder was observed in the first and second generation of offsprings. From the results of immunostaining method, reduction in the number of cells that contain serotonin was observed in all mice. Furthermore, the increase in CRF expression level was found only in the first generation of offsprings.

(COI: No)

P3-217

DL-/PO-phosphatidylcholine restores restraint stress-induced depression-related behaviors and spatial memory impairment

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The present study investigated the effects of 1, 2-dilinoleoyl-*sn*- glycero-3-phosphocholine (DL-PC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PO-PC) on depression-related behaviors and spatial memory impairment in mice subjected to restraint stress. The immobility time in forced-swim and tail-suspension tests for mice subjected to restraint stress was significantly longer than that for nonstressed control mice, and oral coadministration of DL-PC and PO-PC (DL-/PO-PC; DL-PC : PO-PC=1 : 1) shortened the prolonged immobility time in a dose (0.1-5 mg/kg)-dependent manner. In the water maze test, the retention latency for stressed mice was significantly longer than that for control mice and DL-/PO-PC (1 mg/kg, *per os*) reversed the prolonged latency to control levels. Phosphorylation of Akt and glycogen synthase kinase 3β (GSK-3β) in the hypothalamus of stressed mice was significantly reduced compared with that for control mice, and DL-/PO-PC (1 mg/kg, *per os*) recovered the reduced phosphorylation of Akt and GSK-3β. The results of the present study indicate that DL-/PO-PC has the potential to ameliorate stress-induced depressionrelated behaviors and memory impairment, possibly by activating Akt and inhibiting GSK-3β.

(COI: No)

P3-218

Time-dependent effect of viral infection during pregnancy on brain 5-HT content using poly I:C

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It is well known that virus infection during pregnancy induces malformation(s) to the fetus including fetal cataracts, cardiac defects and deafness. However, little is known about influence of maternal virus infection to fetal brain development. Recently, we have shown that viral infection during pregnancy impairs fetal serotonergic development which may affect emotional or psychological status on offspring (Ohkawara et al, Brain and development, *in press*). At the last meeting of The Japanese Association of Anatomists, we reported rough critical period in impairment of serotonergic system on offspring by maternal viral infection. Now, we performed a detailed analysis of critical period and found that impairment of serotonergic system depend on at least two critical periods of viral infection during pregnancy and neonatal period. An early period is between gestation day (GD) 5 to GD17 and a late period is between GD21 to postnatal day 5. We will discuss the reason why impairment of serotonergic system has two critical periods of viral infection.

(COI: No)

P3-219

Ex-vivo MRI morphometric analysis of gray matter volume alterations upon rat PTSD-model stress

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Post-traumatic stress disorder (PTSD) is a mental disorder that occurs after exposure to severe stress. Voxel-based morphometry (VBM) is a comprehensive gray matter volume analysis by normalizing with a standardized template on brain MRI images. Several VBM studies were carried out in PTSD patients; however, it is unclear whether the neurological change is caused by the stress or by the patient's genetic disposition. In order to verify a causal relationship between severe stress and atrophy, VBM analysis in rats upon PTSD-model stress was carried out. Rats (male, 7 weeks old) in the experimental group were exposed to Single Prolonged Stress (SPS); 1) immobilization for 2 hours, 2) forced swimming for 20 min, 3) ether anesthesia. The rats were fixed after 7days, and their brains with skulls were removed. 3D-T2WI animal brain MRI images were then acquired. Gray matter regions were segmented using the standardized anatomical template, and VBM analysis was performed using Statistical Parametrical Mapping (SPM) 8. As a result, significant atrophy was detected in the thalamus, and right visual cortex (SPS: n=17, Sham: n=15, uncorrected $P<0.001$). These results suggest that thalamus and visual systems might be involved in the severe stress response and pathogenesis of PTSD.

(COI: No)

P3-220

Optogenetic activation of dorsal raphe serotonergic neurons produces antidepressant-like effect in mice

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It is unclear whether the activation of 5-HT neurons in the dorsal raphe nucleus (DRN) per se is sufficient for the treatment of depression. Recently, optogenetics using light-activated ion channels or pumps makes possible the modulation of target neurons in which these tools are specifically expressed. Here, we constructed a lentiviral vector (LVV) that induces expression of a channel rhodopsin 2 variant, ChETA, or light-activated proton pump for optogenetic inhibition, eArchT3.0, conjugated with EYFP under the control of tryptophan hydroxylase (TPH) promoter. One week after the injection of LVV into DRN of mice, we observed many EYFP-expressing neurons merged with TPH immunostaining. In whole-cell recordings in acute brain slice, photostimulation evoked single action potentials in ChETA-EYFP expressing neurons and inhibited neuronal firing in eArchT3.0 expressing neurons. Furthermore, in vivo photoactivation of the mouse DRN 5-HT neurons decreased immobility duration in the tail suspension test, while it had no effect on the anxiety-like behaviors in the elevated plus maze or open filed tests. On the other hand, photoinhibition of the DRN 5-HT neurons had no effect on these behavioral tests. These results suggest that the activation of DRN 5-HT neurons is sufficient to elicit antidepressant-like behavior in mice.

(COI: No)

P3-221

GABAergic interneurons in the spinal cord are increased in a chick model of spina bifida aperta

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Spina bifida aperta (SBA) is a complex congenital disorder with different neurological complications such as spinal ataxia, paralysis of the legs, and a lack of bowel and bladder control. We developed a chick model of surgery-induced SBA that shows spinal ataxia after hatching. However, the underlying pathophysiological mechanisms of SBA remain largely elusive. In this study, we examined the changes in inhibitory interneurons, GABA, glutamate decarboxylase 67 (GAD67), parvalbumin (PV), calbindin-D28K (CB), and calretinin (CR) in the spinal cord of surgery-induced SBA chicks on embryonic day 18. An immunohistochemical analysis showed increased levels of GABA and its synthesizing enzyme, GAD67, throughout the gray matter of the spinal cord in SBA chicks compared to normal chicks. We also found increased calcium-binding interneurons, PV, CB, and CR in the spinal cord of SBA chicks. The overexpression of calcium-binding proteins in the SBA chicks may have occurred to attenuate injury-mediated calcium excitotoxicity. Furthermore, the overexpression of GABA may represent at least a partly regenerative process following spinal cord injury as GABA influences the cytodifferentiation of developing neurons. In conclusion, we detected an increased level of GABAergic inhibitory neurons in the spinal cord of SBA chicks. This increase may have altered the inhibitory functions and, subsequently, muscle innervation of these chicks.

(COI: No)

P3-222

Autoantibody mediated CNS myelin morphology in the acute phase of experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the CNS. Demyelination and axonal damage are responsible for neurological deficits in MS. However, the mechanisms of demyelination and axonal damage have not been fully understood. To clarify the mechanism of demyelination in experimental autoimmune encephalomyelitis (EAE), we examined myelin morphology during the course of MOG35-55-induced EAE in the C57BL/6 mice. Osmium-maceration scanning electron microscopic (SEM) analysis displayed ultrastructural abnormalities of myelin structure in the white matter of the EAE spinal cord. In addition, abnormal morphology of myelin was observed at early stages of EAE. While infiltrating immune cells into the CNS were not observed in the spinal cord, anti-MOG autoantibody was observed in the CNS at this point. These observations suggest that anti-MOG antibody plays an important role in the pathogenesis at the acute stages of EAE.

(COI: No)

P3-223

Evaluation of Motor Cortex Myelination in Developmental White Matter Injury Model Rat by Electron Microscopy

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Perinatal hypoxia-ischemia (HI) causes developing white matter injury (DWMI), which induces neurodevelopmental disabilities. We previously established a DWMI model rat (HI in postnatal day 3) that shows the deficits of hindlimb motor function and motor coordination. Electrophysiological change in motor cortex (M1) by intracortical microstimulation and weaker staining of myelin basic protein in the area were observed in our model. In this study, we challenged to know whether myelination was changed in M1 by electron microscopy. The DWMI rats were perfused with the fixative of 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, and the brain coronal sections were soaked into the fixative, and processed for electron microscopy (EM) including fixation with 2% osmium tetraoxide. Ultrathin section of the motor cortex of hindlimb area were made and observed under EM. We counted the number of myelinated axons and their g-ratio in surface (layer II-III) and deep layer (layer V) of the cortex and corpus callosum (CC). No significant difference was observed in the number of both myelinated axons and its g-ratio in the layer II-III and the layer V. There was also no significant difference of those parameters in the CC. Data suggested that myelination in M1 is not related to the behavioral deficits in our DWMI model.

(COI: No)

P3-224

Analysis of oligodendrocytes in CD38 knockout mouse, a model associated with Autism Spectrum Disorder

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CD38, a type II transmembrane protein with ADP-ribosyl cyclase activity, is involved in Ca2+ -induced Ca2+ -release for Oxytocin (OXT) secretion in hypothalamic OXT neurons. In CD38 knockout (KO) mouse, a model associated with Autism Spectrum Disorder (ASD), decreased level of central Oxytocin secretion causes impaired social behavior, such as social recognition, pair bonding and maternal behavior. In this study, we investigated effect of CD38 on development of neurons and glial cells using CD38 KO mice from postnatal day 7 to 70. We found that MBP, MAG and CNP mRNA, markers of oligodendrocytes, were significantly decreased at postnatal day 7 to 14 in the cerebral cortices of CD38 KO mice. Furthermore, we confirmed that MBP and CNP proteins were also significantly decreased at postnatal day 14 to 21 of CD38 KO mice by western blotting and immunohistochemistry. These results suggest that deletion of CD38 cause inhibition of oligodendrocyte development in the cerebral cortex.

(COI: No)

P3-225

Maturation processes of developing prefrontal cortex of normal and disease model mice

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Altered development of prefrontal cortex (PFC) circuitry caused by a combination of genetic and environmental factors may underlay phenotypes of developmental psychiatric disorders like autism and schizophrenia. To gain insights into a sequence of biological events during PFC maturation, we performed gene expression, morphological, and drug sensitivity analyses. Expression of oligodendrocyte/myelin genes and a gene encoding fast-spiking interneuron marker parvalbumin were dramatically increased between postnatal days 7 (P7) and P21, and peaked at P21 and P35, respectively. Appearance of parvalbumin-positive interneurons increased drastically when we analyzed transgenic mice that express GFP under the parvalbumin gene promoter. Measurement of extracellular glutamate using in vivo microdialysis after an administration of MK-801, an NMDA antagonist, into the mouse PFC found that most responsive period for MK-801 administration was at around P42 and suggested that maturation of PFC circuit may take place after P42. These data suggest that circuitry maturation is a result of coordinated biological processes including synapse formation, myelination, and interneuron maturation and that many biological processes may be affected by diverse genetic and environmental insults, each having different sensitivity and time window. We are now characterizing genetically modified animals for genes that are associated with autism and schizophrenia to determine how circuitry maturation patterns are altered in these mice.

(COI: No)

P3-226

Intrathecal injection of mesenchymal stem cells ameliorates neurodegeneration of spinocerebellar ataxia type 1 mice

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No effective treatments have been developed for spinocerebellar ataxia (SCA). However, some studies have shown that mesenchymal stem cells (MSCs) are partially effective for Lurcher mutant mouse, a spontaneous genetic mouse model with cerebellar ataxia. Here, we tested the usefulness of intrathecal injection of MSCs for the treatment of SCA1 transgenic mice (SCA1-Tg). We observed that MSCs greatly mitigate cerebellar neuronal disorganization seen in the SCA1-Tg. Although Purkinje cells (PCs) of 24-week-old SCA1-Tg display multi-layer arrangement, MSCs-injected SCA1-Tg at the similar age showed mono-layer PCs. Furthermore, MSCs suppressed atrophy of PC dendrites in the SCA1-Tg. Finally, rotarod tests revealed that progressive deficits in motor coordination were significantly suppressed in the MSC-treated SCA1-Tg. However, we have not yet identified the mechanisms by which MSCs successfully ameliorated the neurodegeneration seen in SCA1 mice. Since recent studies have indicated the beneficial effects of factors released from MSCs through paracrine-mediated actions, we tested the hypothesis that some unknown factors released from MSCs are sufficient to prevent neurodegeneration of SCA1 mice. This notion was proven to be true. Upon injection of MSC conditioned medium into the SCA1-Tg, the progress of the behavioral defects were markedly mitigated. These results indicate an availability of a cell-free therapeutic approach against the SCA1. There are no potential conflicts of interest in the content of this study.

(COI: No)

P3-227

Mesenchymal stem cell-conditioned medium reduces the peripheral pathology in spinocerebellar ataxia type 1-knockin mice

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Spinocerebellar ataxia (SCA) is a major neurodegenerative disorder, in which autosomal-dominantly inherited polyglutamine diseases are the most frequent types and are caused by the expansion of a CAG trinucleotide repeat in the coding region of causative genes. SCA type 1 (SCA1) is caused by ataxin-1 protein (ATXN1) with an abnormally expanded polyglutamine stretch and is characterized by neurodegeneration in the nervous system. Mesenchymal stem cell (MSC) are defined as multipotent progenitor cells that can differentiate into mesenchymal lineage cells, such as osteoblasts, adipocytes, and chondrocytes, and into other cell lineages, such as glial cells and hepatocytes. We previously verified that MSCs reduce neurodegeneration seen in cerebellar Purkinje cell-specific SCA1-transgenic mice. Because MSCs are known to secrete a variety of growth factors that have both paracrine and autocrine activities in the damaged brain, we tested if MSC-conditioned medium can prevent the spinal motor neurons from neurodegeneration in SCA1-knockin mice. Application of the conditioned medium suppressed the neuro degeneration and consequently, maintained the conduction velocity in the spinal motor neurons of SCA1-knockin mice. These results suggest that unknown factors released from MSC mitigate progress of the functional disturbances seen in SCA1 knock-in mice in a paracrine manner. There are no potential conflicts of interest in the content of this study.

(COI: No)

P3-228

Cytoarchitecture of the cerebellum in the *laggard* mutant mouse

Yunus, Junaedy; Setsu, Tomiyoshi; Kikkawa, Satoshi; Sakisaka, Toshiaki; Terashima, Toshio (*Kobe Univ. Grad. Sch. Med., Kobe, Japan*)

The *laggard* mutant mouse, characterized by hypomyelination and cerebellar ataxia, is a spontaneously occurring mutant mouse caused by mutation in Kif14 (Fujikura et al., 2013). In this mutant, the laminated structures are cytoarchitecturally abnormal. Macroscopically, the cerebellum of this mutant mouse is smaller in size than the normal counterpart. Hematoxylin-eosin staining reveals that the mutant cerebellum has conserved foliation pattern, although they are rudimentary and the depths of the folds are markedly reduced. The lamination of the mutant cerebellar cortex is normal in general, but detailed analysis has demonstrated that granule cell layers are dramatically reduced, especially the internal granule cell layer that is almost absent. In the mutant, the Purkinje cell layer is cytoarchitecturally disorganized and arranged in a multiple cell layer instead of being arranged in a single line. The dendritic harborization of Purkinje cells is severely underdeveloped, as revealed by anti-calbindin immunostaining. TUNEL-positive cells in the external granule cell layer are increased in number, suggesting that the decreased population of granule cells in laggard mutant mouse is caused by the increased apoptotic cell death. In conclusion, the cerebellum of laggard mutant mouse is cytoarchitecturally affected, suggesting that the causal gene for the laggard mutation has multiple effects on the development of the laminated structures in the central nervous system in addition to the myelin formation.

(COI: No)

P3-229

Localization of huntingtin-associated protein 1-immunoreactive stigmoid bodies in the spinal cord of adult rat

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Huntingtin-associated protein 1 (HAP1) is a neural huntingtin interactor and is considered to be a determinant marker of the stigmoid body (STB). STB/HAP1 has putative protective functions against neurodegeneration. Although the expression of STB/HAP1 has been well described in the brain, little is known about its localization in the spinal cord. We immunohistochemically determined the distribution of STB/HAP1 in the spinal cord of adult Wistar rats in light (and fluorescence) and electron microscopy. HAP1-ir cells were abundantly expressed in the lamina I, II, III, V, sympathetic and parasympathetic preganglionic neurons of lamina VII, and lamina X, whereas HAP1-ir cells were relatively sparse in lamina IV and VI. In contrast, no HAP1-ir cells were found in the motoneurons of the lamina IX. Our present study suggests that STB/HAP1 in the spinal cord might play an important role in diverse spinal sensory and autonomic functions. Sensory and autonomic neurons in the spinal cord should be stable against stressful conditions as inducing neurodegeneration due to putative STB/HAP1 protectivity, whereas the motoneurons might be vulnerable to such stresses due to the absence of STB/HAP1 in lamina IX. Current results might explain why the spinal motoneurons are the constant target in certain neurodegenerative diseases.

(COI: No)

P3-230

Hyperphosphorylation of Tau at Ser396 occurs in the much earlier stage than appearance of learning and memory disorders in 5XFAD mice

Kanno, Takeshi; Tsuchiya, Ayako; Nishizaki, Tomoyuki (*Div Bioinform, Dept Physiol, Hyogo College of Med, Nishinomiya, Japan*)

The present study investigated the relation of age-dependent spatial learning/memory impairment and Tau phosphorylation in 5XFAD mice, a model of Alzheimer's disease. In the water maze test, the acquisition and retention latencies for 5XFAD mice at 6 months, but not 2 months, of age was significantly longer than those for wild-type mice at the same months of age, without difference in the swim speed and visual acuity between two groups. The level of glycogen synthase kinase 3β (GSK-3β) phosphorylation at Ser9 in the hippocampus for the 5XFAD mice at >4 months of age was significantly lesser than that for wild-type mice at the same months of age, while a robust increase in the Tyr216 phosphorylation of GSK-3β was found both in wild-type and 5XFAD mice at 6 months of age, without no significant difference in the extent between two groups. There was no significant difference in the Tau phosphorylation at Ser202/Thr205 in the hippocampus between two groups, but Ser396 phosphorylation for 5XFAD mice was significantly higher than that for wild-type mice at ages ranging from 2 to 6 months. The results of the present study indicate that Tau hyperphosphorylation in the brain for 5XFAD mice precedes high activation of GSK-3β and occurs in the much earlier stage than appearance of learning and memory disorders.

(COI: No)

P3-231

Whale meat extract improves learning memory in Alzheimer's disease model mouse

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Introduction: Whale meat extract (WE) is known to strong anti-fatigue effects. Recently, we discovered that whale meat also have robust anti-oxidative effects. However the effects on the central nervous system, remain unclear. The Senescence-accelerated prone 8 (SAMP8) strain has been characterized by accelerated impairment of learning and memory, as used model mouse of Alzheimer's disease. We hypothesize the anti-oxidative effect of WE could affect on the prevention of pathogenesis of Alzheimer's disease.

Material & Method: In the present study we investigated the effects of whale meat extract, chronically administrated in the diet, and examine the behavioral test in the SAMP8 mouse.24-weeks age SAMP8 mouse fed the low-safflower oil diet (LSO) as control diet, or whale meet extract containing diet (WE) for 30 weeks. Then, we have investigated behavioral test to examine learning and memory including open-field test, Y-maze test, new object recognition test, and multiple-T-maze test.

Results: The WE group was significantly improved the learning memory in Y-maze test and multiple-T-maze test compared to LSO group.

Discussion: Our data revealed that whale meat extract improved the learning memory in Alzheimer's disease model mouse. These results suggest that the continuous eating of whale meat is able to prevent the dementia including Alzheimer's disease and senile dementia.

(COI: Properly Declared)

P3-232

Differential expression of alpha-synuclein with a cell-type dependent manner in vivo

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α-Synuclein is physiologically localized at presynapses and is also known as a major pathological component of synucleinopathies including Parkinson's disease and dementia with Lewy bodies. However, the precise relationship between the physiological functions and the pathogenicity of α-synuclein remains to be elucidated. To address this issue, we investigated the subcellular localization and the expression property of the protein. Recently, we reported that α-synuclein is localized at excitatory synapses, but not at inhibitory synapses in the hippocampus. There was a differential expression of α-synuclein between excitatory and inhibitory neurons. Here, we further investigated the expression profile of α-synuclein in the mouse whole brain. Localization of α-synuclein was similar to that of vesicular glutamate transporter-1. However, the protein was exceptionally colocalized with glutamic acid decarboxylase in the external plexiform layer (EPL) of olfactory bulb, lateral globus pallidus (LGP), medial globus pallidus (MGP), and substantia nigra pars reticulata (SNR). Now, we further study the relationship between the expression of α-synuclein and each neuronal cell-type. Regulation mechanism of the protein expression will be discussed.

(COI: No)

P3-233

Involvement of excess neuronal nitric oxide synthase in the dopaminergic neurodegeneration in Zitter rats

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Excess nitric oxide (NO) results in the formation of toxic peroxynitrite and causes neurodegeneration. Moreover, inhibition of inducible NO synthase forming NO in glial cells has been shown to display neuroprotective effects. However, little is known whether neuronal NO synthase (nNOS) forming NO in neurons affects the neurodegeneration. Here we focused on Zitter rat (Zi) which is autosomal recessive mutant rat and shows the dopaminergic neurodegeneration with age. Using the mutant rats, we investigated the distribution of nNOS in the nigrostriatal dopaminergic system and the effect of chronic administration of nNOS inhibitor, 7-nitroindazole (7-NI), on the neurodegeneration. The high levels of nNOS-expression were observed in the basal brain and midbrain of Zi. Furthermore, the increased number of nNOS immunoreactive (nNOS-ir) cells was observed in the substantia nigra pars compacta (SNc). In addition, chronic 7-NI administration significantly prevents the reduction of TH-ir cells in the SNc and fibers in the caudate putamen (CPU). Using HPLC reveals the protection of 7-NI against the reduction of dopamine in the CPU of Zi. These results indicate that excess nNOS is involved in the slowly progressive dopaminergic neurodegeneration in Zi, and the inhibition of nNOS prevents the degeneration.

(COI: No)

P3-234

Regulation of tau phosphorylation at the Alzheimer-specific AT100 sites by tau phosphorylation protein complex

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Tau, a microtubule-associated protein, is essential to the integrity of microtubules in neuronal tissues. Interestingly abnormally hyperphosphorylated tau is found as a characteristic component of paired helical filaments and neurofibrillary tangles in Alzheimer's disease (AD) brain. It has been speculated that hyperphosphorylated tau dissociates from microtubules, impairs microtubule (MT) function and then induces neuronal cell death. However a key determinant of abnormal phosphorylation of tau in AD is still to be clarified.

We have been proposing that degree of tau phosphorylation in neuronal cells is regulated by not kinases or phosphatase but the functional unit associated with MT as tau phosphorylation protein complex (TPPC). Multiple tau kinases, CDK5, GSK3beta, PKA, and the chaperons like 14-3-3 protein are the major participants of TPPC.

We focused on kinase-kinase activities on tau phosphorylation by the members of TPPC and conducted *in vitro* kinase assay by multiple combinations of TPPC-kinases (CDK5, GSK3beta, PKA). We found that phosphorylation of AT100 specific sites by PKA were enhanced with preincubation of CDK5 or GSK3beta. The simultaneous applications also induced enhancement of phosphorylation of AT100-specific sites. These results suggest achievement of phosphorylation on AT100-specific sites may the key regulation on abnormal phosphorylation of AD.

(COI: No)

P3-235

Neuronal damage after bone marrow transplantation in a mouse model of Krabbe disease

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Bone marrow transplantation (BMT) and umbilical cord blood transplantation are the only therapies available to date for Krabbe's disease (globoid cell leukodystrophy). BMT cross-corrects the activity of galactocerebrosidase, the missing enzyme in myelinating cells, via donor-derived monocytes that enter the nervous system. However, BMT does not cure the disease. To determine why BMT is not curative in the long run, we investigated the pathology of twitcher mice (twi), a model of Krabbe's disease, that lived over 200 days after BMT. Besides severe demyelination both in the central and peripheral nervous systems long-term after BMT, neurons appeared to be affected as evidenced by 1) sporadic axonal spheroids present in the spinal white matter and 2) bilateral defect in neurons of the ventrolateral thalamic nuclei. This study demonstrates that enzyme replacement by BMT is not sufficient in the long term. It will be necessary to supplement BMT perhaps with other interventions such as transplantation of glial progenitors and gene therapies.

(COI: No)

P3-236

Synaptic dysfunction and abnormal social behavior in mice with knockdown of autism susceptibility genes in the prefrontal cortex

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Autism spectrum disorder (ASD) is characterized by deficits in social interaction and communication, and stereotyped and repetitive behaviors. Recent studies have identified hundreds of genes whose mutations are found in patients with ASD. However, roles of such ASD susceptibility genes in synapse development/function and behavior, and brain regions in which they function remain largely unknown. We have developed an experimental system to examine the roles of ASD susceptibility genes in the development and function of synapses in the prefrontal cortex (PFC) and ASD-like behavior in mice. We performed RNAi knockdown of ASD susceptibility genes in pyramidal cells of the mouse PFC at embryonic day 14-15 by in utero electroporation. We examined contactin associated protein-like 2 (CNTNAP2) whose mutation is reported to be implicated in the development of ASD and whose knockout mice show ASD-like behavior (Penagarikano et al., Cell. 147, 235-246, 2011). We found that knockdown of CNTNAP2 decreased excitatory and inhibitory synaptic transmission in pyramidal cells of the PFC. Moreover, mice with CNTNAP2-knockdown in the PFC showed impaired social interaction and communication. These results clearly indicate that our system is useful for evaluating the contributions of ASD susceptibility genes to the development of synaptic function in the PFC and to ASD-like behavior in mice. Our preliminary data suggest that decreased excitatory synaptic transmission in the PFC may be related to ASD-like behavior.

(COI: No)

P3-237

Toll-like receptors control neuronal cell death via regulating the activation and production of nitric oxide in microglia cells of cathepsin D-deficient mice

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Cathepsin D (CD) deficiency induces ceroid-lipofuscin storage in lysosomes of mouse CNS neurons. We have previously reported that CD-deficient (CD−/−) mice die approximately postnatal day (P) 26 accompanied by not only intestinal necrosis but also neuronal degeneration. In addition, we showed that chemical inhibition of nitric oxide (NO) production reduces neuronal cell death. To understand the relationship of neuronal cell death and NO production, we investigated the role of Toll-like receptors (TLRs). For this, we generated triple knockout (tKO) mice of TLR2, TLR4 and CD. The tKO mice increased lifespan to approximately P 29. Although no difference was observed in aggregate formation positive for ubiquitin and p62/Sqstm1 in neurons, i) accumulation of activated microglia, ii) expression of iNOS in microglia, and iii) neuronal cell death were all delayed in the hippocampal pyramidal cell layer. These data suggest that the activation of microglia followed by the production of NO is triggered through TLRs.

(COI: No)

P3-238

Polarized localization of p62 and NBR1 regulates selective autophagy in cathepsin D-deficient neurons

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Cathepsin D (CD) deficiency is known to induce autophagy and accumulate abnormal lysosomes called granular osmiophilic deposits (GRODs) in mouse brain neurons that are often up-taken by autophagosomes. We show here that p62 and NBR1 are required for selective autophagy to eliminate GRODs. Immunocytochemistry and morphometry revealed that p62, NBR1 and ubiquitin were positive in neurons deficient in CD, while autophagosomes with GRODs disappeared from neurons deficient in CD, p62, and NBR1. Moreover, immunosignals of p62 and NBR1 were observed only in somatodendrites but not in axons of CD-deficient neurons, where no GRODs were detected. This localization pattern of p62 and NBR1 was confirmed in primary cultured cortical neurons. N-terminal specific domains of these proteins are responsible for the polarized localization. These results suggest that polarized localization of p62 and NBR1 regulates the degradation system through autophagy. This work is supported by JPSP KAKENHI Grant numbers 23110571, 23111004 and 25670099

(COI: No)

P3-239

Histological analysis of the brain in Dystonin-deficient mice

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Dystonia musculorum (*dt*) is an inherited mouse neuropathy characterized by progressive motor disorders. *Dystonin* (*Dst*) is a causative gene for *dt* mice. Although degeneration of the peripheral nervous system during early postnatal stage is well-recognized in *dt* mice, histological appearance in the central nervous system (CNS) responsible for motor disorders are still unclear. We generated a novel *Dst* gene trap mice, Dst^{Gt}, in which actin-binding domain-containing isoforms are disrupted. The gene trap allele encodes for a mutant Dst-LacZ fusion protein, which is detected by X-gal staining with high sensitivity. Homozygous mice showed typical *dt* phenotypes with progressive neurological symptoms, such as severe motor disorders in their limbs and twisted postures. Electrophysiological study showed abnormal co-contractions of agonist and antagonist muscles in Dst^{Gt} homozygotes. In histological analysis, abnormal neurofilament immunoreactivity was found in both somatosensory pathway and motor-related reticular nucleus, which showed high Dystonin protein expression. These results raise the possibility that cell-autonomous primary CNS defects contribute to *dt* phenotype.

(COI: No)

P3-240

Characterization of model mice for nuclear envelopathies

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Mutations encoding nuclear envelope proteins can cause variable human diseases including muscular dystrophy, cardiomyopathy with conduction defects, central and peripheral nervous system disorders, skeletal deformities, metabolic disorders, and premature aging, and they are so-called "nuclear envelopathy". Emery-Dreifuss muscular dystrophy (EDMD) is the firstly identified human nuclear enevlopathy, clinically characterized as muscular dystrophy, cardiomyopathy with conduction defects, and early joint contractures. Several causative genes for EDMD have been identified. Mutations in the *EMD* gene that encodes emerin, an inner nuclear envelope protein, cause X-linked EDMD and limb girdle muscular dystrophy (LGMD). Mutations in the *LMNA* gene, encoding A-type lamins of nuclear lamina, are known to cause various human diseases including autosomal forms of EDMD, LGMD, dilated cardiomyopathy with conduction defects, lipodystrophy, premature aging syndromes, and so on. We have previously produced emerin knockout mouse (EKO), that show mild conduction delay and locomotion abnormality. Lmna H222P knock-in mice (H222P) produced by the other group show severe cardiomyopathy. Here, we produced double mutant mice of EKO and H222P (EH), and found more severe skeletal muscle involvement. Surprisingly, cardiac muscle from EH mice show milder fibrosis compared to H222P mice. In this paper, we will show the gene expression profiles of each mutant mouse to consider tissue-specific roles of emerin-lamin A interaction.

(COI: No)

P3-241

Rbm24 is involved in tissue-specific aberrant splicing of Familial dysautonomia

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Tissue-specific alternative splicing is a pathway where complex networks of RNA-binding proteins (RBPs) are involved. The IKBKAP gene, whose mutation is found in almost all patients with Familial Dysautonomia (FD) exhibit aberrant splicing in neural tissue and fibroblasts, while others such as lymphoblasts and muscle tissue show a preference to normal splicing in the presence of the FD mutation. We were prompt to seek for tissue-specific regulators of FD aberrant splicing. We constructed a splicing reporter with the genomic region of the IKBKAP gene surrounding the FD mutation. We found this reporter recapitulates the abnormal splicing in fibroblasts of FD patients as well as HeLa cells. Using this bichromatic fluorescent reporter and cDNA library of RBPs, we found Rbm24 corrects exon-skipping caused by the FD mutation. Rbm24 functioned through an intronic sequence downstream of the FD mutation. RNA electromobility shift assays show that Rbm24 binds to and recruits U1 snRNP to this region and induces normal splicing in spite of the FD mutation. Since Rbm24 is expressed mainly in muscle tissue and scarcely expressed in neural tissue, we believe Rbm24 is involved in tissue-specific aberrant splicing of FD and possible therapeutic implications can assessed by its ectopic expression.

(COI: No)

P3-242

Schizophrenia brain elucidated by T1w/T2w ratio MRI

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MRI T1w/T2w ratio signal intensity (calculated by T1 weighted image signal intensity divided by that of T2 weighted image) cancels the receiver coil bias and increases the contrast related to myelin content. We used T1w/T2w ratio signal intensity to investigate whole-brain voxel-wise differences between 29 schizophrenia patients and 33 healthy controls and compared the results with those for T1w image. Normalized T1w/T2w ratio images were created for all the subjects and both gray matter (GM) and white matter (WM) components of T1w/T2w ratio image were smoothed with Gaussian kernels of 8mm full width at half maximum. Similar processes were also performed to T1w image. A two-sample t-test was undertaken using SPM8 with age and gender as nuisance covariates. Multiple comparison correction was performed by setting the family-wise error (FWE) at a threshold of $p < 0.01$. In GM we identified bilateral insula, right olfactory cortex, right putamen and right parahippocampal gyrus to be significantly decreased using the ratio image whereas only bilateral insula were found in T1w image. In WM we identified abnormal signal intensity in the right superior medial frontal white matter using the T1w/T2w ratio image while no regions were found in the T1w image. These results indicated that T1w/T2w ratio image enhances the pathological changes in the SZ brain due to reduced myelin contents and that it is useful to map the myelin-related changes in SZ brain.

(COI: No)

P3-243

The astrocyte-specific promoter for transgene expression in the marmoset brain

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Glial fibrillary acidic protein (GFAP) is the major intermediate filament protein specifically expressed in astrocytes. We examined a mouse and marmoset GFAP promoter region necessary and sufficient for the robust promoter strength and astrocyte specificity. At first, mouse GFAP promoter segments of 5 different sizes (1.9, 0.6, 0.3, 0.2 and 0.1 kb) were examined in their promoter strength and astrocyte specificity in mouse cerebellum in vivo, using lentiviral vectors expressing GFP under the control of one of the 5 mouse promoters. We found that the GFAP promoter of 0.6 kb in length showed robust promoter strength and astrocyte specificity, whereas the promoter of less than 0.3 kb was inferior to the promoter strength and/or cell specificity. Then, we cloned the GFAP promoter of 0.6 kb in size from marmoset genome, which showed in a mouse cerebellum a similar promoter strength with a mouse GFAP promoter of 0.6 kb and highly specific expression in astrocytes including Bergmann glia. Our results suggest that 0.6 kb of the GFAP promoter region is necessary and sufficient for the promoter strength and astrocyte specificity in mouse and presumably marmoset brains. We are examining properties of the marmoset GFAP promoter of 0.6 kb in marmoset brain.

(COI: No)

P3-244

Glia-tumor interaction in microenvironment of brain metastases

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Interaction between tumor cells and glial cells are important in the brain microenvironment. In the previous study, we observed that microglia and astrocytes accumulated around human lung cancer-derived (HARA-B) cells in a rodent model of brain metastasis. In vitro experiments showed that tumor cells and astrocytes stimulate each other by releasing cytokines. On the other hand, tumor cells were harmful for neurons. Interaction between tumor-microglia and astrocyte-microglia, or tripartite system of tumor-astrocyte-microglia remained elusive. In the present study, we investigated tumor-microglia interaction. We have found that tumor cell-derived factors suppressed microglial activation and the expression of antigen presentation. These results suggest that tumor cells and astrocytes stimulate each other but tumor cells suppressed immune responses induced by microglia, causing immune evasion.

(COI: No)

P3-245

Age-related changes in brain cytokine profile associated with enhanced recruitment of bone marrow-derived cells into the brain

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Neurodegeneration is associated with altered immune-brain interaction. The senescence-accelerated mouse prone 10 (SAMP10) is a model of early onset brain aging following immune senescence. We hypothesized that the brain-immune interaction is perturbed in SAMP10 mice. We created 4 groups of radiation chimeras by bone marrow transplantation using young and aged SAMP10 and B6 mice as recipients with 5-week-old GFP transgenic B6 mice as donors and analyzed chimeras immunohistochemically. Donor's marrow-derived cells of the myeloid lineage entered discrete brain regions through the attachments of choroid plexus. In chimeric mice with aged SAMP10 mice being used as recipients, larger numbers of marrow cells entered more brain regions than the other groups in the diencephalon. We performed multiplex cytokine assays to determine tissue concentration of 10 cytokines in the diencephalon prepared from young and aged SAMP10 and B6 mice. Aged SAMP10 mice exhibited higher concentrations of IL-6, G-CSF, CCL11, CXCL1 and CXCL10 than the other groups of chimera. Immunohistochemistry revealed that these cytokines were expressed in astrocytic processes of the attachments of choroid plexus, periventricular astrocytes, tanycytes, and hypothalamic neurons. Therefore, the enhanced recruitment of bone marrow-derived cells into the brain may be associated with region-specific changes in cytokine microenvironment in SAMP10 mice.

(COI: No)

P3-246

The cervical intramedullary sudomotor pathway speculated from the pathophysiology of hemifacial dyshidrosis caused by cervical disc hernia in humans

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To elucidate the cervical intramedullary sudomotor pathway, we analyzed 11 patients aged 37-74 years with hemifacial hyperhidrosis compensatory to the anhidrotic area caused by cervical disc hernia. Lesion estimation was performed via simultaneous qualitative sweat testing (Minor's method) and infrared thermography in an artificial climate chamber at 40℃ and 50% relative humidity. Neurological examination, and magnetic resonance imaging (MRI) were also performed. Hemilateral and segmental hyperhidrotic patterns were identified. MRI showed disc protrusion near the midline (median type) in the hemilateral sweat pattern, and approximately 3 mm lateral to the midline (paramedian type) in the segmental sweat pattern with no intramedullary lesion. In 80% of the latter, the disc protrusion was ipsilateral and corresponded to the anhidrotic segment. In the median type, the protruded disc may compress the central artery and cause insufficient peripheral perfusion of the sudomotor pathway around the anterior horn, causing ipsilateral anhidrosis without motor or sensory disorders. In the paramedian pattern, the disc may compress the sympathetic premotor neuron in the dorsolateral fasciculus, and spare the upper segments synapsing the spinal segmental autonomic interneurons and propriospinal neurons projecting to the intermediolateral nucleus. In conclusion, analysis of hemifacial hyperhidrosis could help clarify the cervical intramedullary sudomotor pathway.

(COI: No)

P3-247

Protective efficacy of the acupuncture stimulation for paclitaxel-induced peripheral neuropathy

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Paclitaxel (PTX) is a mitotic inhibitor used in cancer chemotherapy, but it develops chemotherapy-induced peripheral neuropathy (CIPN). Acupuncture is effective in the pathogenesis of pain. Therefore, we tested the influence of acupuncture stimulation (ACU) with PTX-CIPN model rats. The SD rats were randomly divided into 4 groups: PTX group, ACU of PTX pre-treatment (A-prePTX; ACU started on day 0), ACU of PTX post-treatment (A-postPTX; ACU started on day 14), and control group. All rats were injected intraperitoneally on 4 alternate days (days 1, 3, 5, and 7) with vehicle (saline) or 2.0 mg/kg PTX. Electro-ACU which caused slight muscle twitch was applied to ZuSanli acupoint (ST36) in the limbs on every other day (right side, 1Hz, 20 min., 3-5V). Behavioral assays were carried out by mechano-hypersensitivity von Frey hair test in the feet, sciatic nerve territory. All rats were sacrificed on day 35, and the lumbosacral spinal cord was collected for microscopy examination. PTX and A-postPTX group produced significant mechano-hypersensitivity in the feet, but A-prePTX group did not show any decrease in the mechanical threshold. In the PTX and A-postPTX, P2Y12 receptor a large amount of microglia appeared. In conclusion, our study indicates that the satellite cells in the dorsal horn of spinal cord cause PTX-CIPN. However, applying ACU stimulation before PTX administration relieves it. Therefore, ACU stimulation is effective in preventing PTX-CIPN, but any delay in the start of ACU treatment may decrease its effect.

(COI: No)

P3-248

Identification of novel target genes of mood stabilizer treatment

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Bipolar disorder (also known as manic-depression illness) is a severe and chronic psychiatric disease. The pathogenesis of bipolar disorder is not well understood. Medical treatments effect partially, and relapses are often experienced in spite of medications. There are needs for better therapeutics and elucidation of pathological mechanism. Some of antiepileptic drugs (mood stabilizers) have clinical efficacy in bipolar disorder, but there is a preliminary understanding of the mechanism of the treatment effect. Interestingly, mood stabilizers each have their own therapeutic properties. Among them, valproate (VPA) has preponderance of mania treatment, while lamotrigine (LTG) appears effective for prevention of depressive episodes but not manic. We hypothesized that VPA involves in the regulation of mania specific genes and LTG does in depression specific genes. To explore novel target genes of mood stabilizer treatment, we conducted a comprehensive analysis using microarray. We used C57BL6J mice for primary cerebral cell cultures. On the 10th day in vitro cultured cells were treated with 1mM VPA, 0.1mM LTG or control medium. We extracted total RNA from cultured cells. We analyzed gene expressions by microarray. 100 candidate genes were validated by quantitative real-time PCR, WB and immunohistochemistry. Finally we identified several specific genes regulated by VPA or LTG treatment.

(COI: No)

P3-249

Effect of systemic angiotensin II on exercise-enhanced neurogenesis in adult rat hippocampus

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Physical exercise enhances adult hippocampal neurogenesis via cell proliferation, which is promoted by direct action of growth factors on neuronal stem cells. However, the mechanisms between exercise and growth factor-dependent hippocampal neurogenesis are not yet fully understood. We found that exercise-enhanced hippocampal neurogenesis is cancelled by treatment of an angiotensin II (Ang II) type 1 receptor antagonist, losartan, suggesting that Ang II is involved in this enhancement. Here, we examined the role of systemic Ang II in exercise-induced hippocampal neurogenesis in adult rats. Plasma Ang II concentration increased rapidly in response to 30 min of treadmill running. After undertaking this exercise once daily for a week, the number of proliferating cells, identified by 5-bromo-2′-deoxyuridine (BrdU) incorporation, had increased approximately 1.5-fold in the hippocampus compared with controls. To mimic the increase in plasma Ang II concentrations brought about by exercise, rats were injected with 10^{-5} M Ang II once daily for a week. The number of BrdU-incorporating cells and of doublecortin-expressing newborn neurons, in the hippocampus rose approximately 1.5 and 1.9-fold compared with controls, respectively. The effects were completely abolished by losartan. These findings suggest that an increased levels of systemic Ang II during exercise may enhance neurogenesis in the adult rat hippocampus.

(COI: No)

P3-250

Neuroregenerative Effect of Conditioned Medium of Adipose-Derived Stem Cell on Cerebral Infarction in Mice

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Accumulating evidence has pointed that adipose-derived stem cell or its conditioned medium (ADSC-CM) might be available for neuroregeneration after cerebral infarction. The present study investigated the neuroregenerative effect of ADSC-CM and the underlying mechanism. We made a model of cerebral infarction by ligating the middle cerebral artery of CB17 mice. ADSC-CM or DMEM as a control was intravenously injected 1 h after infarction. Mice were sacrificed after periods of time, and the brain was removed and fixed for immunostaining with an anti-MAP2 antibody. Then, we measured the infarct volume and the ratio of the left hemisphere size relative to the right one (L/R ratio). We also carried out immunohistochemical analysis using antibodies against BrdU, Tuj1, and DCX, to evaluate regenerative neurons. There was no significant difference in the infarct volume between ADSM-CM and control groups. The L/R ratio for ADSM-CM group was significantly higher than that for control group ($P<0.05$), indicating that ADSM-CM alleviates atrophy of the ipsilateral hemisphere. Tuj1- and DCX-positive neurons were found along the border of infarction in ADSM-CM group, while little is detected in control group. Taken together, these results show that ADSC-CM has the potential to promote neuroregeneration and therefore, could be developed as a beneficial treatment of cerebral infarction.

(COI: No)

P3-251

Physiological analysis in *Drosophila* circadian pacemaker neurons with Ca^{2+}/pH-sensitive fluorescent proteins

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Since circadian oscillations in the concentration of cytoplasmic Ca^{2+} have been observed in the mammalian master clock suprachiasmatic nuclei as well as plants, intracellular Ca^{2+} may act as a messenger to link molecular clock oscillations with cellular physiological rhythms. However, in *Drosophila*, no circadian change in any ion concentrations in master clock lateral neurons (LNs) has reported. In this study, we first analyzed physiological activities in LNs in organotypic cultures of *Drosophila* central nervous system using Ca^{2+}-sensitive fluorescent protein Yellow Cameleon (YC2.1). As a result, we observed parallel circadian oscillations in both YC2.1 donor and acceptor fluorescence intensities, suggesting the possibility that cytosolic pH in LNs may oscillate in a circadian manner. To verify this hypothesis, we generated transgenic flies expressing ratiometric dual emission pH sensor deGFP4 and monitored intracellular pH in cultured LNs. As a result, we observed circadian pH oscillations in LNs. The pH values range from about 6.5 to 7.7 and the amplitude range of each oscillation were about 0.5 pH unit. In summary, LNs exhibited circadian oscillations in intracellular pH but not in intracellular Ca^{2+} concentration in the sensitivity range of YC2.1.

(COI: No)

P3-252

Oxtr expressed in GABA neurons at the MeA are suspected to control social memory

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OXT/OXTR system is well known as one of the regulating mechanism for social behavior, and thus OXTR deficient mice are considered as a useful model to study the neural mechanisms which govern social memory. We used this model to reveal regions and neuronal subtypes which control the social memories. In this study, we found that OXTR positive GABAergic neurons in Medial Amygdaloid nuclei (MeA) play an important role for constructing social memories. First, we found social stimulation induced neural activation in MeA. This finding implies that MeA contributes important role in constructing social memory. We next analyzed histologically to clarify neuronal subtypes of OXTR positive neurons in MeA. Interestingly, in situ hybridization analysis reveals that 30% of GABAergic neurons in MeA were expressing OXTR. It was acceptable because previous studies suggest that loss of GABAergic neurons causes dysfunction of social memory as same as OXTR deficient mice. Then we hypothesize these OXTR positive GABAergic neurons are necessary for social memory function. To test this hypothesis, we generated GABAergic neuron specific OXTR deficient mice by crossing loxP flanked OXTR mice and vesicular GABA transporter locus cre recombinase knocked in mice. This conditional knockout mouse showed social memory abnormality in our behavioral analysis. Taken together, these findings propose that OXTR positive GABAergic neurons in MeA play an important role in social memory, and it might be potential mechanism of pathology of mental disorder such as autism.

(COI: No)

P3-253

Coffee polyphenol chlorogenic acid protects neurons against glutamate neurotoxicity

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Stroke remains the leading cause of adult disability. Involvement of various neurotransmitters and neuromodulators have been shown to contribute to the ischemic damage and neuronal cell death associated with stroke. The roles of glutamate release, glutamate receptor-mediated Ca^{2+} influx, production of nitric oxide (NO) by activation of nitric oxide synthase, and oxidative stress in the pathogenesis of ischemic brain injury have been well established. Recent epidemiological studies suggested that moderate coffee consumption might reduce the risk of neurodegenerative diseases such as stroke. Coffee contains a larger amount of coffee polyphenol (chlorogenic acid) than caffeine, however, the roles of chlorogenic acid in the prevention of ischemic injury have not been fully examined. In the present study, we investigated the protective effects of chlorogenic acid on cell death using primary neuronal cultures of mouse cerebral cortex. Glutamate-induced neuronal cell death was inhibited by pretreatment with chlorogenic acid. On the other hand, there was little effect of chlorogenic acid on NO-induced cell death. Our results suggested that coffee polyphenol chlorogenic acid protects neurons from glutamate neurotoxicity. They provide a basis for the therapeutic target for ischemic stroke.

(COI: No)

P3-254

Transcriptional and post-translational regulation of transmembrane protein 132A

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Transmembrane protein 132A (TMEM132A) was first isolated from rat brain using PCR-selected cDNA subtraction, and it was found to be predominantly expressed in the brain. However, the transcriptional regulation of the TMEM132A gene has not been fully characterized. In this study, we characterized the promoter activity of the 880 bp region upstream of the mouse TMEM132A, identifying several putative sites recognized by transcription factors, which are highly conserved between the mouse and human TMEM132A genes. A mutational analysis of the TMEM132A promoter identified a critical region for its activation just upstream of the transcriptional start site. We also found that this region could be bound by the transcriptional factor MAZ, which overexpression resulted in downregulation of the TMEM132A promoter activity. Finally, we investigated the levels of TMEM132A mRNA and protein after exposure to five different neurotoxic stimuli, including thapsigargin, tunicamycin, serum starvation, homocysteine and hydrogen peroxide. Treatment with thapsigargin, a calcium modulating agent, markedly attenuated the levels of TMEM132A mRNA and protein in NSC-34 cells. These results give new insight into the mechanisms involved in regulating TMEM132 expression, and suggest that several transcriptional and post-transcriptional pathways regulate TMEM132A expression under developmental and pathophysiological conditions.

(COI: No)

P3-255

Neuroprotective effects of zonisamide against oxygen glucose deprivation

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Recently, zonisamide, a well-known antiepileptic drug, is also used in therapy for Parkinson's disease. However, roles of zonisamide in various aspects of neuroprotection mostly remains to be elucidated. Previously we demonstrated neuroprotective effects of zonisamide against oxygen glucose deprivation (OGD) using rat hippocampus slice culture and reported it at the 119th Annual Meeting of the Japanese Association of Anatomists. In this study, to analyze the molecular mechanisms of neuroprotective effects of zonisamide, we screened the genes, the expressions of which were changed by zonisamide, using microarray system. We prepared RNA from rat hippocampus slice cultures, which were assigned to 4 groups: control, zonisamide-administered, OGD-treated, and OGD-treated and zonisamide-administered groups (all groups were duplicated.). We performed gene expression profiling of the RNA samples using microarray system and compared the gene expression patterns among these groups. The results showed that among 40 genes which changed their expression more than 4 times by OGD treatment, 31 genes tended to be suppressed their changes by zonisamide-administration. These genes included inflammatory protein and cellular stress response protein. Furthermore, we detected so far unknown changes in several gene expressions induced by zonisamide alone. We will also discuss the functions of these genes in neuroprotection.

(COI: No)

P3-256

Differential effect of peripheral administered kainic acid on *vasopressin* and *oxytocin* mRNAs in the supraoptic and paraventricular nuclei of the hypothalamus

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The supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus contain two types of magnocellular neurosecretory neurons: arginine oxytocin (OXT)-producing and vasopressin (AVP)-producing neurons. We have previously described that, by electrophysiological recording, kainite receptors (KARs) may be more highly expressed in OXT neurons than in AVP neurons in the SON neurons using transgenic rat lines. Here we examined the *in vivo* effect of kainic acid (KA) on OXT- and AVP-producing meurons in the SON and PVN using adult male Wistar rats. After 3h, 6h, 12h, 24h, 48h and 1 week after subcutaneous administration of saline or KA (4mg/kg), the gene expressions of the *OXT* and *AVP* in the SON and PVN were measured by in situ hybridization histochemistry. The gene expression of the *OXT* was significantly increased 3h, 6h, 12h and 24h after the administration of KA in the SON, magnocellular and parvocellular division of the PVN, and 48h after the administration of KA in the SON compared to saline, while, the gene expression of the *AVP* in the SON and the PVN did not differ among the groups. These results suggest that KARs are highly expressed in the OXT neurons in the SON and PVN, and that OXT neurons are more highly affected by peripheral administered KA than AVP neurons in the SON and PVN, which is consistent with our previous *ex vivo* study.

(COI: No)

P3-257

Selective retention of value representation in hippocampal CA1

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Hippocampus plays an important role in the formation of memories for space and events. However, it is not well understood how neuronal circuits are reorganized during the formation and retention of memory. To address this issue, we imaged neuronal activities during the learning using transgenic mice that express a fluorescent calcium sensor protein G-CaMP7 in hippocampal CA1 pyramidal neurons. Mice head-fixed under a two-photon microscope performed a memory task in virtual reality. Three target zones were placed along a virtual linear track. Mice need to remember correct target and stay there for 2 sec to receive reward. While the animals navigate through the track, a population of neurons exhibited place-cell like activity. In addition, another group of neurons fired while the mice stayed at the target zone. Over days, whereas the place-specific activities turned over to form new patterns, the representation of target was more stable: 2 % of all identified cells remained multiple days and still responded even reward zone were relocated. Therefore, these results indicate external representation has different stability in hippocampus. Information with higher value is more stably represented in subpopulations of pyramidal neurons in CA1 hippocampus than that without value.

(COI: Properly Declared)

P3-258

Total number of neurons of the hypoglossal nucleus after repeated crush injuries on the hypoglossal nerve in adult rats

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It is well known that peripheral nerve crush injury in neonatal animals causes retrograde neuronal cell death and thus results in drastic decrease in the total number of affected neurons. Although it is generally believed that this phenomenon does not occur in adult animals, a previous study reported substantial decreases in the total number of neurons of the hypoglossal (XII) nucleus by repeated crush injuries on the XII nerve in adult rats. Therefore, we re-examined the number of neurons in the XII nucleus after repeated XII nerve crush injuries by using stereological sampling, the most reliable counting method for whole quantification. Triple nerve crush injuries of the XII nerve were inflicted on adult rats at 1-week intervals, and the brainstem containing the XII nucleus was removed 4 weeks after the last crush. Frozen sections were cut at 50 μm, collected at 300 μm intervals, and stained with Nissl. The number of neurons in the XII nucleus was measured using an optical fractionator with the Stereo-Investigator software and total number of neurons in the XII nucleus was estimated. We report the effects of repeated XII nerve crush injuries on the number of XII neurons in adult rats.

(COI: No)

P3-259

Active participation of vasculature in immune-to-brain communication in the subfornical organ

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Inflammation in the body generates fever and activates the hypothalamic-pituitary-adrenal axis. Peripherally-released proinflammatory cytokines act on the brain and thereby cause these sick signs. Increased release of the cytokines into the general circulation can be experimentally achieved by systemic injections of lipopolysaccharide (LPS). Although LPS and cytokine cannot pass the blood-brain barrier (BBB), there are some small brain areas that lack a typical BBB, so-called circumventricular organs (CVOs). Among them, the subfornical organ (SFO) is a key site for immune-to-brain communication. Recently, we reported the occurrence of continuous angiogenesis in the CVOs of adult mice, suggesting that the vasculature of the CVOs has more dynamic property. In the present study, we showed that the administration of LPS decreased proliferation of endothelial cells and vascular permeability in the SFO. We focused on Platelet-Derived Growth Factor-B (PDGF-B) signaling in this system, because PDGF-B regulates vascular remodeling. After single LPS administration, PDGF-B protein levels increased in the SFO. Repeated LPS injection attenuated LPS-induced nuclear STAT3 translocation and c-Fos expression in the SFO. These data suggests that vascular remodeling in the SFO play an important role in immune-to-brain communication.

(COI: No)

P3-260

Distinct post-transcriptional regulation of monocarboxylate transporter 1 expression between neurons and non-neuronal cells in the adult mouse brain

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Rapid transport of monocarboxylates is essential for the carbohydrate, fat, amino acid metabolisms. The transport is facilitated by proton-linked monocarboxylate transporters (MCTs). In the present study, cellular expression of MCT1 in the adult mouse brain by fluorescent in situ hybridization and immunohistochemistry. In the hippocampal CA1, high neuronal expression was shown by intense MCT1 mRNA signals in pyramidal cells expressing vesicular glutamate transporter-1 (VGluT1) mRNA. Low expressions were also found for GABAergic interneurons expressing 67 kDa-glutamatic acid decarboxylase (GAD67) mRNA, astrocytes expressing plasmalemmal glutamate transporter GLAST mRNA, and capillary endothelial cells expressing vascular endothelial growth factor receptor-1 (VEGFR1) mRNA. By immunofluorescence, however, MCT1 immunoreactivity was intense in astrocytes expressing 3-phosphoglycerate dehydrogenase and capillary endothelial cells expressing glucose transporter-1, but negative in pyramidal cell dendrites and somata expressing microtubule-associated protein-2. Such a dissociated transcription and translational control in neurons was also found in Purkinje cells in the cerebellum and cholinergic neurons in the dorsal motor nucleus of vagus nerve. Therefore, neuronal expression of MCT1 is transcriptionally active, but suppressed at the post-transcription levels. Though this mechanism, predominant MCT1 expression in astrocytes and capillary endothelial cells is constructed in the adult brain.

(COI: No)

P3-261

Estrogen increases the expression of platelet-derived growth factor receptor alpha (PDGFRα) in NG2-positive oligodendrocyte precursor cells of the hypothalamus in rats

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NG2 cells are thought to differentiate into oligodendrocytes which have ability to maintain myelin structure. Therefore, they are known as oligodendrocyte precursor cells. However, their functions are no only limited as oligodendrocytes: it is suggested that they develop into neurons. It was also discovered that they express PDGFR α for their survival. In the present study, we examined whether estrogen induced PDGFR α expression in the rat hypothalamus. Firstly, we confirmed NG2 cells also expressed PDGFR α in the rat hypothalamus. Microglia (CD11b, Iba 1), astrocyte (GFAP), immature oligodendrocyte (O4), and mature oligodendrocyte (NS) did not co-localized in PDGFR α. This indicates that PDGFR α -positive cells are NG2 cells and oligodendrocyte precursor cells in the rar hypothalamus. Rats were ovariectomized and used for 2 weeks later. Estrogen treatment with a silicone tube implantation decreased body weight gain by decreasing the amount of food consumption. Also, this dose of estrogen was capable to increase the uterus weight. Importantly, estrogen treatment significantly increased the expression of PDGFR α protein in the hypothalamus revealed by western blotting. We suggest from the present study that estrogen induces PDGFR α signals in oligodendrocyte precursor cells and contributes to decrease in body weight controlled by the hypothalamus.

(COI: No)

P3-262

Regionalization of the Lamprey Telencephalon by *Foxg1*; Evolution of DV Patterning of the Vertebrate Telencephalon

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The telencephalon is the most complex and divergent structure of the brain. During development, the telencephalon can be divided dorsoventrally into a pallium (cortex) and a subpallium (basal ganglia). Regarding its regionalization, *Foxg1*, a downstream of FGF signaling, is thought to be a key mediator by suppression of *Wnt8b* in the pallium and promotion of subpallial fate by studies in mice and teleosts. However, the evolutionary history of the telencephalic DV patterning is less understood. Lamprey is only one of two living jawless vertebrates diverged from jawed vertebrates over 500 million years ago. In this study, we identified three *Foxg1* homologous genes from Japanese lamprey, (*L. japonicum*). Of those, *LjFoxg1b* is widely expressed in the lamprey telencephalon as in jawed vertebrates, whereas *LjFoxg1a* and *c* are expressed only in the subpallium. Functional assays utilizing FGF inhibitor resulted in significant reductions of *Foxg1* as well as subpallium markers (*Dlx* etc.) by contrast with expansion of pallium markers (*Wnt8*, *Pax6* etc.). We also identified three *Six3/6* homologous genes, required for the *Fgf8* induction at the anterior neural border in jawed vertebrates. The overall expressions of *LjSix3/6s* are similar with that of *Six3* and *6* in mice. Altogether, we point out that, those developmental mechanisms might have been established by the last common ancestor of vertebrates.

(COI: No)

P3-263

Reproducing Retinal Rod Bipolar Cell Light Response by Mathematical Model Including Neurotransmitter Receptors

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Electroretinogram (ERG) is clinically used for diagnoses of retinal diseases. However, detailed mechanism of the ERG wave has not been clarified yet. Therefore, it is useful to understand the quantitative physiological characteristics of the cellular functions of retinal neurons and the connections among these cells. In 1997, Ishihara et al. proposed a mathematical model of bipolar cell body. Although the model contains ion channel models, existing on cell body, neurotransmitter receptors, which are essential for reproducing the retinal light response are not included. Here, we propose a retinal rod bipolar cell model which can reproduce voltage response of light. This model is constructed by introducing two neurotransmitter receptor models, TRPM1 and GABA C receptor, and a simple amacrine cell model to the electrophysiological model of bipolar cell body proposed by Ishihara et al. TRPM1 channel functions as light signal source which is produced by photoreceptor cell, while GABA C receptor functions as lateral inhibition signal produced by the surrounding amacrine cells. Resulting action potential of the model were evaluated by providing several light signals, where experimentally obtained photoreceptor membrane potential shapes were used as input to TRPM1 channel. The resulting membrane potential shape showed good agreement with the experimentally obtained data. Additionally we considered to reproduce b-wave of ERG by using this model.

(COI: No)

P3-264

Effects of electrical microstimulation to the primate cerebellar dentate nucleus on the detection of stimulus omission in the missing oddball paradigm

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We recently found that neuronal activity in the dentate nucleus (DN) of the cerebellum exhibited temporally-specific firing modulation when monkeys performed the missing oddball task (Ohmae et al., J Neurosci, 2013). In this task, an audiovisual stimulus was presented repeatedly at a fixed interstimulus interval (ISI), then one stimulus in series was omitted. Monkeys were required to predict the timing of each next stimulus so as to make a saccade in response to the stimulus omission. We applied electrical microstimulation (200-333 Hz for 100-200 ms at 100 μA) to the DN 100 ms before the stimulus omission to test if neuronal activity played a role in temporal prediction. Electrical stimulation significantly shortened the reaction time by 67.8 ± 57.2 ms and 50.5 ± 73.3 ms (SD, n = 41, ISI = 400 ms, t-test, $p < 0.05$) for contraversive and ipsiversive saccades, respectively. The same stimulation current delivered before the second audiovisual stimulus in the sequence or during intertrial interval failed to evoke saccades, suggesting that neuronal activity in the DN might not simply represent saccadic motor commands. The effects of electrical stimulation varied depending on its timing during the ISI just before the oddball, indicating that the stimulation effects were modified by the existing neuronal activity. These results suggest that neurons in the DN may carry signals related to the prediction of stimulus timing that could be advanced by electrical stimulation.

(COI: No)

P3-265

Dose-related changes in hindbrain of prenatally X-irradiated rats

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Pregnant SD rats were exposed to a single whole body X-irradiation at 0.5, 1.0 or 1.5 Gy on gestational day 15, and their offspring at 4 weeks of age were intracardially perfused with 4% paraformaldehyde solution under deep anesthesia. T2-weighted MRI at 11.7-tesla was acquired from fixed brains, and then sagittal sections of the cerebellum and coronal sections of the brainstem were made. By MRI-based volumetry revealed dose-dependent reduction in the volume of cerebellar cortex. However, there were no alterations in the cerebellar lobulation and cortical cytoarchitectures of prenatally X-irradiated rats. Regarding the brainstem, immunohistochemical analysis was performed using anti-heat shock protein 25 (HSP25), a maker for cranial nerve motoneurons. In control rats, HSP25 immunostaining appeared in the motor and mesencephalic nuclei of the trigeminal nerve, facial nucleus, abducens nucleus, accessory facial nucleus, the magnocellular region of medial vestibular nucleus, the ambiguous nucleus, dorsal nucleus of vagus nerve, hypoglossus nucleus, the spinal tract of the trigeminal nerve, and facial nerve tracts. In prenatally X-irradiated rats, HSP25 staining in those neurons was enhanced with increasing doses of prenatal X-irradiation. The results suggest a dose-related cerebellar cortical hypoplasia and an increased expression of HSP25 in cranial nerve motoneurons and their related fiber tracts in prenatally X-irradiated rats.

(COI: No)

P3-266

The morphological differences between the right and the left cerebral hemispheres relating to the dominant hand observed with the naked eye and MRI

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The morphological differences between the right and the left hemispheres of the human brain have been remained to study. In the present study, using 25 brains for training of the medical students, in order to clarify the differences the following 2 points, which can be seen relatively easily with the naked eye, were observed with the naked eye and MRI: 1) the length from the frontal pole to the occipital pole (A-P), 2) the length from the cerebral longitudinal fissure to the most lateral part of the lateral surface of the hemisphere (M-L). Eighty-four % of all A-P cases were longer in the left hemisphere, about 80% of all M-L cases were longer in the right hemisphere. These results show that the left hemisphere is elongated anteroposteriorly and the right hemisphere is widened mediolaterally by the three dimensional pressure of the cranium and that the left hemisphere develops slightly earlier than the right hemisphere.
It is assumed that the earlier development of the left hemisphere relates to the right hand dominance. Observing MRI from the 7 left handed persons, it became clear that results for the above 1) and 2) cases in MRI was very similar to those of the naked eye observation. This means that the morphological differences between the bilaterality hemispheres do not correspond to the laterality of the dominant hand.

(COI: No)

P3-267

Structural changes in pericyte may cause the dysfunction of BBB in rat gliomas

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Blood-brain barrier;BBB is composed of endothelial cells, pericytes, astrocytes, and their basement membranes. Among them, pericytes have been attracting attention as one of the main contributors for construction and maintenance of BBB. Meanwhile, gliomas are characterized by angiogenesis of leaky blood vessels, which BBB is not properly functioned. We speculated that in gliomas, certain structural changes or scantiness of functional pericytes might be involved in the formation of disfunctional blood vessels and performed morphological examinations to elucidate the possible involvement of pericytes using a rat glioma model(RG2 glioma line). RG2 cells(1×106) were stereotactically injected in the right striatum of female Fischer 344 rat brains. After two weeks, animals were injected intravenously with tomato lectin in order to evaluate vessel structures and leakiness, and sacrificed by fixative perfusion. Glioma tissue sections were prepared for immunohistochemical examinations. Desmin+ and PDGFR β + pericytes were abundantly found on the leaky vessels characterized by extravasated lectin. Besides, they were covered by a type-IV collagen+ basement membrane together with endothelia similar to those in normal brain vessels. However, they typically showed various shapes, and projected multiple cytoplasmic processes into the stroma, which is not usually obserbed in normal brains. The new formation of dysfunctional vessels in gliomas might not be related not to scarceness of pericytes but to their certain phenotypic changes and dysfunction.

(COI: No)

P3-268

Visualization of cell division in neurons isolated from the rodent central nerveous system

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It has long been recognized that neurons in mammals complete cell division during embryonic and neonatal life and thereafter they differentiate and do not divide any more. However, current evidence shows that neural stem or progenitor cells exist in the postnatal brain and produce neurons *de novo*. The present time-lapse imaging shows that cultured rodent neurons are dividing. Cell division occurred in neurons derived from the various central nervous system (CNS) regions (cerebral cortex, hippocampus, hypothalamus and spinal cord) of rats at all ages from fetus to adult. Regardless of the CNS region and age, approximately 15% of neurons divided during 12 h and the mean division interval was 21 h. The divided cells were identified as neurons since they were positive for neuronal markers but not for glial markers, and showed action potentials. The cells identified as neurons by live-cell immunocytochemistry, expressing neuronal cell surface antigen Thy1.1 but not neuronal stem cell surface antigen prominin-1, were dividing. Cell division was also found in neurons of the Thy1-yellow fluorescent protein (YFP) transgenic mouse, which can be identified as neurons by fluorescence emission. The present study further indicated that some neurons in culture or immediately after isolation from new-born or adult rats were in cell cycle and showed mitotic figures, DNA precursor incorporation and DNA replication. These results suggest that rodent CNS cells showing neuronal characteristics have the ability of cell division under physiological culture conditions.

(COI: No)

P3-269

Distribution pattern of hydra synapsin revealed heterogeneity of synapses in the diffuse nervous system

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The diffuse nervous system (DNS) in cnidarians and ctenophores is composed of diffusely distributed and loosely connected neurons. The DNS is believed to be the most primitive form of the nervous system. Though the DNS appears to be homogenous neuronal network, conspicuous nerve bundles have been observed. These nerve bundles would be the phylogenetically oldest neuronal circuits. To understand the evolutional origin of neuronal circuits, we are screening for genes that are expressed in the nerve bundle around the mouth, the nerve ring, of Hydra. Among the candidate genes, we found that a Hydra gene homologous to synapsins in the other animals is expressed in the nerve ring. Hydra synapsin (HySyn) was generally detected as punctate staining along the neurites by immunostaining. At the ultrastructural level, HySyn immunoreacitivity was associated with synaptic vesicles. In addition to the nerve ring, a large number of HySyn-positive neurites were detected in the tentacles and the head region. In the other regions such as the body and peduncle, a small number of HySyn-positive fibers were observed. Our results indicate that HySyn is produced and utilized in the synapses of subpopulations of neurons in the Hydra DNS. Synapsin-expressing neurons in the DNS may have more elaborate synapses compared with the other neurons. The function of HySyn is currently being examined by in vivo transgenic approaches.

(COI: No)

P3-270

Autonomic nervous response and subjective sleep quality for sleep in older adults

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This study is to investigate the autonomic nervous response for 2 hours from onset of sleep and subjective sleep quality (SQ) for sleep in elderly The seven subjects aged 64-82 years participated in this study from three to six times during sleep, each in separated days. The subjects measured the ECG using heart rate monitor and the total sleep time (TST) using sensor mattress, while asleep and answered the questionnaires about SQ (VAS), the sleep onset, and so on. Autonomic function was estimated by the Lorenz plot and time domain analysis for RR interval (RRI). In this study, data were collected from 7 older adults over 21 nights in their own home. The TST and SQ in older adults were 363.5 ± 78.4 (min) and 67.9 ± 20.1. The mean RRI after the onset of the sleep first became longer, and then gradually became shorter, compared with during awake. The index of parasympathetic function (rMSSD) showed also a change similar to that of the RR interval. Our subjects showed that the SQ had positive significant correlation either with the TST ($r=0.610$, $p<0.01$), or with the sleep efficiency ($r=0.485$, $p<0, 05$). Number of nights lengthened in RRI was 16 of the all the experimental nights 21. In case of 16 nights, SQ showed positive significant correlations either with the TST or with the sleep efficiency. We concluded that although the SQ was influenced by the TST, it can be also related to other factors, such as sleep latency and number of awakenings. This work was supported by JSPS KAKENHI Grant Number 23593466.

(COI: No)

P3-271

Possible involvement of glucocorticoids in the inhibition by stress of sweet/umami receptor induction in rodents

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Chronic exposure to stress reportedly inhibits induction of common receptor (T1R3) for sweet and umami taste in rats. Here, we investigate whether endogenous glucocorticoids (GCs) are responsible for this receptor inhibition. In addition, mouse taste bud cells (TB cells) expressing T1R3 were used to examine the effect of exogenous GC on the induction of T1R3. Both adrenal glands were removed from rats [adrenalectomized (ADX) rats] and expression of T1R3 mRNA in fungiform papilae was examined by real time RT-PCR. The expression of T1R3 mRNA in the sham-ADX rats was significantly reduced in the ADX rats. The reduced mRNA expression was restored to the level seen in the sham-ADX rats by administration of the smallest dose (0.1 ng/kg, i.p.) of dexamethasone (DEX). However, the larger doses of DEX (10 and 1000 ng/kg, i.p.) conversely inhibited the enhancement of mRNA expression seen in the ADX rats given smallest dose of DEX. The mRNA expression for GC receptor (GR α) was detected in the mouse TB cells by RT-PCR. Significant reduction of T1R3 mRNA expression, as measured by real time RT-PCR, was observed in the TB cells at 24 or 48h after application of three doses of DEX (0.1, 1.0 and 10 μM). These results suggest that small dose of endogenous GC is necessary for the expression of T1R3, while the larger doses inhibit the expression, and that this inhibitory effect exerted by GC might be, at least in part, due to its direct action on the taste cells in rodents.

(COI: No)

P3-272

Regulatory role of AMPK in hypothalamic CRH neurons in social stress-induced alteration of feeding behavior

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Corticotropin releasing hormone (CRH) secreting neurons in the paraventricular nucleus of the hypothalamus (PVH) have important roles for the stress responses. Recently, we revealed that preferential activation of AMPK in CRH neurons in the PVH enhanced carbohydrate intake but not fat intake. This alteration of food selection behavior was caused by the activation of a subset of CRH neurons induced by AMPK activation. However, the physiological relevance of AMPK in stress induced alteration of feeding behavior has still been unclear. In the present study, the objective is to unravel whether AMPK in hypothalamic CRH neuron regulates stress-induced alteration of food selection behavior.

We subjected C57BL/6J mice to social defeat stress and examined the alteration of food selection behavior. To investigate the role of AMPK in the CRH neurons, we examined the effects of preferential expression of shRNA for AMPK in CRH neurons in the PVH. We constructed lenti virus vector expressing shRNA for AMPK in Cre recombinase (Cre) dependent manner, and injected into the PVH of CRH neuron-specific Cre expressing knock-in mice.

We found that social stress increased carbohydrate selection in C57BL/6J mice. This alteration of food selection behavior was completely blunted by expression of shRNA for AMPK in CRH neurons in the PVH. This result suggests that AMPK in CRH neurons in the PVH is important for stress-induced carbohydrate eating.

(COI: No)

P3-273

Influence of roller coaster boarding on human beings

Tsuda, Mayuko; Toshima, Hiroko (*Department of Nutrition, Ciba Prefectural University of Health Science*)

Purpose: The roller coaster of amusement park is popular. I evaluated the influence which roller coaster boarding has on the autonomic function, and considered the popular reason of the roller coaster.

Subject: Twelve young healthy male (23.5 ± 2.8years old). All the subjects never had ridden on the roller coaster which I used for loading. Eight subjects were fearful during boarding, and four were not. Ten subjects wanted to board once again, but two never wanted to board.

Method: I measured the plasma epinephrine concentration before and after roller coaster boarding. I recorded subject's holster ECG during roller coaster boarding and calculated HR. Sympathetic function (LF/HF) was calculated by using frequency analysis of RR intervals of ECG.

Results: 1. Plasma epinephrine concentration (1) Increase group (pre, post, No.) (40.2 ± 15.5, 58.5 ± 19.5, 4) (2) Slightly increase group (38.7 ± 16.6, 44.3 ± 17.5, 6) (3) No change group (63.5 ± 8.8, 62.1 ± 9.0, 2) 2. HR and Sympathetic function. (1) In epinephrine increase group, HR and LF/HF ratio increased (70 ± 20, 130 ± 25, 6) (3.5 ± 4.0, 20 ± 8.9, 6) (2) In epinephrine no change group, they did not change (80 ± 12, 110 ± 15, 6) (7.5 ± 5.8, 7.8 ± 7.5, 6)

Discussion: Six subjects whose epinephrine and LF/HF increased were considered to feel the fear of roller coaster as pleasure. Four subjects whose epinephrine slightly increased and LF/HF did not change were considered to feel the change of gravity as a pleasant sensation.

Conclusion: It was thought that there were two type roller coaster lover, those who like a fear feeling, and those who like gravity change.

(COI: No)

P3-274

Physical exercise reduces social avoidance induced by defeated stress

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Psycho-social stress is one of the important risk factor for depression. Recent studies reported that physical exercise eases depressive behavior. But, effect of exercise on psycho-social stress is unclear. In this study, we investigated the effect of exercise on social interaction behavior using social-defeat stress (SDS). Male C57BL/6J mice were attacked by retired ICR mice for 2.5 min. After the SDS, mice were transferred to another cage with (Ex) or without (St) freely accessible activity wheel for 2 hours. After then, mice were housed with the same ICR mouse resident preventing physical contact using acryl central plate during stress period. We also made control (Con) or exercise (N-Ex) group without SDS. The SDS was performed for 5 days followed by 2 days of no SDS. Two weeks later, we examined some behaviors in mice and monoamine level in brain. St group showed negative social interaction with ICR mice compared to Con group while Ex group did not show the negative social interaction. In contrast, N-Ex group showed positive social interaction. There were no significant differences in other behavioral tests among these groups. We observed that exercise decreased the elevated monoamine turnover by SDS in amygdala, which is critical site for fear memory. These results suggest that physical exercise reduces the SDS-induced social avoidance behavior with decrease of monoamine turnover in amygdala.

(COI: No)

P3-275

Effects of lactational perfluorooctanesulfonate exsposure on visual discrimination learning in adult male mouse offspring

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Perinatal perfluorooctanesulfonate (PFOS) exposure has been suspected to affect brain fuctions. However, little is known on the neurotoxic effects of PFOS on the cognitive functions. Here, we examined whether lactational exposure to PFOS affect the performance of visual discrimination learning and hippocampal development. For PFOS esposure, post pertum C57BL/6J mouse dams received 1 mg/kg b.w. of PFOS via gavage from post natal day 1 to 14. Control dams received water as a vehicle. After mice progeny reached adulthood, the visual discrimination learning task was conducted. After the completion of behavioral tests, extracellular amino acids levels in the dorsal hippocampus were assessed using *in vivo* microdialysis. The performance of PFOS-exposed group was significantly lower than that of control group. In addition, PFOS-exposed group showed higher extracellular glutamate levels in the dorsal hippocampus compared to those of the control group. These results suggest that lactational PFOS exposure affects learning ability and hippocampal development.

(COI: No)

P3-276

Peripheral clock gene expression by bright light exposure during daytime in humans

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Light is most strong synchronizer for control of circadian rhythm. The light intensity and the duration of light are changed through a year, affecting the body weight, food preference and melatonin secretion in humans and animals. We investigated that the effect of bright light exposure during daytime on clock gene expression using hair follicular and root cells. Seven healthy men participated in this study. The participants completed two 3-day experimental sessions in 1 month. The sessions consisted of a period of dim light on the first day, followed by a period of bright light exposure during daytime on the 2nd day instead of a bright light exposure. Other session consisted of a period of dim light exposure during daytime through the experiment for 3 days. Hair samples were taken at 3 pm, 6 pm, and 9 pm on the 2nd day and 3 am and 7 am on the 3rd day. We assessed mRNA changes in levels of *Per1, 2, 3, Cry1, 2, Rev-erb-α, Rev-erb-β, Dec1* using branched DNA probes. The clock gene expression of *Per 3* and *Rev-erb-β* were significantly increased through 3 pm and 7 am. However, the clock gene expressions were not significantly different between bright and dim light exposure in humans. It suggested that bright light exposure during daytime did not effect on the clock gene expression in humans.

(COI: No)

P3-277

Variations in endothelial function after mental stress during the menstrual cycle in young women

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The changes in the hormonal milieu throughout the menstrual cycle have direct actions on arterial wall physiology in women. However, very limited human data are available regarding the alterations in endothelial function at baseline and after mental stress during the discrete phases of the menstrual cycle. We examined whether the menstrual cycle influences the endothelial function after mental stress in young women. Female university students were tested during three phases of a normal menstrual cycle. Delineation of the three phases occurred as follows: (1) the early follicular phase; (2) the late follicular phase; (3) the middle luteal phase. Non-invasive measurement of peripheral endothelial function was determined by flow-mediated dilation (FMD) testing in the brachial artery during reactive hyperemia using echo and Doppler ultrasound. After the measurements of basal levels, they were subjected to mental stress evoked by the modified STROOP Color Word Test (CWT) in 10 min. The CWT induced arterial pressure (MAP) and heart rate (HR) elevations, and a slight vasoconstriction of brachial artery. The measurements of flow-mediated dilation (FMD) and maximal blood flow (%) were repeated at 5 min and 30 min after the CWT. Basal FMD varied during the three phases cyclically. In addition, the effect of CWT on FMD was changed during the menstrual cycle. These findings suggest the importance of menstrual phase in the interpretation of data on endothelial function.

(COI: No)

P3-278

5HT1A receptors in orexin neurons play an important role in regulation of REM sleep

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Orexin A and orexin B are lateral hypothalamic neuropeptides. A series of studies have suggested that orexin-deficiency causes narcolepsy in humans and other mammalian species, highlighting roles of this hypothalamic neuropeptide in the regulation of sleep and wakefulness. Orexins were shown to have a strong excitatory influence on serotonergic neurons in the raphe nuclei through both orexin 1 and orexin 2 receptors. Conversely, orexin neurons receive abundant input from the serotonergic neurons in the raphe nuclei. We also found serotonin potently inhibited orexin neurons through 5HT1A receptors, implying the negative feedback regulation. This linkage might play an important role in the regulation of sleep/wakefulness. To evaluate this hypothesis, we generated mice in which orexin neurons specifically lack expression of 5HT1A receptors utilizing Cre-loxP mediated deletion of 5HT1A gene. We examined sleep/wakefulness characteristics of these mice, and found that these mice exhibited several abnormality in sleep/wakefulness architecture. Also, these mice exhibited increase REM sleep amount after applying restraint stress. These observation suggests that serotonergic inhibitory regulation of orexin neurons play an important role in normal maintenance of sleep/wakefulness behavior.

(COI: No)

P3-279

A Single Nucleotide Polymorphism in the Human neuropeptides B/W receptor-1 Gene Affects Amygdala Function and Social Behavior

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Neuropeptides B/W recetptor-1 (NPBWR1) expressed specifically in lateral part of the CeA (CeL) plays a role in Amygdala function and social behavior. We found that a genetic variation of gene encodes a G-protein coupled receptor NPBWR1 might be one of the key factors that contribute to variation of social behavior in humans. As assessed by functional MRI, individuals carrying the loss-of-function SNP (404T>A) in the human NPBWR1 gene showed a stronger activation of the amygdala during passive viewing of faces expressing various emotions. On the other hand, we previously reported that NPBWR1-deficient (Npbwr1$^{-/-}$)mouse showed dramatic increase in social contact number in resident-intruder test, and decrease freezing behavior in contextual fear conditioning test. This time, we revealed that focal expression of hNPBWR1 gene in GABAergic neurons in the central amygdala in the Npbwr1$^{-/-}$; Gad67-Cre$^{+/-}$ mice reversed the abnormal social behavior of these mice, but that of hNPBWR1 with the SNP did not. Also, decreased freezing behavior in Npbwr1$^{-/-}$ recovered to the level comparable to WT mice with hNPBWR1 gene expression. These observations suggest that the SNP affects the function of hNPBWR1 and machinery that regulates amygdala function in response to various kind of social interaction in humans and mice.

(COI: No)

P3-280

Involvement of estrogen in the wheel running induced by oxytocin injection into the rat ventromedial hypothalamus

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Estrogen acts on the central nervous system and induces several behavioral changes. Some of those changes are mediated by the ventromedial nucleus of the hypothalamus (VMH). Estrogen is also known to facilitate wheel running. Our previous study has demonstrated that excitation of neurons in the rat VMH increase in wheel running. There exists oxytocin receptor (OTR) in the VMH and OTR is up-regulated by estrogen. Thus we hypothesized that OTR in the VMH regulate wheel running and estrogen modify this behavioral change. Microinjection of oxytocin into the rat VMH induced a dose-dependent increase in the wheel running. On the other hand, simultaneous injection of OTR antagonist, d(CH2)5-Tyr(Me)2-Orn8-Vasotocin inhibited the oxytocin-induced wheel running. Oxytocin administration into the VMH of ovariectomized (OVX) rats also increased in wheel running. In the estrogen-treated OVX rats oxytocin injection into the VMH further increased the running behavior than control OVX rats. Nocturnal wheel running in proestrus increased compared with other stage of estrus cycle. Injection of OTR antagonist into the VMH just before the onset of proestrus nocturnal period inhibited the following nocturnal wheel running. These findings suggest that oxytocin receptor in the VMH is involved in the induction of the wheel running. In female rats estrogen facilitate the effect of oxytocin on wheel running.

(COI: No)

P3-281

A new simple method for analysis of sleep by use of a subcutaneously implanted accelerometor in rats

Igarashi, Ayako; Omura, Natsumi; Miura, Megumi; Mima, Nanako; Nishimura, Yuri; Mabuchi, Kaori; Takamata, Akira (*Dept Environ Health, Nara Women Univ, Nara, Japan*)

Recording electroencephalogram (EEG) and electromyogram (EMG) is the standard method for evaluating sleep-wake state in rats. The wired EEG/EMG measurement restricts animal's activity, and telemetry system is relatively expensive. In addition, EEG/EMG-based methods are often unsuitable for use in high-throughput screens because they are time-consuming and involve invasive surgery. In the present study, we propose a new simple method for evaluation of sleep/wake using a subcutaneously implanted acceleration sensor. Rats were implanted with an acceleration sensor subcutaneously for measurement of the amount of activity every 2 min. We simultaneously measured the activity using an infrared sensor and also recorded EEG/EMG. The accelerometer can detect small movement more sensitively compared with the infrared sensor. Evaluation of sleep by the subcutaneous accelerometor after setting the threshold showed a good correlation with the evaluation of sleep using EEG/EMG, while the evaluation of sleep with the infrared sensor relatively overestimated sleep. Further, the assessment of sleep in multiple free-moving rats in the same cage can be performed with this method. Thus, this method could be employed for a screen for assessment of sleep.

(COI: No)

P3-282

Estradiol replacement attenuates osmoregulatory and angiotensin II-induced central body fluid regulatory responses in ovariectomized rats

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Estrogen replacement reportedly attenuates the drinking response to hypertonic saline infusion and i.c.v. angiotensin II (ANGII) administration in ovariectomized rats. In order to elucidate the site of the action of estradiol, we examined the effect of estradiol replacement on c-Fos expression in the central body fluid regulation-related sites in response to systemic hypertonic saline and i.c.v. ANGII administrations in ovariectomized rats. We also examined the ANGII type I (AT1) receptor expression in the hypothalamus by use of western blotting in estradiol replaced and not replaced ovariectomized rats. Estradiol replacement attenuated c-Fos expression at the organum vasclosam laminar terminalis (OVLT), supraoptic nucleus (SON) and paraventricular nucleus (PVN) in response to hypertonic saline infusion, and also attenuated c-Fos expression at the OVLT, subfornical organ, median preoptic nucleus, SON and PVN after i.c.v. ANGII administration. We also found that the hypothalamic AT1 receptor expression was less in estradiol-replaced rats than estrogen deficit rats. Our data suggest that estradiol possibly attenuates AT1 receptor expression in the hypothalamus, and the attenuated AT1 receptor expression may be involved in the attenuated body fluid regulatory responses to systemic hyperosmolality and central ANGII.

(COI: No)

P3-283

The effect of the mating and yeast ingestion on the salt ingestion behavior of *Drosophila*

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It have been reported that the fly show "specific hungers" for protein-rich food to produce eggs, and the mating augment egg-laying rate and food consumption. While protein ingestion or mating affect the subsequent intake of protein, it is not investigated whether the mating and protein intake elicit behavioral changes in salt intake. In this study, we measured intake volumes of salt (NaCl) solutions after mating and yeast ingestion. The mating and yeast ingestion caused males to reduce NaCl intake, on the other hand, females to consume increased amount of NaCl solutions. The inverse relationship between behavioral change of male and that of female led to consequent increase of female/male ratio of salt intake. The female/male ratio of 80 mM NaCl solution intake was 2.2 ± 0.9 in mated and yeast deprived flies, while 7.9 ± 0.9 in mated and yeast fed flies. It may be concluded that mating and yeast intake enhance sexually dimorphic intake of salt. It is interesting how the enhancement of sexual dimorphism of salt intake was induced. The decreased salt intake of male flies might be understandable since yeast contains salts and adult male flies do not require large amount of salt because they do not produce eggs. Female flies, by contrast, might require larger amount of salts than those contained in ingested yeast, because the egg laying rate might be increased by mating and yeast ingestion. The effect of mating and yeast ingestion on egg-laying rate should be investigated to confirm this assumption.

(COI: No)

P3-284

Modulatory mechanism of autonomic system that induced by acquired auditory experience in mice and rats

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Hearing a sound often elicits an emotion that is induced by not instinct but experience. In light of this sound effect, we tried to examine whether or not the hearing experience actually evokes emotional response using an experimental mouse model, in which mice were exposed to environmental sound stimulation coupled to different housing conditions. We exposed mice to an artificial sound stimulation under each of the pleasant (16 hours) and the unpleasant (8 hours) conditions in a day. After mice were spent in each of the two conditions for several days, we analyzed some physiological parameters of mice while exposing to a sequence of sound stimulations. Among the parameters, presenting a sound coupled to the pleasant housing condition significantly decreased hart beat rate. But in the model study have some unresolved issues, i.e., how long the autonomic effect remained, what evidence showed the mice feels pleasant or unpleasant, what is the difference point between this model and well-known conditioned reflex response. To resolve these question, we explored difference of signature of acquired brain function between the model mice and control mice with physiological, morphological and biochemical method. In this study, we tried to reveal the neural mechanism of this experience dependent autonomic nerve modulation. At first, we shut down the amygdala that involved in fears and some sound induced autonomic responses. Second, rats were checked by this model study, to get the full time autonomic response recording by telemetory apparatus.

(COI: No)

P3-285

Inhibitory effects of melatonin on age-related memory impairment in mice

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Deficits of learning and memory are one of the most striking phenomena of senescence. Recent studies have suggested that oxidative stresses are involved in the aging processes. Melatonin, a hormone secreted mainly by pineal gland has been well documented to have anti-oxidative effects. Melatonin secretion declines during aging, implying that the reduction of melatonin levels with age contributes to the aging process. The aim of this study is to investigate the anti-aging effects of melatonin on deficits in learning and memory during natural aging process. BALB/c mice were received long-term administration of either melatonin or vehicle in their drinking water from the age of 10 months (middle age) until 18 months (old age). We examined the effects of melatonin on spatial memory and object recognition memory using object location test and object recognition test, respectively, and on neurogenesis in the dentate gyrus (DG) of hippocampus and neuron sizes and numbers in the DG and the perirhinal cortex. Oxidative stress was measured by accumulation of 8-hydroxy-2'- deoxyguanosine (8-OHdG) in the hippocampal CA1 region and the peririhinal cortex. The data obtained in the present study suggested that the long-term administration of melatonin from middle age attenuates the age-related deficits of learning and memory via attenuation of age-related changes in neural structure and function, which would be mediated by melatonin at least in part through its anti-oxidantive effects.

(COI: No)

P3-286

Spatio-temporal dynamics of calcium activity in the cortex of naturally sleeping and awake mice

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Sleep is essential not only for the rest of body, but also for the maintenance of brain function. Electroencephalography (EEG), the summed activity of a large number of neurons in the cortex, shows that the slow wave activity (SWA) occurs during NREM sleep (also known as slow wave sleep). The SWA is presumed to reflect synchronous up and down states in cortical neurons and generate locally on a macro scale. However, it is unknown in what way sleep alters spontaneous activity of cortical individual neuron, especially their spatio-temporal pattern on a microscopic field. To address this question, we performed two-photon Ca^{2+} imaging of cortical neurons in naturally sleeping and awake mice. The mouse head was restrained with the head plate under the objective while the mouse could move limbs freely on the spherical treadmill. The mice were habituated to the experimental circumstances for five days. Using this method, we observed spontaneous calcium dynamics in the layer 2/3 of primary motor cortex during wakefulness, NREM sleep and REM sleep. The synchronicity of spontaneous calcium signals among neurons changed in response to the sleep stages. The synchronization did not depend on the distance between neurons up to micrometer-order. Our results, taken together with other studies, suggest that cortical activity synchronize regionally but not locally during sleep.

(COI: No)

P3-287

The pontomedullary tegmentum GABAergic neurons are involved in the regulation of sleep and wakefulness

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Sleep is regulated by several subcortical regions including the brainstem. However, the detailed locations and functions of the brainstem sleep-related neurons are incompletely identified. To explore novel neurons regulating sleep and wakefulness, we systematically manipulated firing of neurons in the pons and medulla oblongata, and investigate if their firing affects sleep. Region- and cell-type specific gene delivery was achieved by local injection of Cre-inducible AAV vectors into the GAD1-Cre mice. To silence their firing, the inhibitory DREADD hM4Di was targeted to them. Histological studies showed that 99.3% of hM4Di-expressing neurons were GABAergic. Patch-clamp recordings revealed that the hM4Di agonist CNO suppressed their spontaneous discharge. We discovered that a subset of pontomedullary tegmentum GABAergic neurons regulate sleep and wakefulness. Suppression of GABAergic neuron firing in the dorsal area of pontomedullary tegmentum increased the total amount of NREM sleep, whereas inhibition of the medial GABAergic neurons had no effect on sleep and wakefulness. These results suggest that GABAergic neurons in the dorsal area of pontomedullary tegmentum contribute to the regulation of sleep.

(COI: No)

P3-288

Modulation of masseter activity by vigilance states and circadian rhythm

Mochizuki, Ayako[1]; Katayama, Keisuke[2]; Kato, Takafumi[3]; Ikeda, Minako[2]; Nogawa, Yasuha[4]; Nakamura, Shiro[1]; Nakayama, Kiyomi[1]; Kiyomoto, Masaaki[1]; Wakabayashi, Noriyuki[4]; Baba, Kazuyoshi[2]; Inoue, Tomio[1] ([1]*Dept Oral Physiol, Showa Univ Sch Dent, Tokyo, Japan;* [2]*Dept Prosthodont, Showa Univ Sch Dent, Tokyo, Japan;* [3]*Dept Oral Anat Neurobiol, Osaka Univ, Osaka, Japan;* [4]*Removable Partial Prosthodont, Tokyo Med Dent Univ, Tokyo, Japan*)

Bruxism is associated with an increase in the activities of the jaw-closing muscle; however, the alteration of the jaw-closing muscle activity among vigilance states is unclear. We examined the influences of dark/light and sleep/wake cycles on the activity of the masseter muscle in comparison to those of the neck muscle over a 24-h period in mice. The mean EMG activities of the masseter and neck muscles during wakefulness (W) were much larger than those during non-REM sleep (NREM) and REM sleep (REM). In contrast, the mean EMG activities of the masseter and neck muscles during W and NREM were significantly smaller during the transition period from dark to light. During NREM, the masseter EMG activity was moderately correlated with the neck EMG activity in both dark and light periods, whereas there was no correlation between two muscles during W or REM. During W and NREM, bimodal distributions were found in the masseter EMG activity, whereas the neck EMG activities were unimodal distributions in any state. These results suggest that the activities of the masseter and neck muscles are modulated by both sleep/wake and dark/light cycles. Furthermore, even during NREM, the masseter muscle is activated bimodally, which may contribute to the occurrence of raised masseter muscle activity such as sleep bruxism.

(COI: No)

P3-289

Circadian profiling of an interaction between BMAL1 and CLOCK by FRET bioimaging

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A circadian rhythm is a crucial factor in the regulation of a wide range of physiological processes that are involved in biological systems such as the endocrine system and the sleep-wake cycle. The systemic circadian system can be broken down into cellular rhythms, which are maintained by periodic change in the status of a set of clock genes and proteins. Such proteins oscillate not only at the levels of their expression but also at those of posttranslational modification.
FRET (Förster resonance energy transfer) is a phenomenon of radiationless energy transfer between a pair of fluorophores, where wavelengths of fluorescence emission are altered in a manner dependent on the distance between them. Therefore, in combination with a color pallet of different fluorescent proteins, it enables us to examine protein-protein interaction, protein conformational change, and enzymatic activity in living cells or organisms. In this study, we have constructed fluorescent biosensors for clock proteins, BMAL1 and CLOCK, and performed qualitative and quantitative observation of circadian rhythms of protein dynamics, including their subcellular localization, mobility, and interaction, in living cells.

(COI: No)

P3-290

Analysis of diurnal yawning rhythm in Wister rat

Ikegami, Keisuke; Shigeyoshi, Yasufumi (*Dept Anatomy Neurobiol, Kinki Univ Fac Med, Osaka, Japan*)

Yawning is known to have the important physiological function because yawning is observed in multiple species, not only mammal but also birds and reptilian. Yawning has been thought to be happen when we feel sleep, but resent studies suggest that yawning has the arousal effect. In addition, brain disease or stress may induce the yawning with typically arousal effect. However, the mechanism and physiological functions of yawning remain unclear. To clarify it, it is important to confirm the diurnal rhythm of yawning and the relationship with sleep-awake patterns. However it has been unknown in rat, which has been a nice model and contributed to the studies of yawning. In this study, we has performed the time-course monitoring of 10-weeks-old male Wister rat under individual and group housing. We suggest the time-dependent yawning pattern and the relationship with another rat.

(COI: Properly Declared)

P3-291

AVP-releasing rhythm of SCN and SON in culture

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In mammals, circadian rhythms are driven by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Recordings of neuronal firing of dissociated SCN cells suggest that single SCN cells are competent circadian oscillators. The SCN cells also show clear circadian rhythm of arginine vasopressin (AVP) release in cell culture when they are plated at high density. Both the amount of AVP release and amplitude of the rhythm depended on the plating density. Co-culture with cortex cells could not restore the loss of rhythmicity in low-density culture. AVP is also produced in the supraoptic nucleus (SON). However, SON did not show any circadian rhythm in culture. When SON cells were added to low-density cell cultures of SCN, the amount of AVP release, but not the rhythm amplitude was increased. These results suggest that SON cells do not show AVP-releasing rhythm even in the presence of rhythmic SCN cells.

(COI: No)

P3-292

Development of the animal model of shift work using the mouse

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In the shift work, there are a lot of health damage that are not only the acute such as complained of insomnia and decrease of alertness but also chronic risk such as hypertension and diabetes. The purpose of this study was to develop the animal model of shift work (SW) using a wild type mouse (C57BL/6) and we investigated the effect of the separation between biological rhythms and light-dark cycle on the animals. To create the SW mouse, we permitted the mouse to run on the running wheel and to eat the food during only light period and limited both of them in the dark period. In control group, we allow them during the dark period and permit them during the light period. After one week of baseline recording, body temperature, amount of spontaneous activity and running wheel activity were measured for two weeks. As a result, in SW group, the phase of body temperature, spontaneous activity and running wheel activity was shifted to the light phase. These results suggested that we successfully created an animal model of shift work as we observed the apparent separation between biological rhythms and light-dark cycle.

(COI: No)

P3-293

Insulin resistance in heart-specific *Bmal1* knockout mice

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The regulation of mammalian energy balance such as glucose and lipid metabolism is influenced by function of the circadian clock, which is composed of a set of core clock genes. Since functional clock genes are widespread throughout the body, diverse organs might participate in clock-controlled energy metabolism. However, the impact of altered clock function in specific organs on the regulation of glucose metabolism remains unclear. Here, we demonstrate that heart-specific disruption of the circadian clock gene *Bmal1* not only results in significant reduction in cardiac function but induces impaired glucose metabolism in mice. The glucose tolerance test showed that glucose tolerance was significantly impaired in heart-specific *Bmal1* knockout mice. In addition, the insulin tolerance test revealed a decrease in insulin sensitivity in the knockout mice, indicating that hyperglycemia observed in these animals was due to systemic insulin resistance. Although glucose metabolism may have been affected by increased body mass, body weight was not different between control and heart-specific *Bmal1* knockout animals. Our results suggest that functional clock in the heart is an important component of the circadian clock network that maintains mammalian glucose metabolism.

(COI: No)

P3-294

Aging Dissociates Circadian PER2 Oscillations of Individual Cells in the Suprachiasmatic Clock

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In aged C57BL/6J mice, decreased amplitude and increased fragmentation of the wheel running rhythm and lengthened circadian free-running period have been observed. Evidences indicating that aging impacts the neural activity rhythms in the suprachiasmatic nucleus (SCN) were reported by many laboratories. However, there are few reports that show circadian oscillations of clock genes in the SCN was disrupted by aging. To explore the contradiction between neural activity and clock gene rhythms in aged SCN clock, we have carried out ex vivo bioluminescence recordings from cultured SCN slice of young and aged PER2::LUC mice. As previous reports, there was little change in the amplitude of PER2::LUC rhythm between the young and aged SCN explants from animals that were housed in a normal light/dark condition. However, PER2::LUC rhythm in the aged SCN taken from animals that were housed in a constant dark condition for 10 days showed longer circadian period with lower amplitude. Results from recording PER2::LUC rhythm of SCN individual cells using the electron multiplying CCD camera, individual cells of aged SCN showed longer circadian period of PER2::LUC oscillation and desynchronization between individual cells. These data suggest that the molecular clocks in individual SCN cells are also degraded by aging.

(COI: No)

P3-295

Role of estrogen receptor β in the medial preoptic area in the regulation of aggressive behavior in male mice

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The expression of male-type social behaviors such as sexual and aggressive behavior highly depends on the action of testosterone (T) in which T plays central role in both the facilitation of behaviors and the development of their neural bases. Moreover, T is known to activate estrogen receptors (ER) α and β after being aromatized to estradiol in the brain. We have shown that the activation of ERα in the medial preoptic area (MPOA) is absolutely necessary for the facilitation of male sexual, but not aggressive behavior (Sano et al. EJN, 2013). However, the contribution of ERβ in the MPOA to the regulation of male-type social behavior has yet to be determined. Thus in this study, we site-specifically knocked down ERβ in the MPOA and examined its effect on the expression of male sexual and aggressive behaviors. At the age of 21 days, gonadally intact male mice (ICR/Jcl) were bilaterally injected either with adeno-associated viral vector silencing ERβ or a control vector in the MPOA. Starting at the age of 12 weeks, all mice were tested for their sexual and aggressive behaviors. Surprisingly, knocking down of ERβ in the area reduced the expression of aggressive, but not sexual behavior. Our results suggest that ERα and ERβ in the MPOA may be responsible for the differential regulation of male sexual and aggressive behavior by testosterone.(KAKEN #23240057 to SO)

(COI: No)

P3-296

Monitoring of circadian rhythm in arginine vasopressin expression by a bioluminescence reporter

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Arginine vasopressin (AVP) is a major neuropeptide in the suprachiasmatic nucleus (SCN), where the master circadian pacemaker is located in mammals. AVP is a plausible transmitter of circadian signals from SCN to other areas, but the role of AVP in clock functions is not well understood. AVP is also expressed in the paraventricular nucleus (PVN) and supraoptic nucleus (SON). Due to a lack of analytical tool, dynamics of AVP production with high temporal and spatial resolution are not well understood. In the present study, we produced knock-in mice carrying an Emerald-luciferase reporter (AVP^{ELuc}) to monitor AVP expression in cultured brain slices. Bioluminescence (AVP::ELuc) was measured either from a whole tissue with a photomultiplier tube or from individual cells with an EM-CCD camera. SCN slices showed robust circadian rhythms for more than 10 days in AVP::ELuc bioluminescence. The peak phase of the rhythm was located at the middle of the day. AVP::ELuc bioluminescence in the PVN and SON exhibited a huge peak on the first day of culturing, thereafter, rhythms with significantly low amplitude persisted in the subsequent days. AVP^{ELuc} knock-in mice are useful not only for circadian but also for neuroendocrinological studies.

(COI: No)

P3-297

A GABAergic mechanism is indispensable for Per2-suppressing effect in the rat SCN by sevoflurane

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The inhalation anesthetic, sevoflurane, have the suppressive effect on the clock gene *Per2* expression in the rat suprachiasmatic nucleus (SCN). We examined intra-SCN spatial susceptibility to sevoflurane and the involvement of GABAergic signal transduction on suppressive effect of sevoflurane on *Per2* expression. Sevoflurane was applied to SCN slice cultures from Per2-dLuc transgenic rats, and luciferase bioluminescence was monitored using a microscope equipped with a CCD camera. To investigate a detailed spatial property of sevoflurane effect, acquired time lapse images of the SCN were divided into small regions of interest (ROIs). The bioluminescence in the most of ROIs showed a clear circadian pattern, and the bioluminescence was repressed by sevoflurane application. We also examined the possibility that sevoflurane suppresses *Per2* expression through the modulation of GABA receptor activities. To investigate the role of GABA receptors in suppression of *Per2* expression by sevoflurane, we applied GABA receptor blockers to the SCN cultures. The suppressive effect of sevoflurane was totally diminished in the presence of GABA receptor blockers. These results suggest that GABAergic mechanism is indispensable for sevoflurane to suppress *Per2* expression in the SCN, indicating that sevoflurane may act via GABA receptor systems in the SCN.

(COI: No)

P3-298

Close relationship between histamine H1 receptor-expressing neurons and CRH neurons in the mouse hypothalamic paraventricular nucleus

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Paraventricular nucleus of the hypothalamus (PVH) is a satiety center that inhibits feeding. Several types of neurons have been found in the PVH that regulate feeding, including CRH, oxytocin (OXT), TRH, and Nesfatin-1 neurons. In addition to these types of neurons, we showed that histamine H1 receptor (H1R)-expressing neurons are also involved in feeding regulation (J Physiol Sci. 62:S209, 2012). In this study, we tested whether H1R neurons had something to do with these types of neurons. First, we examined whether H1R neurons coexpressed CRH, OXT, vasopressin (AVP) or TRH in the mouse PVH by use of double *in situ* hybridization (ISH) method. Secondly, we examined the effect of the ablation of H1R neurons (ibid.) on other types of neurons in the PVH. The ISH study showed that about half of the H1R neurons expressed CRH, but less than ten percent of them expressed OXT and/or AVP, and no H1R neurons expressed TRH. Secondly, the ablation of H1R neurons greatly decreased the number of CRH neurons, but had little or no effect on the number of OXT, AVP and TRH neurons. These results suggest that the PVH neurons can be classified into two groups, H1R/CRH group and OXT/AVP group. Although OXT neurons have been reported to regulate feeding, the present study indicates that H1R an/or CRH neurons also regulate feeding by a mechanism distinct from that of OXT neurons.

(COI: No)

P3-299

Dopamine release in the nucleus accumbens of estrous female rats during exposure to male odors

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In seminatural conditions, estrous female rats actively pace the timing of coital stimulation from male rats. During such copulation under female preferred pace, female rats show increased extracellular concentration of dopamine (DA) in the nucleus accumbens (NAcc), implying the anticipation of rewarding sexual stimuli. It has been known that estrous females show preference for odors from males over estrous females irrespective of presence or absence of prior sexual experience. A previous study in our laboratory, however, demonstrated that Fos expression in the NAcc core was increased after exposure to soiled bedding from male rats only in sexually experienced estrous females (Hosokawa and Chiba, 2007). These results may suggest that odors from males are not intrinsically rewarding for female rats despite the existence of male-directed odor preference in sexually naïve females. In the present study, we examined the change of NAcc extracellular DA concentrations during exposure to male odors in both sexually experienced and sexually naïve estrous female rats using in vivo microdialysis combined with HPLC. DA concentration in the NAcc significantly increased after exposure to male odors, not only in sexually experienced but also in sexually naïve estrous females. However, the magnitude of the increase in DA during exposure to male odors was greater in sexually experienced females than that in sexually naïve ones. These date suggest that male odors are intrinsically rewarding for females and that sexual experience contributes to increase the value of the reward.

(COI: No)

P3-300

Administration of NMDA antagonist shifts the interval timing peak rightward in rats

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Timing and time perception are fundamental to survival and goal approaching in all animals. It is known that animals have some special timing ability of intervals. However, neural mechanisms of time perception are still unknown. The purpose of this study is to investigate the effects of NMDA antagonist on timing behavior. Firstly, using six male rats of Wistar strain, approximately 3 month-old at the beginning of the experiment, we examined psychological expectation of the interval timing in laboratory experimental settings with the peak-interval (PI) procedure. Interval-timing refers to time estimation in the second-to-minutes range. In the PI procedure, rats were trained on a fixed interval schedule to press lever for food after a specified interval (30 seconds in this experiment) as signaled by a certain stimulus. The rats received reinforcement only for desirable response. Though with some individual variations, the distribution of the lever press responses eventually showed an apparent peak in the vicinity of 30 seconds. Secondly, after 30 sessions of trainings, NMDA antagonist was administered directly into the septum region of the brain via microinjection. As a result, the peak time shifted rightward and lever press responses increased. This result of this study suggests that the comparison between the rats administered with NMDA antagonist, NMDA agonist, dopamine agonist and antagonist may clarify neural mechanisms of the interval timing.

(COI: No)

P3-301

Differential effects of propofol and etomidate on hypnotic electroencephalogram stage and sleep-wake cycle in mice

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General anesthesia is the clinical state comprising multiple components such as sedation, hypnosis and immobility. $GABA_A$-R is the major target of general anesthetics which mainly responsible for sedation and hypnosis. Propofol and etomidate exert anesthetic actions via the same $GABA_A$-R $\beta 3$ subunit. Thus, we examined whether propofol and etomidate possess the similar of hypnotic action and the same effects on sleep-wake cycle or not, by analyzing EEG and EMG. We found that both anesthetics induced significant increases in theta EEG power after anesthetics administration. Interestingly, the occurrence rate of slow wave burst of propofol is significantly lower than that of etomidate. As for sleep-wake cycle, propofol extended the duration of no-rapid eye movement sleep compared with etomidate. We discuss the differential effects of propofol and etomidate on hypnotic-EEG-stage and sleep-wake cycle.

(COI: No)

P3-302

Establishment of an in vitro experimental system using a cell line to investigate the mechanisms of anesthesia

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The molecular mechanisms of the general anesthesia have still remained to be clearly elucidated. The inhalational anesthetic, sevoflurane, is the most used general anesthetics in human. Our recent studies revealed the following evidence: 1) Administration of sevoflurane reversibly suppressed expression of the clock gene Per2 in the suprachiasmatic nucleus (SCN). 2) The suppression of Per2 expression in the SCN was mediated via histone deacetylation in the Per2 promoter. 3) Sevoflurane altered the phase of Per2 expression rhythm in the SCN slice culture. 4) Inhibition of GABA receptors blocked the sevoflurane-induced phase shift of Per2 expression in the slice culture. To further investigate the molecular mechanisms and target sites of the general anesthesia, it was required to develop an in vitro experimental system. We developed a GT1-7 cell line stably expressing the luciferase gene under control of the mouse Per2 promoter (GT1-7:6D3) and compared the response to sevoflurane with that of the other stable cell line (RS182). GT1-7:6D3 cells showed luciferase activity in a circadian manner as well as RS182 cells. GT1-7:6D3 cells responded to sevoflurane and showed a decrease in the activity leading to phase delay. Whereas treatment of RS182 cells with sevoflurane induced no change in the phase of luciferase expression. Now, we are examining the epigenetic events under anesthetic treatment.

(COI: No)

P3-303

A variant right hepatic artery with caterpillar hump formation: A case report with surgical implications

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Pub Med search reveals the presence of a few reports regarding the caterpillar right hepatic artery (CPRHA) (right hepatic artery with caterpillar hump configuration) in spite of its clinical relevance to laparoscopic cholecystectomy. During the dissection of abdominal cavity in 27 cadavers, we detected a case of trifurcated common hepatic artery into gastroduodenal artery, left hepatic artery and CPRHA in old man. After forming its characteristic caterpillar hump while passing ventral to the terminal part of common hepatic duct, the CPRHA passed through Calot'triangle giving its large cystic branch, then left the triangle deep to the cystic duct and gall bladder neck before its termination into the cystic plate. This case of CPRHA was associated with accessory left gastric artery stemming from left hepatic artery. The latter artery also gave rise to the right gastric artery. The accessory left gastric artery terminated at the gastroesophageal junction with terminal esophageal and fundic branches. The branching pattern of celiac artery was not typical; the splenic and common hepatic arteries arose from a common hepato-splenic trunk while the left gastric artery originated separately at more proximal level. The clinical relevance of the present case report to hepatobiliary surgery will be discussed.

(COI: No)

P3-304

The Observational Study of the Knee Articular Cartilage among Cadavars in S University

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We performed the observational study of the cadavars for medical training from 2012 to 2014 in S university, faculty of medicine as subjects. Our aim is to grasp the condition of the knee joint cartilage in the general elderly. The 81 cadavers, with the approval of the bereaved, were observed. The years of age were 85.15 ± 9.92 years (minimum 61 year-old, Max 103 year-old). The subjects were 38 males, and 43 females. The male cadavars were statistically younger than female cadavars by Welch t-test ($p<0.0001$) (80.47 ± 8.94 years in male vs 89.28 ± 8.94 years in female). To observe the knee joint cartilage, the femoral articular surfaces were divided 5 areas, the tibial articular surfaces were divided 3 areas, and the patella articular surfaces were divided 2 areas. We assessed the articular cartilage by each area, as following four steps, which were "1"= normal, "2"= fibrosis, "3"= ulcer-like, "4"= missing. Total score is up to 40 points, and a minimum 10 points by each knee. We also recorded for the presence of osteophytes of each area. Though the score in the right knee has positively related with those in the left knee (peason $r = 0.83$), the scores in the right knee were significantly higher than those in the left knee by paired t-test ($p = 0.015$). Comparing the total scores between in the presence and in the absence of femur osteophytes, the scores in the presence group are higher than those in the absence group by Welch t-test ($p<0.0001$).

(COI: No)

P3-305

Observation of the bipennate portio anterior of the soleus muscle using ultrasonic image

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The bipennate portio anterior of the soleus muscle is unique to the bipedal walking humans. We examined donated cadavers to investigate the types of the positional correlation between the calcaneal tendon and the sagittal tendon of the bipennate portio anterior. The purpose of this study is to determine whether or not it is possible to observe the deepest layers of the soleus muscle using ultrasonic image on donated cadavers and comparing those images with visual observations during a dissection. Two cadavers fixed with 10% formalin solution were observed in this study. After conducting sonograms on the four lower extremities to confirm the sagittal tendon, dissections were performed and visual observations were compared with the image-based observations of the sagittal tendon. Ultrasonic images of the posterior surface of the legs of five adult males and females were also used to compare the length and direction of travel of the sagittal tendon. It was suggested that when the sagittal tendon was present, the direction of travel of the band can be confirmed using ultrasonic images. However, using ultrasonic images, it is difficult to identify where the sagittal tendon begins and the point of transition to the Achilles tendon. It was also suggested that there was a tendency for the tendon to be identified as shorter than was actually apparent during visual observation.

(COI: No)

P3-306

Morphological analysis of the trapezius: muscle fibers in the three divisions

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The trapezius is diverse in shape and molds the outline of shoulder by keeping scapula in position. The 15 trapezius muscles specimens were photographed before and after removal of the fascia. The shape of trapezius was quite diverse at first, but became similar after removal of the fascia. The trapezius was divided into three parts depending on the insertion on the clavicle, on the acromion and scapular spine, and via distal tendon on the medial part of the spine. The proximal tendon was developed in the superior and middle part. In the superior part, the tendon protruded more on the superficial side, whereas in the middle part it protruded widely on the deep side to provide wide attachment for the muscle. In the superior part, the muscle fibers in the higher location were more inclined and longer and those in the lower location were nearly horizontal and shorter. The relation was reversed in the lower part. In the middle part the muscle fibers were almost horizontal and homogeneous in length, since the shape of the protrusion of proximal tendon and of skeletal insertion were fit well. The superior and inferior parts were slender sheet and the middle part was thick and voluminous. The middle part was most well developed and functioned as main sustainer of the scapula, whereas the superior part was poor and ancillary, and the inferior part function as abductor of the scapula.

(COI: No)

P3-307

Functional anatomy of the acromioclavicular ligament based on its macroscopic fiber analyses

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Introduction: The acromioclavicular (AC) ligament connects between the acromion and the lateral clavicle. The ligament has been illustrated as running nearly vertical to the AC joint surface, and has been described to interlace with the aponeuroses of the trapezius and deltoid muscles. While there are a lot of researches regarding measuring dimensions of the AC ligament for a distal clavicle resection technique, detailed macroscopic researches have not been done. Our objective for this study was to investigate the morphology of the ligament in detail.
Methods: We used 20 shoulder girdles of 11 cadavers in the anatomical practice at Tokyo Medical and Dental University. After extracting the scapula and the lateral half of the clavicle en bloc, we observed the ligament macroscopically.
Results: The AC ligament could be divided into two parts, posterior and anterior bundles. The well-developed posterior bundle ran obliquely from the anterosuperior part of the acromion to the posterior part of the lateral clavicle. In contrast, the anterior bundle was poorly developed and connected between the anterior surface of the acromion and the clavicle. The ligament was clearly separated from the muscles.
Discussion: Orientation of the posterior bundle passed obliquely over the joint surface, which might act as a constraint against the posterior translation of the clavicle in relation to the acromion.
(COI: No)

P3-308

Morphometric study of molar root furcation area and its relation to periodontal tissue destruction in Japanese populations

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Furcation involvement in periodontal disease has long been a challenge for dentists. As the destruction of the periodontium progresses apically, the furcation of multirooted teeth is exposed, leading to irreversible bone loss. Therefore, a thorough understanding of root anatomy is essential for proper diagnosis. However, little is known about three-dimensional (3D) morphology of molar root furcation (MRF) area and its relation to periodontal disease. The aim of this study was to establish a 3D measurement technique of MRF in 20 mandibular molars. In addition, 19 extracted molars of Japanese patients were investigated to evaluate their relation to periodontal parameters, including probing pocket depth, attachment level and bleeding on probing. Virtual images were generated from micro-CT imagery to quantify the MRF area. Variables such as root trunk length, furcation entrance, root separation and cervical enamel projection were evaluated. Our result showed similar values for the variables measured by conventional two-dimensional methods. In conclusion, 3D measurements of MRF area were successfully established. Additional data are still needed to assess relationship between 3D morphology of MRF and periodontal tissue destruction.
(COI: No)

P3-309

Anatomical variations of the lingual artery: a case report

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Recently crucial bleeding is the focus of preventive care in dental implant surgery. On the anatomical survey of blood vessels in oral floor, we newly found bilateral variations in arising pattern of the lingual artery in a case. On the right side, the deep lingual artery and the sublingual artery divided from common trunk which ran forward lateral to the hyoglossus, and this trunk arose from the facial artery just proximally to the position at which the submental artery arose. At the external carotid artery level, the original but remnant lingual artery arose just below the facial artery to distribute only the tongue root covered by the hyoglossus, entering to this area just above the greater horn of the hyoid bone. On the left side, the sublingual artery arose from the facial artery just proximally to the position at which the submental artery arose. The deep lingual artery, on the other hand, was the continuation of the lingual artery which arose from the external carotid artery far below the facial artery, making anastomosis with the sublingual artery in the sublingual region. The lingual artery of this side ran laterally to the hyoglossus during coursing the posterior half of this muscle, then penetrated the muscle and ran medially to the hyoglossus up to the anterior border of this muscle to continue to the deep lingual artery. In this side, there seemed to be no remnant lingual artery seen in opposite side. Providing the detailed portrait of arterial variations may be of clinical importance in dental implant surgery and preoperative radiologic examinations.
(COI: No)

P3-310

A morphological study of the maxillary anterior wall in Japanese population

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Maxillary bone between a nasal cavity and the maxillary sinus is available for the dental implant to bury. Since the bone around the maxillary sinus is thin, the dentist should be care for the dental implant placement. We observed the maxillary bone thickness in the cross-section of the alveolar process of the maxillary bone.
The head of the 23 cadavers (male 12 bodies 20 side 101-52 years old, female six bodies 11 side 100-71 years old) for of the dissection training at the Nihon University School of Dentistry at Matsudo in 2013. We cut the horizontal section on the upper part of 1cm from the alveolar crest at the maxillary canine part by the belt saw, and in addition 1cm upper part of the section was cut off. The cross-sections were photographed with a digital camera and drew a circle on the bone of the point of intersection of the canine fossa, the maxillary sinus and the nasal cavity with image analysis software and calculated the diameter of the circle.
The size of the circle did not have the significant sex differences, the significant difference by having tooth or not. As for the average of the diameter of the circle, in the 1cm upper part, in 6.1mm, the 2cm upper part, it was 4.7mm from the alveolar crest. The standard of the diameter of an implant is 3.75 mm and the frequency of the size not more than it is 9 sides in 1 cm and 13 sides in 2cm. In these areas, the dental implant may perforate the maxillary sinus, the nasal cavity and the canine fossa, and therefore attention is necessary.
(COI: No)

P3-311

Morphological analysis of the vastus lateralis and intermedius of the quadriceps femoris

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Anatomy of the vastus lateralis (VL) is complicated. VL is clinically divided into the long and oblique heads (VLL, VLO) and in addition its border toward the vastus intermedius (VI) is frequently blurred because of partial fusion origin's form these muscle. Eighteen specimens of the quadriceps femoris were detached from the skeleton of cadavers with preserving the periosteum and intermuscular septum. The field of origin of VL and VI was demarcated on the deep surface of specimens. The insertion of VLL and VLO was also analyzed as regards to the insertion tendon.
The origins of these muscles were classified into 3 types. In type I, the three origins were continuous (9/18). In type II, the origin of VI was separated from the origins of VLO and VLL (8/18). In type III, the origin of VLL was separated from the origins of VLO and VI (1/18). The insertion of VLL was classified into 3 types; type A with wide insertion tendon on the deep surface (10 cases), type B with intermediate insertion tendon (2 cases) and type C with narrow insertion tendon at the lateral and medial border of the muscle (6 cases). VLO inserted on the tendon of VLL either without additional insertion (type X, 7 cases) or with insertion tendon onto the patella (type Y, 11 cases). It was revealed that VL and VI made a single muscular body in most of the cases, and the separation of this muscular body occurred in various places. This fact indicated that the quadriceps femoris had three heads instead of four.
(COI: No)

P3-312

Comparison of vapor levels of formaldehyde from embalmed human cadavers between males and females

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Formaldehyde (FA) is soluble compound, and used to embalm human cadavers for gross anatomy laboratory. It has been documented that FA vaporize from embalmed cadavers in laboratory. However little is known about evaporation level of FA in each cadaver and dissecting process. FA vapor levels have been compared among non-dissected (ND), skin-incised (SI), subcutaneous fat-removed (FR) and thoracoabdominal cavity-opened (TO) cadavers in our previous studies, and our results have shown increased FA levels in SI, FR and TO cadavers compared to that of ND. In this study, we evaluated the FA levels between male and female cadavers. FA was collected by active sampling method and evaluated by high performance liquid chromatography. Our data showed that the FA level increased in SI, FR and TO cadavers compared to that of ND in both of male and female cadavers. In particular, such increase was significant in SI and FR cadavers in males. In addition, we found that the FA levels are higher in female cadavers than in male cadavers. This sexual difference was significant in FR and TO cadavers. These data provide new knowledge about difference in vapor levels of FA from embalmed cadavers.
(COI: No)

P3-313

Right external iliac venous ring lacking the right common iliac vein

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Preaortic iliac venous confluence, also known as marsupial vena cava, is a rare congenital anomaly in which inferior vena cava or left common iliac vein is located anterior to aortic bifurcation or right common iliac artery. A very rare case of preaortic iliac venous confluence was found in a 84-year-old female cadaver. In this case, the confluence originated from right external iliac vein and drained directly into inferior vena cava. This confluence and right external iliac vein surrounded right common iliac artery to form "external iliac venous ring". In addition, right internal iliac vein drained into left common iliac vein and right obuturator vein draining into right external iliac vein. Embryogically, external iliac venous ring in our case may represent a form that developmental preaortic and postaortic iliac veins both persist. The external iliac venous ring has clinical importance especially during central venous cauterization from right external iliac vein. The other venous variations are no less clinically important in retroperitoneal surgery respectively.

(COI: No)

P3-314

Anomalous course of the external carotid artery

Kawai, Katsushi (*Grad. Sch. Med. Sci. Kumamoto Univ., Kumamoto, Japan*)

After the external carotid artery begins at the bifurcation of the common carotid artery, at the level of the upper border of the thyroid cartilage, it ascends usually medial to the stylohyoid muscle. It reaches to the region between the neck of the mandible and the mastoid process, where it ends by dividing into the superficial temporal and maxillary arteries. However, it is known that this artery sometimes runs between the posterior belly of the digastric and stylohyoid muscles, rarely lateral to the digastric muscle. So the incidence and the branching patterns of such anomalous external carotid artery were investigated in a total of 550 bodies or 1100 head sides of Japanese subjects, donated for student dissection at Kumamoto University from 1994 to 2014. With the exception of 3 head sides in which the course of the external carotid artery was not clear, the external carotid artery running between the digastric and stylohyoid muscles were found in 42 (3.8%) out of 1097 head sides. Further, in 23 out of them, the external carotid artery ran between the stylohyoid branch of the facial nerve and stylohyoid muscle. In the remaining 19 head sides, the stylohyoid branch has been cut and there was no instance in which the stylohyoid branch of the facial nerve ran obviously medial to the external carotid artery. The external carotid artery running lateral to the digastric muscle were found in 4 (0.4%). In this research, I examined which branch has a possibility of being a vestige of such anomalous external carotid artery and discussed its process of formation.

(COI: No)

P3-315

The morphologic study on a course of the maxillary and the posterior deep temporal arteries

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Objectives: The distribution of the maxillary artery (Mx) is important landmarks for surgery. The course of Mx was classified into lateral type and medial type. The lateral type runs superficially to the lateral pterygoid muscle (LP), and the medial type runs deeply to the LP. The order of divergence of the Mx in the lateral type was the middle meningeal artery(MM), the inferior alveolar artery (IA), the posterior deep temporal artery (PDT). On the other hand, the order of divergence of the medial type was common trunk of the MM and IA, the PDT.
Material and Methods: We studied the course and order divergence of the Mx at the dissection training in 2013. We compared the data which was obtained at the Nippon Dental University with the present study. We injected resin to the external carotid artery (EC) after dissection. After the injection, we confirmed previous EC distribution.
Results and Discussion: The course of the Mx in the lateral type was 90%, in the medial type was 10%. Double maxillary arteries were observed in the most deeper medial type of the Mx. It was very rare case. The PDT which deeply run to the LP was found. A study of arteries in the maxillofacial region is clinically very important. We believe the study of blood vessels will be useful for many clinical fields.

(COI: No)

P3-316

The relationship between age-related changes in the lumbar spines and sacroiliac joints

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Back ground: The purpose of this study was to clarify the relationship in the age-related change of lumbar spine (LS), these of sacroiliac joints (SIJ) and other major joints, and the shape of the SIJ surface.
Method: SIJ and LS (osteophyte of vertebral body and degenerative changes of zygapophysial joint) and periarticular osteophytes of six major joints in 42 modern male skeletons were quantitatively examined macroscopically. A corrected index of age-related change, which was the gap value (Gap), was calculated. Moreover, the degree of curvature in the posterior border line around the iliac auricular surface was calculated as a quantitative indicator, constriction ratio (CR). Some correlation coefficients were examined with Gaps and CR.
Results: Certain degree of positive relationship between Gap in the major six joints and those in SIJ was indicated. Furthermore, there was a certain positive correlation between left SIJ and LS. There was a tendency of negative correlation between Gap in the vertebral body and the mean value of CR of both sides.
Conclusion: It was suggested that these results were relevant to the age-related changes of SIJ and LS. The relationship between the degree of osteophytes in the vertebral body and shape of the auricular surfaces indicated that the difference in stability of SIJ might affect the extent of the mechanical stresses occurring in the lumbar vertebral bodies.

(COI: No)

P3-317

Muscle Architecture of the Triceps Surae Muscle

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The major ankle plantar flexor is the triceps surae muscle (TSM), which comprises the medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (SOL) muscles. The MG and LG cross both the knee and ankle joints, whereas the SOL is a plantar flexor that crosses only the ankle joint. The SOL has a unique architectural feature characterized by a pinnated portion on its ventral surface. The present study was performed to quantitatively evaluate the muscle structure and compare the composition of muscle fiber types of the TSM.
The gross anatomical features of 138 formalin-fixed cadavers (83 males and 55 females) at our laboratory were studied from 2009 to 2014. The MG, LG and SOL were observed from both the dorsal and ventral sides, and each part of the muscles and all tendons were measured. Additionally, the histological features of the TSM were observed in five human cadavers. The muscle fibers were categorized into slow- and fast-twitch fibers by immunohistochemical staining.
The LG was a bipennate muscle that contained an intramuscular tendon, while the MG was a unipennate muscle with no intramuscular tendon. Moreover, a bipenniform muscle structure was mostly present in the deep ventral region of the SOL. The mobile end of this part of the tendon of the SOL is called the sagittales sehnenblatt. The minimum width of the Achilles tendon was positively correlated with the maximum width of the belly of the TSM. The percentage of slow-twitch fibers was higher than that of fast-twitch fibers in the MG, LG and SOL.

(COI: No)

P3-318

Ethics Subcommittee on the Cadaver Study in Kyorin University

Matsumura, George (*Dept. Anat., Sch. Med., Kyorin Univ., Tokyo, Japan*)

Presently, in contributing the findings of cadaver study to most medical journals, the deliberation and approval by the Medical Ethics Committee is an essential requirement. In 2012, to carry out surgical training and research using donated cadavers lawfully, "the guideline of the autopsy in education and research of clinical medicine" was presented by Japan Surgical Society and the Japanese Association of Anatomists. The guidance specifies the Ethics Committee of each medical school should recognize implementation of the cadaver study, after examining the legitimacy of the research procedure sufficiently. However, most of the Ethics Committee were organized for deliberating whether the patients' human rights, dignity, and their personal information are protected appropriately in clinical and/or hospital studies. Conventionally, cadaver studies were performed according to "Autopsy Conservation Act", and not regarded as deliberation matters by the "Clinical" Ethics Committee. For this reason, many cadaver studies were not able to undergo deliberation of the Ethics Committee. In Kyorin university, the Ethics Subcommittee of the Cadaver Study was organized to examine the ethicality of research using cadavers. The requirements for undergoing examination are reported.

(COI: No)

P3-319

The great cardiac vein and the anterior interventricular branch of left coronary artery covered with myocardium: A case report

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Myocardial bridges (MBs) are one of the anatomical variations of the coronary artery that is covered by myocardium (Myo). We previously reported that the cardiac veins and autonomic nerves located on the surface of the Myo in the case of the MB. In the present study, we show a rare case that the Myo enveloped not only the anterior interventricular branch (AIB) but also the great cardiac vein (GCV) in 75-year-old male Japanese cadaver at the anatomical dissection in the Nippon Dental Univ. Niigata. The proximal part of the AIB and GCV was covered with Myo. The Myo in this part covering the GCV was thinner than that of the AIB. The fine vein penetrated the superficial layer of this overlying Myo along this muscle bundle. In the middle part, the AIB ran deep to the Myo but the GCV passed the external surface of the Myo. In the distal part, the AIB and GCV lied on the Myo. A branch of the autonomic nerves entered Myo along with the AIB, and the remaining nerves ran over the surface of the Myo. Both nerves passed along the distal AIB. Coronary vascular precursor cells derive from the epicardium in the early stage of development (reviewed by Brade et al., 2013). Hypoxia inducible factor1 α (HIF1 α) regulates migration of these cells into the Myo (Tao et al., 2013). Taken together with the previous data, the present case suggests that HIF1 α may affect not only the coronary artery but also the cardiac vein.

(COI: No)

P3-320

Morphological study of the lateral occipital nerve using a technique of the nerve fiber analysis

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Current anatomy textbooks describe that the greater occipital nerve (C2:posterior ramus), the minor occipital nerve (C2:anterior ramus), the third occipital nerve, and the great auricular nerve (C3:anterior ramus) are distributed from medial to lateral side in the occipital region, and the third occipital nerve is distribute in lower region to the greater occipital nerve. Especially, the cutaneous branch of C1 is not generally distributed in the region. However, the lateral occipital nerve (including C1/C2 cutaneous branch) is rarely distributed in the region between the greater and lesser occipital nerves. The lateral occipital nerve was recognized as the lateral branch of the posterior ramus of the C1/C2, and ran upward outside of the occipital artery. The lateral occipital nerves at right and left sides were observed in macroscopic anatomy seminar at Niigata in 2013. This nerve had a similar feature as we mentioned before. On the other hand these nerves were characterized that was not branched from the lateral branch of the posterior ramus of the C1. However, the nerve originated from the anterior ramus of the C1. To sum up our study, we confirmed the root of the lateral occipital nerve, by using the technique of nerve fiber analysis.

(COI: No)

P3-321

Variations in the vessels connecting the posterior tributary of the left renal vein to the left ascending lumbar vein without communicating with other renal veins in a Japanese cadaver

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A rare variation was found in one of the two left renal veins in a 94-year-old male cadaver undergoing routine dissection. The characteristic findings in the cadaver included, in addition to the primary left renal vein, the presence of a posterior left renal vein draining to the left ascending lumbar vein without communicating with the inferior vena cava and other renal veins. Variations in the number and arrangement of the vessels terminating in the renal veins are common, but to our knowledge, variation similar to our findings has not been previously reported. This variation may represent an immature form of the complicated development of the renal vessels.

(COI: No)

P3-322

An anatomic variation of the coracoacromial ligament: a case report

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We observed a variation of the coracoacromial ligament of the right shoulder in an 82-year-old Japanese female cadaver during dissection at Tokyo Medical and Dental University.

The variant ligament attached to the base of the coracoid process, which was located lateral to the insertion of the superior transverse scapular ligament. It was directed posterolaterally and attached to the anterior border of the acromion. The ligament was 7 mm in width and 32 mm in length. The thickness was 5 mm at the coracoid process and 2 mm at the acromion. The ligament was covered by the clavicle and the trapezoid part of the coracoclavicular ligament. The anterior border was continuous with the proper coracoacromial ligament. The supraspinatus muscle was under the variant ligament.

The variant ligament in this report is similar to the third part of the coracoacromial ligament which was reported by Pieper et al. (1997). However, the attachment at the coracoid process is next to the insertion of the transverse scapular ligament or the coracoclavicular ligament. We consider that the variant ligament might have as close relations with these ligaments as the proper coracoacromial ligament.

(COI: No)

P3-323

Anatomical study on the flexor digitorum superficialis in common marmoset (*Callithrix jacchus*)

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The common marmoset is a New World monkey and uses arboreal locomotion. They can hang vertically from trees and leap between them. Therefore, digital flexors of the forelimb of this species, especially the flexor digitorum superficialis (FDS), are important to perform this style of arboreal locomotion. We dissected the upperlimbs of the adult common mamoset (two cases) and found that the marmoset FDS originated from the medial epicondyle of the humerus as a common origin with the pronator teres, the flexor carpi radialis, and the palmaris longus. The muscle belly for the 5th digit was independent from the other parts of the FDS, and it represented a two-bellied muscle. In one case out of two, the proximal belly of this two-bellied muscle received a twig from the ulnar nerve. The muscle bellies for the 2nd, 3rd, and 4th digits were highly fused each other. The previous study of the human FDS (Ohtani, 1979) showed that the FDS was divided into the superficial layer for the 3rd and 4th digits and the deep one for the 2nd and the 5th digits. The deep layer of the human FDS has the intermediate tendon from which two distal bellies originate and give rise to the tendons for the 2nd and 5th digits. The FDS of the marmoset for the 2nd digit has more fleshy part in comparison with that of human FDS, implying that the marmoset FDS is able to make their digits flex more strongly, which is suitable for climbing on trees.

(COI: No)

P3-324

"The medial cutaneous branches" of the upper cervical dorsal rami do not originate from the medial branches of the dorsal rami of the cervical spinal nerves

Aizawa, Yukio[1]; Takezawa, Kojiro[1]; Tsutsumi, Masahiro[4]; Arakawa, Takamitsu[2]; Kageyama, Ikuo[1]; Kumaki, Katsusji[3] (*[1]Dept Anat, Nippon Dental Univ Sch Life Dentist, Niigata; [2]Dept Rehabilitat Sci, Grad Sch Health Sci, Kobe Univ; [3]Niigata Univ Rehabilitat; [4]Kobe mariners hospital*)

The dorsal cutaneous branches in the upper cervical and the occipital regions have been believed as the homologous nerves with the medial cutaneous branches of the dorsal rami of the upper thoracic nerves, however, the true medial branches of the dorsal rami of the cervical nerves to the transversospinal muscles existed independently of the cutaneous branches. These cutaneous branches were accompanied with the branches to the semispinalis capitis that did not only receive those branches but also the branches from the lateral branches widely with the communications between them. Thus the former were more familiar to the lateral branches than the medial branches. We had reported the details of the courses and the distributions of the lower thoracic lateral branches of the dorsal rami. When the branch to the longissimus muscle takes the most medial course penetrating the intertransverse ligament, it looked like as medial branch. Therefore, we called those branches as "the intermediate branches". The branches to the semispinalis capitis with the cutaneous branches are resembled to the intermediate branches in the lower thoracic region. Therefore, we advocate that the upper cervical dorsal cutaneous branches should be called as the intermediate cutaneous branches, and the semispinalis capitis also should be considered as the medial part of the longissimus capitis.

(COI: No)

P3-325

Re-considering the pathogenesis of Achilles tendonitis based on tendinous-fiber analysis findings

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To re-examine the conventional pathogenesis of based on the structural characteristics, we studied detailed anatomical reviews of the Achilles tendon (AT) by analyzing the fiber bundles that comprise it, and by examining the relationship with the running of the plantaris tendon (PT). We examined 90 lower extremities (47 cadavers). In all cases, we observed inward twists of the comprising fiber bundles beyond the narrow part of the AT. Specifically, we observed regular twists between the fiber bundles originating from the medial gastrocnemius muscle and those from the soleus muscle. We also found that posterior to the tendon, part of the subcalcaneal bursa (synovial fold) fitted between the fiber bundles, which were separable. The most common arrangement of the plantaris tendon was Anson's type III. Around the calcaneus, the AT and PT were covered by a common fascia. These results suggested that fiber bundles may exist when internal rotation occurs in the internal parts of AT. As three functional fiber-bundle areas exist between the fiber bundles in the distal part of AT, the pathogenesis so-called a "saw-like action" may have anatomical basis. In addition, the existence of synovial folds that fit into the interior of the tendon insertion site is considered to be an important finding, because it is closely related to the synovial fringe for causing enthesitis.

(COI: No)

P3-326

A case study of both sides of the vertebral arteries passing through the 3rd transverse foramen and branches from the sympathetic trunk

Arakawa, Takamitsu[1]; Miki, Akinori[1]; Kageyama, Ikuo[2]; Kumaki, Katsuji[3] ([1]*Grad. Sch. Health Sci., Kobe Univ., Kobe, japan;* [2]*Dep. Anat. Fac. Life Dent. Nippon Dental Univ. Niigata;* [3]*Nigata Univ. Rehab.*)

Right side of this case, the vertebral artery originated from the bifurcation point of the common carotid and subclavian arteries. Left side of this artery originated from the aortic arch as the 3rd branch from the arch. Both sides of these arteries ascended anterior of the transverse process, turned backward between the longus colli muscles to pass through the 3rd to 1st transverse foramen. These arteries branched off meningeal branches and passed through the dura mater at C1 vertebra. In addition, both sides of the subclavian arteries branched off additional vertebral arteries passed through the 6th and 5th transverse foramen. The right additional artery distributed the anterior surface of the dura mater of the ventral root for the C5 spinal nerve. The left one divided into medial and lateral branches. The medial branch passed through the dura mater from the anterior surface of the C5 ventral root, distributed the anterior surface of the spinal cord. The lateral branch ascended within the 4th transverse foramen and branched off some twigs to the periosteum. Branches from the sympathetic trunk and ganglion passed among the longus colli muscles segmentally and ran along the upper entering vertebral arteries and additional ones. These pathways of the sympathetic branches were thought to be the route of the intersegmental artery during the developmental period.

(COI: No)

P3-327

A case study of the thymic artery which passed deeply to the left brachiocephalic vein

Miyawaki, Yoshiko[1]; Miyawaki, Makoto[2]; Takezawa, Kojiro[3]; Aizawa, Yukio[3]; Kumaki, Katsuji[3]; Kageyama, Ikuo[3] ([1]*Nihon Institute of Medical Science, Moroyama, Japan;* [2]*International University of Health and Welfare, Ootawara, Japan;* [3]*The Nippon Dental University School of Life Dentistry Niigata, Niigata, Japan*)

The major part of the thymus is located in the superior mediastinum of the thorax and lies superficially to the left brachiocephalic vein. Arterial supplies of the thymus are derived from branches of the internal thoracic and inferior thyroid arteries. It reflects that the thymus descend superficially to the left brachiocephalic vein. However, a case of the thymic artery which passed deeply to the left brachiocephalic vein was observed at the 8th Macroscopic anatomical seminar in Niigata. The arterial supplies to the right lobe of thymus were derived from the branches of the right common carotid and internal thoracic arteries. The arteries to the left lobe were supplied from branches of the left inferior thyroid, left internal thoracic and right common carotid arteries. The branch of the right common carotid artery passed deeply to the left brachiocephalic vein. Other arteries to the thymus passed superficially to the left brachiocephalic vein. Based on the relationship between the thymus and neighboring organs, it is more common the thymic artery runs superficially to the left brachiocephalic vein. The studies of locational relationship between the thymic artery and the left brachiocephalic vein could not found in any previous reports. We will report the rare case of the thymic artery.

(COI: No)

P3-328

What does the recurrent laryngeal nerve of dolphins curve around?

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Background: In mammals, the recurrent laryngeal nerve (RLN) curves around the ligamentum arteriosum (LA) on the right side and around the subclavian artery (SbA) on the left side. This asymmetric travel of the right and left RLN comes about because of different embryological development of each aortic arch.
Materials and Methods: We dissected a rough-toothed dolphin and a Pacific white-sided dolphin which stranded on the beaches of Japan.
Results: In both specimens, the brachiocephalic trunk (BT) gave off the common carotid artery, SbA and costocervical trunk (CT). The SbA was ventral to the subclavian vein and vagus nerve (VN). The right RLN arose from the VN where it crossed in front of the CT. It curved around the CT and then cranially on the right side of the trachea. On the left side, the RLN curved around the LA. The internal thoracic artery (ITA) gave off some thin ventral intercostal arteries and its thick terminal branch supplied the diaphragm.
Discussion: These results suggests that in dolphins the primary (dorsal) SbA disappears remaining the CT at a certain embryological stage and that the flipper receives a secondary (ventral) SbA which passes ventral to the VN like in turtle, crocodile and chick embryo.

(COI: No)

P3-329

Organization of the neck epaxial musculature of fetal pigs

Kojima, Ryuhei (*Saitama Med. Univ., Moroyama, Japan*)

Organization of the neck epaxial musculature was investigated macroscopically in fetal pigs. In the most outer layer of the neck epaxial musculature, m. splenius originated from the median line at the all cervical and upper thoracic vertebrae. It splited to three parts, and inserted onto cranium and the transvers process of the atlas. It was innervated with dorsal rami of C2 to C5. In the middle layer the m. longissimus cervicis, atlantis and capitis existed. In the deep layer the m. biventer cervicis existed dorsomedially, and the m. complexus vetntrolaterally. The m. biventer cervicis originated from articular processes of upper thoracic vertebrae and inserted onto cranium just laterally to median line. It was innervated with dorsal rami of C2 to C6. The m. complexus originated from transverse processes of cervical vertebrae and inserted onto cranium laterally to the m. biventer cervicis. It was innervated with dorsal rami of C1 to C4, In the deepest layer the neck trasversospinalis system existed below 2nd cervical vertebrae and the mm. suboccipitales existed between 2nd cervical vertebrae and cranium. Innervation pattern was investigated in detail at every segment and organization pattern of the neck epaxial musculature will be discussed.

(COI: No)

P3-330

The relationships between the structure of digestive organ and ecotype of Cephalopoda

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Decapodiform cephalopods mainly eat crustaceans, fish and mollusks. In general, digestive organs morphologically be affected by the differences in food. However, morphological differences in digestive organs were shown among cephalopods. Then, it is considerable that lifestyles may affect the morphology of digestive organs. In this study, the relationships between the morphology of digestive organs and ecotypes of decapodiform cephalopods were surveyed.
Six species of adult decapodiform cephalopods representing five families and two different lifestyles were used. Pelagic species included *Todaredes pacificus* (n=4), *Loligo bleekeri* (n=4), *Loligo edulis* (n=4), and *Watasenia scintillans* (n=4), all of which have elongated bodies and swim actively. Benthic species included *Sepia lycidas* (n=4) and *Euprymna morsei* (n=4). Specimens were dissected and digestive organs were exenterated. The following digestive organs were examined: stomach, caecum, digestive gland and digestive duct's appendages. The percentage ratio of each organ's weight to total body weight was calculated by adjusting body mass for all six species.
Pelagic species possessed larger caecum and smaller stomach, digestive gland, and digestive duct's appendages. In contrast, benthic species had larger stomach, digestive gland and digestive duct's appendages, and smaller caecum. The speed of digestion is faster in the pelagic species to swim actively than in the benthic species. Pelagic species encounter more food than benthic species. Then, pelagic species may have smaller stomach for get less food and absorb nutrient faster in larger caecum. Benthic species may get more food in stomach and absorb slower in smaller caecum. And, the nutrient may also absorb in larger digestive duct' appendages and store in larger digestive gland in benthic species.

(COI: No)

P3-331

Dorsal derivative layer of the subcutaneous trunk muscle in the house shrew, *Suncus murinus*

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The house shrew, *Suncus murinus*, is a loose-skinned animal. Although this feature is probably due to the well-developed cutaneous muscles, the skin musculature in the family Soricidae has been described only briefly in earlier works. To understand the multilayered structure of the subcutaneous trunk muscle (STM) in the house shrew, especially focusing on the derivative layer in the dorsum, ten male specimens were dissected. The nomenclature followed that of Ura (1937), who stratified it into two layers: fundamental and derivative. The STM of the house shrew configurated a thin sheet and enclosed the entire body except for the limbs. The nerves supplying the STM were branches of the caudal pectoral nerve. The fundamental and derivative layers could be distinguished based on whether or not it kept the attachment on the humerus. The former, having humeral insertion, was composed of M. humeroabdominalis and M. humerodorsalis. The latter was lost humeral insertion and mainly spread superficial to the fundamental layer. The derivative layer was composed of M. ventralis superficialis, M. dorsalis superficialis, and M. dorsolateralis. M. dorsalis superficialis expanded well in the dorsum and acquired secondary attachment to the humerus and scapula. Only M. dorsolateralis was recognized deep to the supplying nerves. By the innervation and the order of stratification, it is inferred that the nuchal and axillary parts of M. dorsalis superficialis is finally differentiated from the derivative layer.

(COI: No)

P3-332

Relation of segmental variation in the lumbosacral plexus to length of the 12th rib in macaque specimens

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Segmental variation in the lumbosacral plexus has been studied in cadavers, and the evidence suggests that such variation is related to the length of the 12th rib;namely, a short 12th rib exhibits cranial deviation in the plexus and a long rib exhibits caudal deviation. We examined the relation between the length of the 12th rib and segmental composition of the lumbosacral plexus in 10 macaque specimens(20 sides). The furcal nerve(FN)-the boundary between the lumbar and sacral plexus-was used as an index of plexus arrangement. The length of the 12th rib was divided by the width of the proximal tibia-a common indicator of body size-to reduce bias arising from differences in the specimen body size, and the index is thus referred to as the"12th rib/tibia index". Segmental variation in the FN was roughly classified into 3 groups on the basis of whether the FN originated at L5(FN L5 group), L5 and L6(FN L5+L6 group), or L6(FN L6 group). The average 12th rib/tibia index was 2.3 for the FN L5 group, 2.42 for the FN L5+L6 group, and 2.66 for the FN L6 group. The length of the 12th rib was longer when the FN originated at a lower segment. These findings are relevant to the caudal extent of the thoracic region and suggest that variation and the present observed relation are common in primates. This work was supported by the Cooperation Research Program of the Primate Research Institute of Kyoto University.

(COI: No)

P3-333

Comparative anatomy of human posterior auricular muscle and cervicoauricularis muscle in mouse

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In human, the pinna resides just lateral to the head. On the other hand in quadrupedal mammals, the position of pinna usually extend posteo-supero-laterally to overhang neck and shoulder region. This morphological variation is accompanied with the structure of auricular muscle posteriorly attached to the pinna. Compared to the small size of posterior auricular muscle(PAM) in human, the same muscle develops in size in quadrupedal mammals and the origin goes down to the nuchal ligament which is named cervico-auricularis muscle(CAM). This muscle has been reported innervated solely by the posterior auricular nerve(PAN) of facial nerve (VII). In this study, we investigated the innervation of PAM and CAM using human cadaver in systematic anatomy (n=1) and Wistar rat (Rattus norvegicus) (n=1). In Wistar rat, the insertion (pinna) side of CAM is innervated by the PAN, while the origin side is innervated by the second dorsal branch of cervical nerve (DCN). Thus, we confirmed double innervation of CAM with nerves from head and neck, respectively. We affirmed that cutaneous muscle of trunk in the back of Wistar rat was innervated by the DCN in the same manner. These results raise the question whether CAM (PAM in human) is simply a posterior part of facial muscle. On the other hand, human PAM fits in the temporal region and solely innervated by PAN. We discuss the morphological variation of PAM and CAM in line with evolutionary change of head position in human.

(COI: No)

P3-334

Evaluation of PED procedure between surgery for patients and training using fresh cadavers

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Introduction: Although percutaneous endoscopic lumbar descectomy (PED) is the least invasive disc surgery procedure under local anesthesia, this procedure requires skill and experience. There is no doubt that the ideal learning PED procedure is in a real operating room (OR) but the standard surgical teaching of PED procedure in the OR is difficult for three reasons; local anesthesia, injury of lumbar nerve root and the need for advanced technique.
Purpose: The purpose of this study is evaluation of the differences between surgery for patients and training using fresh cadavers.
Methods: Three fresh cadavers underwent the PED procedure at the cadaver laboratory in Taiwan. The procedure included the use of 3 mm cannulas from the posterolateral approach, and removal of the nucleus pulposus with pituitary forceps after dyeing it using indigo methods.
Results: Fresh cadavers still have the same stiffness or viscosity as biological bodies. Discectomy of lumbar disc using fresh cadavers is the nearest simulation of the surgical procedures such as color of the epidural vessel, color of annulus fibers or lumbar nerve roots. Although shortening of the surgical learning curve should be obtained outside the OR, sufficient training using the plastic model or an animal could not be provided.
Conclusion: Training in the PED procedure using fresh cadaver may provide a useful way for surgeons to obtain skill.

(COI: No)

P3-335

Accessory mental nerve found during a gross anatomical dissection course

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Introduction: The incidence of the accessory mental foramen was reported as 2.0% to 11.9%. The accessory mental foramen is diagnosed easily by using three dimensional computed tomography (3D-CT). However, there are few reports of examining the distribution of accessory mental nerves (AMNs) branching off from the accessory mental foramina. In this study, we investigated the distribution patterns of the AMNs using Sihler's staining and transparency technique.
Materials and methods: In a gross anatomical dissection course in our medical school in 2012, we found a case which had right accessory mental foramen and AMN. We performed Sihler's staining technique for the mandibular bone and overlying skin, which makes the soft tissues transparent with staining the nerve deep blue.
Results: The right AMN mainly distributed to the right angular region and the right mental nerve mainly distributed to the right inferior labial and mental regions. Whereas, the left mental nerve distributed to the left angular, inferior labial, and mental regions.
Conclusion: This study revealed that the AMN was a branch to the angular region in this case. We have found some cadavers which have accessory mental foramina in the mandibles and are examining them. To predict the distribution patterns of the AMNs, we have to analyze these mandibles in detail. Then these results will be useful to avoid neurovascular complications during implant surgery, nerve block, and other oral surgery procedures.

(COI: No)

P3-336

Tibial attachments of the lateral meniscus and the anterior cruciate ligament

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Although the laxity of the lateral meniscus (LM) is occasionally observed in anterior cruciate ligament (ACL) deficient patients, the anatomic background of these cases have still remained unclear. To investigate the anatomy of LM, with special reference to the positional relationships to the anterior cruciate ligament (ACL). Twenty four knees from 12 Japanese cadavers (6 males and 6 females) were used in this study. All cadavers were fixed in 8% formalin and preserved in 30% ethanol. ACL were cut off at femoral insertion, and all other supporting tissues including posterior cruciate ligament, capsule and collateral ligament were separated at the attachment of tibia. After macroscopic investigations, six specimens were randomly chosen, and histologic examinations were performed. Outer fibers of the anterior horn of LM extended to ACL, and seemed to be intermingled with ACL fibers in macroscopic observations. However, after histological examinations, a border between LM and ACL were clearly shown. Inner fibers of the anterior horn of LM ran beneath the lateral intercondylar tuberculum, and attached to the lateral intercondylar eminence. Fibers of the posterior horn of LM were separated into the anterolateral crus and the posteromedial crus, and attached to the posterior aspects of the lateral and medial intercondylar eminences respectively. In conclusion, ACL is adjoined with the outer fibers of the anterior horn of LM, and the posterior horn of the LM is firmly attached to the posterior aspects of the intercondylar eminences of the tibia.

(COI: No)

P3-337

Using the petrous part of the temporal bone to estimate fetal age at death

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Little is understood about the age-related changes in the petrous part of the temporal bone in fetal life. The purposes of this study were to examine documented skeletal remains of Japanese fetuses, to measure the length of the petrous part, and to develop diagnostic standards for fetal age-at-death estimation that could be applied to poorly preserved skeletons. The results indicated that it is possible to use regression equations to estimate age at death directly from the length of the petrous part of the temporal bone. The application of the present method to a different population led to a fetal age-at-death estimation with an error of less than 1 month. We also used the Bayesian estimation, which yielded posterior probabilities of age, conditional on being of a particular length of the petrous part. The reference table of estimated gestational age may provide an easy-to-use indicator of the fetal age at death. In conclusion, measurement of the petrous part of the temporal bone may offer a new methodological basis for forensic and bioarchaeological diagnoses of fetuses.

(COI: No)

P3-338

Mutated FcεRI β chain (D234A) affects signal transduction of mast cell but does not affect protein structure and thermal stability of FcεRI β chain protein

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High affinity IgE Fc receptor (Fc ε RI) is expressed on mast cells as a tetrameric receptor composed of the IgE-binding α chain, four-fold membrane-spanning β chain, and disulfide-linked homodimer of the γ chains. Fc ε RI acts as a signal amplifier in mast cells. The β chain contains ITAM, a conserved feature of many antigen receptors that imparts signaling competence.

We revealed the biological functions mutated β chain (D234A) in mast cell activation upon Fc ε RI engagement and demonstrated that D234A severely impaired Fc ε RI-mediated cytokine production (IL-6), however, did not impair degranulation. On the other hands, we previously revealed that β chain ITAM with the replacement of tyrosine to phenylalanine (FFF) impaired degranulation, however, did not impair cytokine production.

In addition, we investigated the structure that is part of Fc ε RI β chain wild type (β-WT, aa:143-235) protein and β-D234A(aa:143-235) protein by circular dichroism spectroscopy (CD). The far-UV CD spectra of β-WT and β-D234A are of an α-helical structure and β-D234A does not have any loss or collapse of α-helical content. Near-UV CD spectroscopy showed that a conformational change has not occurred for β-D234A. The transition curve for the thermal denaturation of β-D234A obtained from ellipticity at 222 nm was almost the same as that of β-WT protein. Gibbs free energy change (ΔG) of β-WT is not different from that of β-D234A. Our results suggest that new signaling pathway through the D234 of the β chain may exist.

(COI: No)

P3-339

The striatal neuronal activity quantified by activation-inducedmanganese-enhanced MRI is related to the severity of Parkinson's disease

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Parkinsons disease (PD) results from degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc), leading to dopamine (DA) depletion in the striatum. This depletion is thought to alter neuronal activity in the basal ganglia, resulting in various symptoms including psychomotor ones such as bradykinesia, rigidity, and tremor. However, the pathological state of PD is associated with activity changes in which basal ganglia areas remains unknown. Here we show a correlation between striatal activity and the pathological state of PD using activation-induced manganese-enhanced magnetic resonance imaging (AIME-MRI). We found that compared to control mice, PD model mice showed significant changes in neuronal activity in the striatum. Moreover, striatal neuronal activity was significantly correlated with tyrosine hydroxylase (TH)-immunoreactivity in the striatum, which is related to motor performance in PD animal models. Thus, our results demonstrated that striatal activity is associated with the pathological state in PD. We think that our findings can pave the way for significant progress in research on PD pathophysiology, since AIME-MRI can be used for non-invasive investigation of whole brain activity. Our results also suggest that AIME-MRI could potentially be utilized for the study and diagnosis of various other neurological disorders

(COI: No)

P3-340

Effects of social isolation on the progression of allergic rhinitis symptoms in mice

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To clarify the relationship between allergic diseases and psychological stress, we investigated the effects of social isolation on the progression of allergic rhinitis symptoms in mice. Female BALB/c mice, aged 3 weeks, were divided into two groups: the group-housed and singly-housed groups. After 6 weeks, mice were sensitized by intraperitoneal injection of saline containing ovalbumin (OVA) and alum, as an adjuvant, on days 0, 5, and 14. Then, local sensitization was performed every day, starting from day 21, by instilling OVA in saline into the bilateral nasal cavities using a micropipette. From day 21, OVA-induced nasal symptoms were observed once every 2 days. Immediately after nasal instillation of the antigen solution into the bilateral nasal cavities, the frequency of sneezing and nasal rubbing was counted for 10 min. After symptoms of allergic rhinitis had progressed, a histamine H_1 receptor antagonist, epinastine, was administered intraperitoneally 60 min prior to the local application of antigen. The nasal symptoms induced by antigen solution were observed for 10 min. In singly-housed mice, the progression of allergic rhinitis was delayed and suppressed compared with group-housed mice. Treatment with epinastine decreased the nasal allergic symptoms in group-housed mice but not in singly-housed mice. These results show that the stress of social isolation partially inhibited the symptoms of allergic rhinitis and weakened sensitivity to epinastine.

(COI: No)

P3-341

Selective blockade of the cortico-rubral pathway masks the recovery of forelimb function by CIMT in capsular hemorrhage rats

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Forced-use of impaired upper limb, such as constraint-induced movement therapy (CIMT), is an effective rehabilitative method after stroke. We reported that CIMT after a capsular hemorrhage resulted in better functional recovery of the forelimb. However, the detailed mechanism of the recovery by CIMT is still unclear. To investigate the CIMT-induced changes of brain circuits and its causality for the recovery, Wistar rats were injected with collagenase to make internal capsule hemorrhage (ICH), followed by CIMT for 7 days from 24 hours after the lesion. As connection between ipsi-lesional motor cortex and red nucleus was enhanced in CIMT-treated ICH rats in biotin dextran amine (BDA) tracing analysis, double-virus vector infection technique was used to block the cortico-rubral pathway selectively (Kinoshita et al., 2012): NeuRet-TRE-EGFP. eTeNT was injected into the red nucleus and subsequent injection of AAV1-CaMKII-rtTAV16 at the motor cortex. It was revealed that blockage of the cortico-rubral tract by doxycycline resulted in deficits of the recovered forelimb function in CIMT-treated ICH group. Data suggest that cortico-rubral pathway is one of essential circuit for CIMT-induced recovery after ICH.

(COI: No)

P3-342

Roles of tPA on the recovery after ischemic stroke

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In ischemic stroke, neurons in the brain cause ischemic death which is associated with neurological dysfunction. This damage is recovered histologically by activation of microglia and astrocytes, and functionally via angiogenesis, remodeling of neural network and neurogenesis. Tissue plasminogen activator (tPA) has important roles on neural function and neuronal death in the brain together with thrombolysis in the vessel. Thus, we studied the roles of tPA on the histological and functional recovery after ischemic stroke. By using photochemically induced thrombosis model, a reproducible brain damage was induced in mice with or without tPA gene deficient (tPAWT or tPAKO) and assessed the neurologic functions by foot fall test, tail lift test and von Fray test, and histological responses by damage size and immunostaining of astrocytes and microglia. It was found that the retracation of damage size and the recovery of neurologic dysfunction assessed by tail lift test and von Fray test was delayed in tPAKO mice compared with tPAWT mice. Furthermore, the number of activated microglia was less in tPAKO mice than tPAWT mice. These findings indicate that tPA is involved in the improvement of histological damage and neurological dysfunction.

(COI: No)

P3-343

Effects of anti-cancer drugs on pain induction in a rat stomatitis model

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Stomatitis is frequently developed as a side effect of chemo-radiotherapy in head and neck cancer patients and induces severe pain during eating and speaking. In this study, to examine relationship between anti-cancer drugs and stomatitis-induced pain, we investigated effects of 5-fluorouracil (5-FU) and cisplatin on stomatitis-induced nociceptive behaviors in male Wistar rats. We intraperitoneally administered 5-FU (40 mg/kg, 3 times), cisplatin (4 mg/kg, 2 times) or saline (as control). After these administrations, rats were treated in the oral mucosa of the mandibular vestibule with 50% acetic acid for 30 seconds under pentobarbital anesthesia to develop stomatitis. Both anti-cancer drugs delayed healing from stomatitis and induced leukopenia, compared with control rats. 5-FU increased stomatitis-induced spontaneous nociceptive behavior, but cisplatin conversely inhibited it, likely anti-bacterial treatment. 5-FU exaggerated stomatitis-induced mechanical allodynia. On the other hand, cisplatin itself induced mechanical allodynia and further decreased mechanical withdrawal threshold was stomatitis induction. These results suggest that 5-FU exaggerates stomatitis-induced nociception due to bacterial overgrowth in stomatitis region. Additionally, cisplatin suppresses spontaneous nociception due to known anti-bacterial effect, but itself induces mechanical allodynia.

(COI: No)

P3-344

Antinociceptive effect of transcutaneous electrical nerve stimulation via an opioid mechanism in rats with adjuvant arthritis

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Objective: The aim of this study was to investigate the effects and mechanism of transcutaneous electrical nerve stimulation (TENS) in patients with chronic inflammatory pain.
Methods: Male Wistar rats were divided into four groups: the Control group, Adjuvant Arthritis rats (AA) group, TENS-treated AA rats (AT) group and TENS- and naloxone-treated AA rats (ATN) group. Arthritis was induced by the injection of complete Freund's adjuvant into the right hind paw. In the ATN group, naloxone, an opioid antagonist, (3 mg/kg, SC) was administered before the TENS treatment. The stimuli (4Hz, 30 min) were applied three times a week for two weeks, after which the mechanical and thermal pain thresholds were detected on days 0, 7 and 14 and the μ-opioid receptor (MOR) level in the spinal cord was analyzed immunohistochemically on day 14.
Results: On day 14, the pain thresholds were significantly decreased and the expression of MOR in the superficial part of the dorsal horn was increased in the AA group versus those observed in the Control group. These changes were inhibited by TENS treatment; however, the effects of TENS were attenuated by the administration of naloxone.
Conclusions: These results suggest that TENS treatment has an antinociceptive effect on chronic inflammatory pain in association with the endogenous opioid system.

(COI: No)

P3-345

Plasminogen Activator Inhibitor-1 Contributes to Glucocorticoid-induced Diabetes, Osteopenia and Sarcopenia in Female Mice

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Glucocorticoids (GC) treatment induces numerous adverse effects, including glucose/lipid abnormalities, osteoporosis and muscle wasting. However, its pathogenesis remains to be fully elucidated. The present study investigated the role of plasminogen activator inhibitor-1 (PAI-1) in GC-induced glucose/lipid abnormalities, osteoporosis and sarcopenia by using PAI-1-deficient mice. The levels of plasma PAI-1 and PAI-1 mRNA in white adipose tissue were markedly elevated in GC-treated wild-type female mice compared with placebo-treated wild-type female mice. PAI-1 deficiency significantly improved insulin resistance and glucose intolerance but not hyperlipidemia induced by GC treatment. In vitro study showed that active PAI-1 treatment attenuated insulin-induced phosphorylation of Akt in HepG2 hepatocytes, but not in 3T3-L1 adipocytes and C2C12 myotubes, indicating that PAI-1 inhibits insulin signaling in hepatocytes. PAI-1 deficiency blunted GC-induced bone loss presumably due to a decrease in apoptosis of osteoblasts. Moreover, PAI-1 deficiency protected from muscle loss induced by GC treatment. In conclusion, the present study indicated that PAI-1 is involved in GC-induced glucose metabolism abnormality, osteopenia and muscle wasting in female mice. PAI-1 may be a novel therapeutic target to reduce adverse effects of GC treatment.

(COI: No)

P3-346

Stress-induced microglial activation may be triggered by noradrenergic neurons

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Microglia has been extensively demonstrated to participate in the neuroinflammatory responses. Recent studies have shown that exposures of animals to stress, either acute or chronic, induce robust microglial activation in the brain. The stress-induced microglial activation has been well documented in the hippocampus, cerebral cortex, thalamus, hypothalamus, and substantia nigra. In the present study, we investigated the mechanism how acute stress could trigger microglial activation in the brain. For this purpose, we studied the spatial distribution of noradrenaline-synthesizing enzyme, dopamine β-hydroxylase (DBH), and activated microglial cells following 2 h period of restraint stress. The results demonstrated that: 1) the microglia activation, as demonstrated with Iba1, occurred in most of these brain regions including the hippocampus and substantia nigra; 2) DBH was densely stained in the neuronal fibers located in most of these brain regions including hippocampus and substantia nigra; 3) The intensity of DBH immunoreactive (IR) fibers and that of DBH-IR cell bodies in the locus ceruleus was significantly increased in the 2 h restraint stress; 4) β1 and β2 adrenergic receptor (AR) are co-localized with microglial cells; 5) The stress-induced microglial activation is significantly inhibited in the double knockout mice that specifically lack β1 and β2 AR. Thus, the present study demonstrates that neuron-microglia may have close interactions through noradrenaline throughout the brain. Noradrenaline may be one of the neurotransmitters that regulate microglial activation in the brain.

(COI: No)

P3-347

Effects of intraperitoneal injection of vasopressin on Oxidative stress in conscious rats

Katoh, Shingo[1]; Hamamoto, Kentaro[1]; Ueda, Naoki[1]; Takeshima, Chiaki[1]; Isaji, Keiyu[2]; Okada, Misaki[1]; Taniguchi, Hiroshi[2]; Taniguchi, Sazu[1]; Kitakoji, Hiroshi[1]; Imai, Kenji[1] (*[1]Dept Clinical Acp Mox, Meiji Univ Integrative Med, Kyoto, Japan; [2]Dept Basic Acp Mox, Meiji Univ Integrative Med, Kyoto, Japan*)

It has been already known that the d-ROM of oxidative stress had been elevated by some kinds of invasion, example for surgical operation, inflammation, ischemia. However, It has not been shown that the physiological and psyclic stress induce the change of d-ROMs or not. Other hand, It has been also shown that the vasopressin can induce the emesis and the excitation of sympathetic activities. The aims of this study were to evaluate the change of oxidative stress [d-ROMs and BAP] under intraperitoneal administration of vasopressin for sympathetic excitation.7-week-old male Sprague-Dawley rats were divided into two groups each of seven control group and vasopressin group. Rats ate the 1.5 g solid food within 10 min after fasting for 24 h. After feeding, the control group was injected the saline (2 ml/kg) and other group war injected the vasopressin (20 μg/kg;2 ml/kg) with intraperitoneally. The stomach was excised 90 min after feeding, the ratio of gastric emptying was calculated from the weight of the contents. No changes had been obtained in this study value of d-ROM and/or BAP in vasopressin group were not significant compare to control group. It had been demonstrated that changes of oxidative stress were not observed under vasopressin injection in conscious rats. From that finding suggest that the sympathetic excitation by vasopressin in injection(i.p.) might not induce the changes of oxidative stress.

(COI: No)

P3-348

Acupuncture related to the vestibular system improves arterial pressure response at the onset of head-up tilt

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Acupuncture has been used for treating multiple kinds of disorders. However, the precise mechanism of acupuncture on such disorders remains unclear. We examined the effects of acupuncture related to the vestibular system for arterial pressure change at the onset of head-up tilt (HUT) in 14 healthy young subjects. Arterial pressure was measured continuously during supine position for 5 minutes, followed by 2 minutes of 60 degrees of HUT with and without acupuncture. With acupuncture, 2 points were tested for each subject using stainless steel acupuncture needles (15mm long, 0.16mm in diameter, 10 mm insertion). One point was TE17 (called *Yifeng*), which is considered to treat inner ear disease. Another point was PC6 (called *Nei Guan*), which is considered to treat nausea and vomiting symptoms. The order was changed randomly. Without acupuncture, mean arterial pressure (MAP) increased or decreased less than 5 mmHg upon HUT in 7 subjects (UP group), however, MAP decreased more than 5 mmHg in 7 subjects (DOWN group). In UP group, no significant difference in MAP was observed between with and without any acupuncture. In DOWN group with acupuncture of TE17, however, the decrease in MAP was smaller than that without acupuncture and with acupuncture of PC6. Therefore, acupuncture of TE17 is considered to be useful for treatment of orthostatic hypotension.

(COI: No)

P3-349

Influence of implantation of slow release corticosterone pellets on the hippocampal neuronal cells in C57BL/6 mice

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The use of exogenously administered corticosterone to experimental animals has some validity to examine chronic stress-induced damage in the brain. In this study, we have examined the influence of treatment with corticosterone on the hippocampal neuronal cells using male C57BL6/J mice, by implanting slow release corticosterone (5 mg). In the control experiment, placebo pellets were implanted. Seven or 21 day treatment with corticosterone resulted in much reduced thymus weight, compared with the control. The rate of increase in body weight was also lowered significantly by the corticosterone treatment. On the 7 or 21 day after implanting the pellets, we anesthetized the animals with intraperitoneal injection of pentobarbital. In some animals, isoflurane was additionally inhaled. Then, we transcardially perfused the heads with 4% performaldehyde solution, and made the Nissl-stained hippocampal preparations. In the preparations from the corticosterone-treated animals, there have been observed pyknosis, and partially degenerated neuronal cells in the region of CA2, CA3 or dentate gyrus. However, similar changes were also observed in the preparations from the control animals. Some of the degenerated changes in the hippocampal neuronal cells might occur due to implantation of the pellet itself, as there is no visible degeneration in the neuronal cells in the preparation from non- treated animals.

(COI: No)

P3-350

CD200-CD200R interaction may play a role in the growth of rat experimental glioblastoma

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CD200 and it's receptor CD200R are a type 1 trans-membrane glycoprotein, and CD200/CD200R interaction transduces suppressive signals to immune cells with CD200R expression. In this study, we investigated the tumor growth and the survival rate of rats that underwent C6 glioma cell transplantation into their forebrains. As revealed by immunohistochemical staining, most endothelial cells of blood vessels in the tumor mass expressed CD200 and tumor associated macrophages (TAMs) expressed CD200R. These results suggest that CD200 on endothelial cells interacts with CD200R on TAMs, causing the macrophage polarization into M2 phenotypes. We have found the expression of a truncated form of CD200 that we call CD200S lacking some amino acid sequences of CD200. CD200S appears to disturb the interaction CD200/CD200R interaction. With an aim to elucidate what kinds of roles the interaction plays on C6 glioma tumor progression, we established rat C6 glioma cells stably expressing CD200 and CD200S using a viral vector. The established cells (C6 transfected with empty vector, CD200, or CD200S) as well as normal C6 glioma cells were transplanted into the brain of neonatal rats. Although the four types of cells did not show any significant differences in the proliferation rate, rats transplanted with CD200S-tranfected C6 cells survived for significantly longer period than the rats transplanted with other cell types. The results suggest that CD200/CD200R interaction aids the progression of glioblastomas.

(COI: No)

P3-351

Monocarboxylate transporter 4 is associated with acidification of synovial fluid pH and synovial fibroblast proliferation in rheumatoid arthritis

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Objectives: Synovial fluid pH is low in rheumatoid arthritis (RA); however, the precise mechanisms are unclear. Here we investigate the correlation between synovial fluid pH and the disease activity of RA. We reveal the mechanisms regulating synovial fluid pH.
Methods: The pH and lactate concentrations in synovial fluid from RA patients were determined. Synovial fibroblasts (SFs) from the inflamed joints of RA patients (RASFs) were examined for the expression of ion transporters that regulate intracellular pH. The ion transporter up-regulated in RASFs was then suppressed by small interfering RNA (siRNA) and the effect of transfection was investigated.
Results: Synovial fluid pH correlated inversely with both the disease activity score using 28 joints and C reactive protein (DAS28-CRP) and synovial fluid lactate levels. RASFs had significantly higher mRNA and protein levels of monocarboxylate transporter (MCT) 4 than osteoarthritis SFs (OASFs). Knockdown of MCT4 induced RASF apoptosis and inhibited their proliferation, but not OASFs.
Conclusion: RA activity correlated with decreased synovial fluid pH. This may be due to increased MCT4 expression in RASFs. Since silencing MCT4 induced RASF apoptosis and inhibited their proliferation, MCT4 may be a potential therapeutic target for RA.

(COI: No)

P3-352

Development of rat diabetic nephropathy is suppressed by voluntary exercise in OLETF rats

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Purpose: The aim of this study was to examine whether voluntary wheel-running (WR) exercise suppresses development of diabetic nephropathy in OLETF rats of a type II diabetes mellitus (DM) model.
Methods: Male OLETF rats of 5 weeks old were reared in cages equipped with wheels (OLETF-WR) or in standard cages (OLETF-SED) for 16 months. These rats underwent urine collection in a metabolic cage, examination for HbA_{1c} and ELISA for renal injury biomarkers.
Results: Creatinine, BUN and urine volume per day were increased in OLETF-SED with high HbA_{1c} as compared with OLETF-WR and LETO. In addition, OLETF-SED and OLETF-WR showed the highest and moderate levels in both kidney/BW ratio and excretions of albumin and total protein into urine, respectively, whereas LETO the lowest levels. ELISA of nephron segment-specific injury indicated that OLETF-SED has injury in both glomerulus and proximal/distal tubules while OLETF-WR has slight injury just only in glomerulus.
Conclusion: Long-term voluntary WR exercise could suppress development of type II DM but not fully diabetic nephropathy.

(COI: No)

P3-353

Fibrinogen gamma-chain peptide-coated, ADP-encapsulated Liposomes Rescue Lethal Blast Lung Injury Hemorrhage via Purinergic Signaling

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Background: Fibrinogen gamma-chain (HHLGGAKQAGDV, H12)-coated, adenosine-diphosphate (ADP)-encapsulated liposomes [H12-(ADP)-liposomes] that accumulate at bleeding sites and release ADP. The aim of the study was to elucidate the effect and the mechanism of H12-(ADP)-liposomes on resuscitation of lethal blast lung injury.
Methods: Mice were pretreated with H12-(ADP)-liposomes, (ADP)-liposomes, (PBS)-liposomes or normal saline, and then received a single shot of Laser Induced Shock Wave (LISW) that caused diffuse alveolar hemorrhage.
Results: H12-ADP-liposomes significantly improved mouse survival and reduced the pathological injury score than normal saline (35 vs 40, p=0.004, n=5). H12-ADP-liposomes reduced the albumin leakage (0.8 vs 1.3 mg/ml, p=0.03, n=6) and MIP-2 levels in the bronchoalveloral lavage fluid (BALF) (74 vs 355 pg/ml, $p<0.01$, n=6) than normal saline. In this setting, exogenous ADP derived from the H12-(ADP)-liposomes not significantly up-regulated the platelet aggregation but was soon metabolized to Adenosine, which has cytoprotective effect.
Conclusion: H12-(ADP)-liposomes may be a safe and effective for acute blast lung injury via hemostatic support and drug delivery system of purinergic signaling for organ protection.

(COI: No)

P3-354

Licarin A is a candidate compound for the treatment of immediate hypersensitivity via inhibition of mast cell activation

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The present study evaluated the pharmacological effects of licarin A, a compound isolated from various plants, on A dinitrophenol-human serum albumin (DNP-HSA)-stimulated rat mast cell line (RBL-2H3). Licarin A (1 - 20 μM) significantly and dose-dependently reduced tumor necrosis factor α (TNF-α) secretion (IC_{50} 12.6 ± 0.3 μM) from DNP-HSA-stimulated RBL-2H3 cells. Furthermore, the secreted levels of prostaglandin D2 (PGD2) of DNP-HSA stimulated cells pretreated with licarin A were lower than those of cells stimulated with DNP-HSA alone (positive control). Treatment with licarin A at 20 μM revealed subtly suppression of the DNP-HSA-induced increase in cyclooxygenase-2 mRNA and protein levels. In contrast to its striking inhibition of TNF-α and PGD2 release, 20 μM licarin A only moderately inhibited histamine release from DNP-HSA-stimulated RBL-2H3 cells, by 28.2%, as compared with the positive control. We identified several signaling pathways which mediate these pharmacological effects. Licarin A treatment reduced phosphorylated protein kinase C alpha/beta II (PKC α / β II) and p38 mitogen-activated protein kinase (MAPK) protein levels. Taken together, our results demonstrate that licarin A reduces TNF-α and PGD2 secretion via the inhibition of PKC α / β II and p38 MAPK pathways, suggesting it may serve to attenuate immediate hypersensitivity.

(COI: No)

P3-355

Rare sugar D-psicose (D-allulose) prevents progression and development of diabetes in T2DM model OLETF rats

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Prevalence of obesity has emerged as life-style-related health problem leading to insulin resistance followed by T2DM. To cope with increased insulin demand pancreas β-cells become injured and failure followed by glucose intolerance. This circumstance demands balanced food intake. We introduce a zero-calorie sweet-taste food additive, D-psicose (also called D-allulose), a rare sugar, have been evaluated effective against hyperglycemia and hyperlipidemia, and represents a safe and non-toxic compound to maintain blood glucose levels through pancreas β-cell preservation in OLETF rats. Treated rats were fed 5% D-psicose. Control OLETF and non-diabetic control, LETO were fed water only. Body weight, food and drink, blood glucose and insulin were measured periodically. Oral glucose tolerance test was performed. Serum and organs were preserved, measured and stained. D-psicose controlled abdominal fat accumulation and prevented body weight increase. D-psicose improved insulin resistance through constant maintenance of blood sugar levels. Oral glucose tolerance test showed reduced blood glucose levels suggesting improvement of insulin resistance. D-psicose attenuated β-cell fibrosis. Serum levels of proinflammatory and antiinflammatory adipocytokines were also controlled well. Rare sugar D-psicose might be a promising strategy for the prevention of obesity, maintenance of blood sugar, and preservation of pancreas β-cells.

(COI: No)

P3-356

Induction of cell death in human cancer cell lines by novel small molecule activator of tyrosine kinase receptors

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Tyrosine kinase receptors (RTKs) mediate a variety of growth factors. Occasionally, RTKs are constitutively activated in malignant cells. Activation of RTKs are correlated with malignant progression of human cancer. Therefore, ATP competitive inhibitors for RTKs have been developed. We identified novel small molecule compound CMB-236 during anticancer drug screening. We observed that CMB-236 markedly induced elevation of kinase activity of a variety of tyrosine kinase receptors, using a TR-FRET kinase assays. In spite of elevated RTKs activity, CMB-236 induced cell death in human cancer cell lines using MTT assay. Significantly elevated caspase-3, -8, and -9 activity was observed in MDA-MB-231 breast cancer cells treated with CMB-236. The cell death induced by CMB-236 was prevented by simultaneously adding of pan-caspase inhibitor Z-VAD-FMK. We found that cell death induced by CMB-236 was strongly dependent on caspase activity. The cell death of MDA-MB-231 breast cancer cells treated with CMB-236 was decreased by combination of CMB-236 with sunitinib, a multitargeted tyrosine kinase inhibitor. Hence, we assumed that unusual activation of tyrosine kinase by small molecule compound disturbed cell homeostasis resulting in induction of cell death. It remains to be determined whether kinase activator will be efficient as a cancer therapy in vivo, but we suggest that such compounds can be considered as potential drug targets.

(COI: No)

P3-357

The development of whitening peptide with peptide percutaneous drug delivery system

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Topical therapy is the most favored form of treatment for whitening against hyperpigmentation and sunburn because it lends itself to self-administration, patient compliance and an absence of systemic adverse effects. However, high-molecular-weight, hydrophilic chemicals are difficult to use as transdermal delivery drugs and the use of topical drugs has been highly limited. Melanogenesis inhibitors from natural sources have great potential, as they are considered to be safe and largely free from adverse side effects. We applied 11-arginine (11R), a cell-membrane-permeable peptide, as a transdermal delivery system with a skin delivery enhancer, pyrenbutyrate. We performed intracellular screening for melanogenesis inhibitors with 11R fused with 28 kinds of tyrosinase inhibitory peptides from natural sources. Peptide No. 10, 8 amino acid, found in gliadin protein, a wheat component, most strongly inhibited melanin production, showed no cytotoxicity and inhibited melanin synthesis as determined through melanin content measured using an absorption spectrometer and observation with a transmission electron microscope. Next, we transduced this 11R-No. 10 into skin with an 11R transdermal delivery system after previous treatment with pyrenbutyrate and performed daily repetitive topical application for two weeks against a UV-induced sun-tanning guinea pig model. We observed a whitening effect and significant melanogenesis inhibition in a model skin sample by Masson-Fontana staining.

(COI: No)

P3-358

A novel methotrexate derivative with intrinsic magnetism

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Background: We have recently reported a novel anti-cancer compound with intrinsic magnetism (EI236). In addition to anti-cancer effect, EI236 has three features 1) EI236 is attracted by a magnet, i.e., magnetic drug delivery, 2) generating heat in an alternating current magnetic field, i.e., hyperthermic effect, and 3) a new contrast agent in magnetic resonance imaging (MRI), because of its magnetism. Based on these properties of EI236, we succeeded in generating a novel methotrexate derivative with intrinsic magnetism (m-MTX). It is well known that MTX is a commercially available and has been used as conventional drug for cancer and rheumatic diseases. In this study, we examined whether m-MTX has an intrinsic magnetism and the anti-cancer effect.
Materials & Methods: The magnetic property of m-MTX was measured by ESR (Electron Spin Resonance) and SQUID (Superconducting Quantum Interference Device). VX2, rabbit squamous cancer cells and MCF7, breast cancer cells, were used. To evaluate the m-MTX-induced cytotoxity, cell proliferation was measured using commercially available kit (ATCC).
Results: m-MTX was easily accumulated by a permanent magnet in water. ESR and SQUID showed that m-MTX has an intrinsic magnetic property. Furthermore, m-MTX inhibited cell proliferation in both cells in a dose dependent manner.
Conclusion: M-MTX may enable us to develop novel strategies in cancer treatment, i.e., chemotherapy with controlled drug delivery with a single drug compound.

(COI: No)

P3-359

Analgesic effect of hangeshashinto on oral ulcer-induced pain in rats

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It is well known that oral pain in head and neck cancer patients treated with chemo-radiotherapy is persistent and intractable, resulting under-nutrition and low quality of life. Recently, it has clinically reported that hangeshashinto (HST), a traditional Japanese medicine, is effective on the oral pain. However, mechanism of the analgesic effect has not been well known. In this study, we investigated the oral ulcer-induced pain and efficacy of HST to the pain in rats using new technique to apply direct stimulations in the oral mucosa. Treatment with acetic acid in the labial fornix region of the inferior incisors developed obvious oral ulcer. Application of HST to oral ulcer region did not change pain-related grooming behavior, suggesting that HST does not have pungent effects. Head withdrawal threshold to mechanical stimulation to the oral mucosa was decreased by oral ulcer development compared to naive. The decrement of mechanical threshold was recovered to naive level from 30 min to 60 min after topical application of HST to the oral ulcer region. These results support that HST is a useful drug to inhibit oral ulcer-induced pain in patients with chemo-radiotherapy.

(COI: Properly Declared)

P3-360

Evaluation of the antinociceptive effect of Uncariae Uncis cam Ramulus (Chotoko) in rat pain models

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Objective: Kampo medicine Yokukansan has been reported to be effective against neuropathic pain, such as that observed in patients with postherpetic neuralgia, central pain and trigeminal neuralgia. The aim of this study was to investigate the antinociceptive effects of Uncariae Uncis cam Ramulus (Chotoko), a crude drug component of Yokukansan on pain.
Methods and Results: 1) Acute study using formalin-induced pain model rats. In the Chotoko-treated group, Chotoko was administered for three days, after which 50 μl of a 5% formalin solution was injected into the right hind paws and the time spent licking the injected paw was recorded. The pre-administration of Chotoko resulted in a decrease in the licking time.
2) Chronic study using the chronic the constriction injury (CCI) model rats. The CCI model rats were prepared according to the model proposed by Bennett. Two weeks postoperatively, a decrease in the pain threshold in the CCI rats was confirmed and then Chotoko had been administered for two weeks. Four weeks postoperatively, the pain threshold significantly decreased and significant spinal astrocytic activation, which is involved in the expression of chronic pain, was noted in the CCI rats. However, the activation of astrocytes was controlled and the decrease in the pain threshold was reduced with the administration of Chotoko.
Conclusions: We therefore conclude that Chotoko can effectively reduce acute inflammatory and chronic neuropathic pain.

(COI: No)

P3-361

Effect of in vivo melanogenesis of Hyugatouki (Angelica tenuisecta var. furcijuga) extract

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Purpose: Angelica furcijuga (AF) is an endemic species and perennial herb of Japanese parsley department that grows wild in the Kyushu island in Japan. It is faced with extinction but its usefulness is attracted attention by success of organic grow. We have ever reported that the AF extracts from lobe and stem, stimulated melanogenesis in mouse B16 Melanoma Cell (B16 cell) and mouse hair, and effect of melanogensis in fraction of water and ethyl-acetate layer. In the present study, on the effects of the each extract solvent fraction of the AF's extracts from lobe and stem, melanogenesis of mouse hair were observed.
Methods: Before and after apllications, change of the melanogenesis of the back hair of the mouse with AF extract was investigated.
Results and Discussion: We observed different effects of each fraction on melanogenesis. This study suggests the certain fraction of AF's extracts contains the melanin production promoting substances.
(COI: No)

P3-362

Effect of pregabalin or pentazocine on restriction of movement and hyperalgesia in an ankle-immobilization rat model

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To evaluate the effect of pregabalin or pentazocine treatment on range of motion (ROM) limitation and the anti-hyperalgesia in ankle-immobilization model. Wister male rats were used. ROM and pain threshold were measured in all rats once a week for the 2 weeks. ROM of ankle dorsiflexion (DF) was measured, and pain thresholds were evaluated by behavioral response with the von Frey test and Hargreaves Assay using a plantar test. All data were shown as % of right limb/left limb. Ankle DF in ankle-immobilization for 14 days (IM) was significantly limited 2 week after immobilization (66%). And Ankle DF in IM+pregabalin (IM+PG) and IM+pentazocine (IM+PZ) also significantly limited 67% and 66% after 2 weeks, respectively. The mechanical hyperalgesia threshold in IM was significantly decreased to 39% after 2 weeks compared to control group, and in IM+PG was also significantly decreased to 58% respectively, but in IM+PZ was not decreased. Thermal nociceptive thresholds were significantly decreased to 61% after 2 weeks in IM, and also significant decreased 82% after 2 weeks in IM+PG, but in IM+PZ was not decreased. These results indicate that PG or PZ treatment was not effect on ROM of ankle DF in this model. On the other, the decrease of mechanical hyperalgesia and thermal nociceptive thresholds in IM+PZ were not observed, and showed a tendency to increase by IM+PG. It might act on central nerve system and/or spinal interneuron. We need further investigation for resolution these mechanisms.
(COI: No)

P3-363

Effectiveness of using specific arrow shapes in illustrations showing lipoprotein dynamics

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Animations showing the basic dynamics of lipoprotein, and then illustrations using specific arrow shapes for movement, changes, and facilitation were presented to 2nd yr students in nutrition, who had learned the topic previously. Results of an anonymous survey were as follows. Compared to other illustrations NOT using specific arrow shapes, for understanding, the presented ones were: easier 35, somewhat easier 30, no different 9, somewhat more difficult 0, more difficult 0. To "get the image" (comprehend the whole idea), the presented ones were: easier 39, somewhat easier 27, no different 8, somewhat more difficult 0, more difficult 0. Regarding memorization, the presented ones were probably: easier 28, somewhat easier 31, no different 15, somewhat more difficult 0, more difficult 0. The high evaluation indicates that presenting animations and then illustrations using specific arrow shapes is effective in showing the basic dynamics of lipoproteins.
(COI: No)

P3-364

Vestibulocochlear organ 3D print model generation trial

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It is very hard for medical students to dissect vestibulocochlear organs and to grasp their complex structures, partially because they are embedded in the temporal bone. We recently developed transparent temporal bone 3D-print-model with vestibulocochlear organs inside(The 119th Annual meeting) Our model worked well as a guidance of whole vestibulocochlear organ dissection. As a guidance of each of vestibulocochlear organs dissection, we developed auditory ossicles (malleus, incus, stapes) models in transparent composition of internal and external acoustic meatuses, and vestibulocochlear organs Since CT value oriented editions of DICOM data images did not always match anatomical structures, the human head CT scan images was edited manually slice by slice based on anatomical features. For example, mastoid cells are topologically identical to middle ear. So, they had to be removed manually. Then auditory ossicles data and surrounding structure data were saved independently to cast them with different resins. The edited DICOM data were then converted to STL data. To determine appropriate size of the model for beginners, double and triple sized models were generated by lamination of 0.2mm thick layers of ultraviolet curable resin with CONNEX 500 3D printer. The new model could be a good guidance for dissection of the vestibulocochlear organs.
(COI: No)

P3-365

Osteology on "Seikotsu Sinsyo", Judo-therapy book at Edo era(1810)

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The present study describes "SeikotsuSinsho" and Kagami Bunken. "SeikotsuSinsho" is a book of Judo-therapy that was written in 1810 by Kagami Bunken. The author was a Judo-therapy doctor of the late Edo period (1755-1819). In this book, general osteology, name of the bone, forms and function of each bone, joints and ligaments have been described. In addition, "KakuKotsu ShinKei Zu", which is a breakthrough skeletal atlas, is a precise sketch of skull, vertebrae, thorax, upper limbs, and lower limbs. The figures of these bones were sketched by Kagami Bunken based on the real bones. "SeikotsuSinsho" has been well known to Judo therapist, but not to Anatomist. We reviewed the terminology of osteology that has been described in this book in the documents from Edo era to early in the Meiji era. There were three types of the terminology of osteology found in "Seikotsu Shinsyo". (1): Terminology used by past orthopedic science, (2): the terms considered to have referred to the KaitaiShinsho, (3): the original terms of Kagami Bunken. However, the original terms have not been reflected in the current terms. As the reason for this, it is suggested that a translation term became mainstream.
(COI: No)

P3-366

Research for characteristics of physiology education in the Saitama Prefectural University and development of efficient educational program for students of several different courses of health sciences

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In Saitama Prefectural University (SPU), we have consistently provided quality education which helps graduates to play important roles in the area of health sciences. Moreover, we have to educate simultaneously basic medical sciences including physiology because we have several different department of health sciences such as nursing or physical therapy. Thus, we have tried to clarify characteristics of physiology education in SPU and development of efficient program of cross-sectional education for students of several different courses of health sciences using by questionnaire method. Firstly, we would analyze characteristics or tendency of needs of physiology education for each department of subjects divided according to specialty in the field of health sciences. Particularly, we would examine the effective cooperation method of three kinds of education subjects of lecture, practice and training in physiology education because we hope to be able to understand an effectual cooperation method among three kinds of educational methods. Data analysis are under way.
(COI: No)

P3-367

The "Hand-made" heart model as an educational tool for three-dimensional cardiac anatomy

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Background: The three-dimensional (3D) anatomy of the heart can be difficult to teach for teachers and to understand for students due to its complexity. We therefore developed a method to simulate the 3D cardiac anatomy using two hands.
Method: Our model is created by folding the two hands together as follows; (1) Make fists using the right and left hands with the thumbs and index fingers straight (a gun gesture). (2) Bring the two hands together to cross each other so that the left index finger is located between the right thumb and the right index finger. (3) Fold the thumbs onto the fist with the right thumb is placed over the left thumb.
Results: The hands together provide a simulated heart view with the cardiac base facing the examiner. The right and left hand represent the right and left heart, respectively. For each hand, the fist and index finger represents the ventricle, and great vessel, respectively. The base of the left and right forefinger correspond to the aortic and pulmonic valves, respectively. The left middle finger and thumb simulates the mitral valve. The base of the right thumb corresponds to the tricuspid valve. The border created by the two fists represents the ventricular septum. Major coronary branches are represented by anatomical landmarks of the hands and borders between the two hands.
Conclusion: Our model represents the cardiac geometry and normal distribution pattern of the coronary artery branches fairly accurately. We propose this model as a useful tool for both self-learning and education on the 3D cardiac anatomy.

(COI: No)

P3-368

Usefulness of fetal pig for understanding orofacial anatomy in anatomical practice

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In recent years, we have known as much about the difficulties of the learning about the anatomical knowledge, especially in orofacial region of oral anatomical education for dental technician and dental hygienist students. We have already introduce the usefulness of fetal pig for understanding tooth development and dental germ in anatomical practice in the 115th and 119th Annual meeting of the Japanese Association of Anatomists. Anatomical practice as a mandatory elective subject using fetal pigs has already been conducted in the first year for two academic years at our school. In the present study, students who took the anatomical practice course using fetal pigs in 2012 and 2013 were asked to complete a questionnaire in order to determine if their level of understanding of anatomy, particularly in orofacial anatomy, had increased or not.

(COI: No)

P3-369

Trial of Anatomical Education by Anatomical Tour Using a Rotation Method for Pharmaceutical Students

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Anatomical tour is an educational method using dissected cadavers mainly for comedical students. In a previous study, we have documented the anatomical tour by using a rotation method is effective in getting good emotion for healthcare students and medical/pharmaceutical graduate students (Master course). In this study, we tried the anatomical tour for pharmaceutical undergraduate students, and their impressions were surveyed by a questionnaire. Half of the pharmaceutical students (n=43) were divided into three groups, and each group attended observational learning at 3 cadavers by rotation (15 min × 3); concerning (1) thoracic viscera and upper limbs, (2) abdominal viscera, and (3) dorsal structures including lower limbs and brain/spinal cord. Finally they observed all cadavers freely for 30 min. Another half of the students (n=44) attended the tour 1 week later. Following impressions were obtained by the questionnaire (n=85); the anatomical tour is very satisfactory (59%), or fairly satisfactory (36%). These data suggest that anatomical tour using the rotation method is also effective in anatomical education for pharmaceutical undergraduate students.

(COI: No)

P3-370

Anatomy Education in the Visual Science Course

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In the Visual Science Course of Tohoku Bunka Gakuen University, we study and teach visual sciences as well as we train students for orthoptists (ORT). The purpose of this presentation is to discuss anatomy education in our course. In education for ORT, subjects about visual organ are dominant, needless to say. However, anatomy and physiology of the whole body are also required because the national license exams for ORT include the area of this study, for example. Among subjects in our curriculum, "Introduction to Anatomy and Physiology I and II" for first year student, "Structure and Function of Sense Organs" and "Seminar for Structure and Function of Sense Organs" for second year student contain anatomy education, respectively. In addition, fourth year students can select anatomical themes for "Graduation Research". In "Seminar for Structure and Function of Sense Organs", students study several area of anatomy, such as dissection of eyeballs of pig, observation and drawing of histological specimens by using a microscope, macro-anatomy by using an anatomical model of human body, study of human skull and skeleton, somatometry and surface anatomy in eye and its surrounding area, for instance. In "Graduation Research", several students supervised by one of the presenters, have studied ocular anatomy such as, vascular system, extraocular muscles and orbital osteology by dissecting fatal pigs for the past three years.

(COI: No)

P3-371

Functional model of Swallowing

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It is difficult to teach students about the mechanism of swallowing. There are three phases of swallowing; oral phase, pharyngeal phase and esophageal phase. The bolus of food is propelled to back of mouth by the tongue and the swallowing reflex happens. After nasopharynx and mouth closure, the glottal closure occurs, then hyoid and larynx are lifted by the contractions of suprahyoid and thyrohyoid muscles. As for the epiglottis, it is compressed by the tongue and inclines downward. As the larynx is lifted upward and antriorly, slight vacuum is caused in the lower pharynx and upper esophagus at the same time, and pharyngeal constrictor compress bolus, therefore, the bolus passes the piriform fossa, and is inhaled into the esophagus. This time, we made a model in order to explain this complicated mechanism. We used three sliding rails. One rail is fixed to the top of backbone horizontally and added two hooks, one hook is for the tongue movement and the other lifting the soft palate. The other two rails are fixed to the backbone vertically and the both tops of these rails are connected by using wire in order to push down the posterior rail and lift up the anterior rail. Wooden chip fixed to posterior rail represented the contraction of pharyngeal constrictor. Hyoid bone and larynx connected to anterior rail can be lifted by pushing down the posterior rail. The mandible is made of paper clay by using a metallic plate in it. The tongue, the soft palate, and the epiglottis are made by using the EVA (Ethylene Vinyl Acetate) sheet. Suprahyoid, thyrohyoid muscles are made of tube using wire in it.

(COI: No)

P3-372

Extraction of narrative intention in medical documents based on morphological analysis

Shimmi, Takahiko; Tatsumi, Haruyuki (*Grad. Sch. Med., Sapporo Medical Univ., Sapporo, Japan*)

In general, documents as the "reification of thoughts, " compound of wording elements such as noun, verb, auxiliary verb, modifier, adjective, post-positional particles, endings and so forth, conforming with its semantic relation and specific stemma. This indicates that the structure of documents and capability/functionality of words are available to approach in morphological (=anatomical) standpoint. Concretely, analysis on words-meanings relations and paragraphs shows its "context." Moreover, context and frequently-appeared word extract the "stressed intention of author."In this study, morphological analysis is implemented on elements and functionality of documents. Based on the analysis, finally, it suggests that the relation between structure and intention are on mutual-supplement: the extraction of intention is possible to be described on the course of context.

(COI: No)

P3-373

Positional relationship between the nerve and vascular of drawing blood, intravenous injection site in the cubital fossa using ultrasonic echo equipment

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Purpose: Drawing blood, intravenous injection in the cubital fossa has been carried out on a daily basis, but complications such as nerve and vascular damage is occurring frequently. This time, the positions were examined about the blood vessel nerve bundle (median nerve (N), brachial artery (A) and brachial vein (V)) that existed in the injection site deeply with a ultrasonic echo equipment.
Method: 202 were examined. It took pictures of the Huter line (cubital fossa horizontal line). The distance and depth of N, A, and V from the middle point of the base line to the ulnar side (inside) were investigated.
Result: The distance and depth from the middle point of the base line to N, A, and V are each 19.1±5.53mm, 8.0±2.17mm, 16.1±5.43mm, 6.6±1.94mm, 16.7±6.28mm, 6.6±2.13mm. As the position of NAV, 6 patterns of NAV, NVA, ANV, AVN, VNA, VAN were seen from ulnar side (inside) to cephalic side (out side).
Conclusion: The distance, depth, and each position to N, A, and V that composed the blood vessel nerve bunch in cubital fossa was obtained. Basilic vein and median basilic vein that exists on the inside(ulnar side) of the cubital fossa, with caution when we puncture them.

(COI: No)

P3-374

Creating electronic materials for the effective teaching of anatomy courses

Inomata, Reiko; Kamezawa, Hajime; Komazaki, Shinji (*Saitama Med. Univ., Saitama, Japan*)

In order for our country to develop and compete internationally, a qualitative change in education is recommended. To this end, we propose that traditional teaching methods be converted to newer, more efficient ones such as active learning and flipped learning. Concurrently, in order to create a foundation for educational reforms, electronic teaching materials should be created or updated and effective teaching methods should be developed using these materials.
In this presentation, we introduce some simple methods for producing practical electronic materials for teaching anatomy courses by using high-functioning free software that is widely available throughout the world. We designed electric teaching materials using our methods, and explore new teaching methods such as active learning using our teaching materials. Descriptions of our electronic materials for teaching anatomy courses and our findings from trials of the new teaching techniques used are included.

(COI: No)

P3-375

RealEEG: a toolkit for medical students' training on EEG recording and analysis

Matsuzaka, Yoshiya (*Dept Physiol, Grad Sch Med, Tohoku Univ, Sendai, Japan*)

An ideal training of electrophysiology for students would be that every student is given the chance to directly experience the recording of bioelectric activity from neuromuscular system. Yet in reality, the high cost of commercial instruments for physiological experiments often precludes the purchase of sufficient number of units, thereby limiting the number of students who experience the recording of neural and/or muscular activity in group teaching. Therefore, I developed a low-cost toolkit for students' training on electrophysiology. The toolkit includes a custom designed amplifier with adjustable gain and two poles band pass filter. It is designed to function even in electrically unshielded environment due to the differential amplification with driven-shield inputs. Further, a PCB (printed circuit board) of this amplifier was developed for easy replication with consistent quality. Using this amplifier, I built a computer-controlled closed loop stimulation and measurement system for the training of electroencephalography (EEG) recording by medical students. Using this system, students succeeded in recording various brain activity with clarity which otherwise would have required costly instruments. The technical information to replicate this resource is freely available for educational as well as research purposes in other institutions.

(COI: No)

P3-376

3D multi-depth dissection atlas as a complementary resource for anatomy education

Murakami, Tohru; Tajika, Yuki; Ueno, Hitoshi; Yorifuji, Hiroshi (*Gunma Univ. Grad. Sch. Med., Maebashi, Gunma, Japan*)

Here we report an anatomical education trial of "MeAV Anatomie," a 3D multi-depth, multi-angle dissection image display system, developed by Panasonic and Okayama University. Anatomy dissection is the most effective method to master in-depth human morphology. Students appreciate human structures by hands-on training of dissection as well as studying textbooks and atlases. The dissection is, though, an irreversible process that makes iteration learning virtually unrealistic. This also means that students have no second chance when challenging difficult structures to dissect. Although photographic anatomy atlases and 3D human models may compensate such difficulties to some extent, these would not cast realistic, detailed 3D structures. MeAV Anatomie includes sets of photographs, or "contents, " of different anatomical regions. A cadaver was dissected to series of depth levels, and photographed at each level from multiple angles around the hemisphere to reproduce a 3D stereoscopic dataset. One can view the cadaver images from arbitrary angles and dissection levels on PCs or iPads. We installed a client PC system with a 3D display in the dissecting room, and distributed an iPad with the viewer app to each dissection group. Students could use the system for their homework, redeeming the irreversibility problems of dissections. They could also use it to simulate dissection procedures during actual dissections. We used some of the contents for lectures and examinations. The academic effects and students' perspectives were reviewed to estimate the validity of the system.

(COI: No)

P3-377

The brain networks underlying the Velvet Hand Illusion: an fMRI Study

Rajaei, Nader[1]; Kitada, Ryo[2]; Aoki, Naoya[2]; Takahashi, Haruka[2]; Miyaoka, Tetsu[3]; Ohka, Masahiro[1]; Sadato, Norihiro[2] (*[1]Dept. Complex Systems Science, Grad Sch Information Science, Nagoya Univ, Nagoya, Japan; [2]National Institute for Physiological Science; [3]Shizuoka Institute of Science and Technology*)

when humans put their hands together with grids of wires between them and wires move, they perceive illusory sensation of velvet between wires (velvet hand illusion, VHI). The VHI has attracted considerable attentions from the engineers for its application to haptic virtual reality. However, it is poorly understood about its underlying neural mechanisms. We experience the VHI by interpreting tactile inputs originated from velvet, even though the inputs are actually originated from wires and skin of the hand. Therefore, the VHI would involve the two brain networks, one related to perception of velvet surface per se and the other that are involved in grouping and segregating tactile inputs. To test this hypothesis, we conducted a functional MRI experiment wherein 30 subjects went through the following four conditions: strong VHI, weak VHI, real velvet and baseline condition involving no stimulation with wires. The contrast of strong VHI (against weak VHI) and the contrast of real velvet (against baseline condition) both activated the postcentral gyrus (PostCG) and cerebellum. By contrast, strong VHI produced greater activation than real velvet in the intraparietal sulcus (IPS) and precentral gyrus (PCG). This result indicates that the IPS and PCG are involved in misinterpretation of tactile inputs, the PostCG and the cerebellum are related to perception of velvet in the VHI.

(COI: No)

P3-378

Effects of the body composition on physiological changes after the Judo match

Takashina, Terue[1]; Kushi, Hidehiko[1]; Fukada, Kihachiro[1]; Onuma, Naoko[1]; Amano, Kiichirou[1]; Yoshida, Akira[2]; Konno, Jun[3] (*[1]Graduate School of Literature and Social Sciences, Nihon University, Tokyo, Japan.; [2]Institute of Humanities and Social Sciences, Nihon University, Tokyo, Japan.; [3]College of Humanities and Sciences, Nihon University, Tokyo, Japan.*)

Introduction: It is reported that body fat percentage increases with increasing weight class, with a specially rapid increase in the >90kg classes. The purpose of this study was to compare physiological changes before and after a Judo match between men < 90kg and >90kg.
Subjects and Methods: This study included 15 men who were divided into 2 groups: one is" < 90kg (n=7)"and ">90kg (n=8)". Body composition (Body fat, skeletal muscle and extracellular water percentages) was measured before the match, while heart rate, blood lactate, tympanic temperature, and skin temperatures were measured before and 1, 10, and 20 min after the match.
Results: Body fat percentage was significantly higher in the >90kg than in the < 90kg. Skeletal muscle and extracellular water percentages was significantly higher in the < 90kg than in the >90kg. Blood lactate were higher in the >90kg than in the < 90kg significantly at 1, and 10 min after the match. Skin temperatures were higher in the >90kg than in the < 90kg significantly at 10 min after the match.
Conclusions: This study revealed that Judo athletes with lower skeletal muscle, extracellular water and higher body fat percentages accumulated more blood lactate and a skin temperatures fall is delayed after the Judo match.

(COI: No)

P3-379

Effects of Trunk Training on Weight-Lifting Performance

Amano, Kiichirou[1]; Kushi, Hidehiko[1]; Fukada, Kihachiro[1]; Takashina, Terue[1]; Onuma, Naoko[1]; Yoshida, Akira[2] (*[1]Graduate School of Literature and Social Sciences, Nihon University, Tokyo, Japan; [2] Institute of Humanities and Social Sciences, Nihon University, Tokyo, Japan.*)

Introduction: Tests were performed to examine whether trunk training improved weight-lifting performance.
Subjects and Methods: The subjects were 30 high-school students (grades 10-12) who were weight-lifting club members. Eight exercises were selected from the trunk training program of university gymnastics club. The subjects trained for 2-4 months, 6 times a week. Before and after the training program, the subjects snatch (S) and clean and jerk (C&J) weight-lifting performances were measured. Changes in the measurements, including growth rate, were compared between the subjects with and those without trunk training.
Results: The results of the trunk training program were as follows: The recorded levels of improvement for the training group were 6.64 ± 1.57 kg for the S lift and 6.68 ± 1.50 kg for the C&J lift, while those for the non-training group were 0.63 ± 0.38 kg and 1.75 ± 0.67 kg, respectively, indicating significant improvements for the subjects who underwent trunk training.
Conclusions: Through this study, the significant contribution of trunk training on the improvement of weight-lifting performance was clarified.

(COI: No)

P3-380

"Info-Medicine" : the Center of Innovative Concept Based on an Anatomical View Point

Tatsumi, Haruyuki; Mizoguchi, Shogo; Shimmi, Takahiko; Sakaki, Fusako; Ninomiya, Takafumi; Ichikawa, Ryoichi; Kikuchi, Shin; Ohta, Shuzo (*Grad. Sch. Med. Sapporo Medical Univ., Sapporo, Japan*)

We define "information" as multimedia stimuli, which move our mind. The mind is the brain function composed of brain cells. Generally speaking, external as well as internal stimuli change the cell status. Some pattern of stimuli becomes a signal, and then information. Therefore, appropriate and timely information exerts therapeutic effects on the cells of our body in addition to our mind. Taking advantage of this nature of the multimedia stimuli, we could develop good medicine in order to make human healthy. So we have coined the concept of "Info-Medicine (Info-Med)." According to this definition, conventional drugs, gene therapies, psychotherapies and Tsubo (acupuncture point) stimulations also come under the scope of "Info-Med". From the points of cell biology, socio-psychology, and "The Theory of Moral Sentiments" written by Adam Smith, we developed "Info-Med" in a much broader sense and proposed "Full-Powered Medicine" utilizing everything good for the health, in contrast to the modern medicine, which is partial. We classified the Info-Med into five types:In-Social, To-Brain, In-Brain, To-Cellular, and In-Cellular. The center of the innovative concept is that "So you believe, you believe so", like "Cogito ergo sum" by Descartes. We have made a proposal of "Strategic Defensive Medical-Care Initiative "(SDMCI)" named after Ex-President Regan's SDI (Strategic Defense Initiative) taking advantages of IT (Information Technology) going to realize the SDMCI by full-powered medicine with "Info-Med".

(COI: No)

P3-381

Sphingosine 1-phosphate receptor-2 plays a protective role against lipopolysaccharide (LPS)-induced acute lung injury

Okamoto, Yasuo[1]; Cui, Hong[1]; Yoshioka, Kazuaki[1]; Takuwa, Noriko[1,2]; Aki, Sho[1]; Zhao, Juanjuan[1]; Pham, Hoa Quynh[1]; Sarker, Mdazadul Kabir[1]; Koizumi, Saori[1]; Takuwa, Yoh[1] (*[1]Dept. of Physiology, Kanazawa Univ. Sch. Med. Ishikawa, Japan; [2]Dept. of Health & Med. Sci., Ishikawa Pref. Nursing Univ. Ishikawa, Japan*)

Sphingosine 1-phosphate (S1P) is a lysophospholipid mediator and plays an important role in the regulation of vascular barrier function. Recently we demonstrated that in a murine anaphylaxis model, S1P receptor-2 ($S1P_2$) plays a protective role against vascular leak, hypotension and lethality through inhibiting endothelial nitric oxide synthase (eNOS). Acute lung injury (ALI) is characterized by leukocyte infiltration into the lung parenchyma, pulmonary vascular permeability increase and edema, and resultant pulmonary dysfunction. However, the role of $S1P_2$ in ALI is still unknown. Here, we explored the role of $S1P_2$ in a murine model of ALI induced by intra-tracheal administration of LPS. $S1P_2$ deletion in mice aggravated leukocyte infiltration in the lung parenchyma, elevation of protein concentrations and neutrophils in bronchoalveolar lavage fluid, and increases in lung proinflammatory cytokine mRNA expression. $S1P_2$ deletion also aggravated LPS-induced increases in vascular permeability and pulmonary edema. Administration of NOS inhibitor, Nω-L-nitro-arginine methyl ester, inhibited exacerbation of leukocyte infiltration and vascular hyperpermeability in $S1P_2$-deleted mice. These results suggest that S1P2 plays a protective role against LPS-induced ALI possibly through inhibiting NOS and is a novel therapeutic target for ALI.

(COI: No)

P3-382

Linking genotype to phenotype in mice molars by means of morphometric mapping

Morita, Wataru[1]; Morimoto, Naoki[2]; Ida-Yonemochi, Hiroko[1]; Takeuchi, Kosei[3,4]; Igarashi, Michihiro[4]; Ohshima, Hayato[1] (*[1]Div. of Anat. & Cell Biol. of Hard Tissue, Grad. Sch. of Med. & Dent. Sci., Niigata Univ.; [2]Lab. of Phys. Anthropol., Grad. Sch. of Sci. Kyoto Univ.; [3]Dept. of Biol., Sch. of Med., Aichi Med. Univ.; [4]Dept. of Neurochem. & Mol. Cell Biol., Grad. Sch. of Med. & Dent. Sci., Niigata Univ.*)

The mouse dentition has been extensively used as a model for the developmental genetic basis of dental morphology. Phenotypic change and disorder have been reported in a variety of mutant mouse strains. In the case of mutant mice showing drastic morphological change in cusp patterns, however, the conventional quantitative approaches, such as landmark-based methods, cannot be used due to the lack of biologically and/or geometrically homologous structures between specimens. Therefore, the phenotype-genotype correlation has remained to be clarified. Here, we applied methods of morphometric mapping (MM), a homology-free method for characterizing the phenotype, to analyze the coronal morphological variation of molars in two strains of wild type mice: ICR and BL6, and two types of mutant mice: CSGalNAcT1 (Chondroitin sulfate N-acetylgalactosaminyltransferase1, a key enzyme for CS synthesis) -null and Msx2-null. Our data showed that the MM enabled to discriminate not only between wild type and mutant, but also between two wild type strains with precise quantification and visualization of the complicated crown surface morphology. Applying this method to various types of mouse mutants representing altered cusp pattern promises well for an elucidation of the genotype-phenotype mapping in more details.

(COI: No)

P3-383

Nucleoprotein affect on Cell-Cycle progression in human cancer cells

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We had previously reported that a nucleoprotein (NP) has an effect of growth suppression for human cancer cells in vitro. We had also showed that NP affected on cell cycle progression of cancer cells, especially delayed the shift to G2/M phase. The event would contribute to the growth suppression of cancer cells. This time, we analyzed the global gene-expression patterns to reveal the molecular mechanism in the anti-cancer effect of NP using human breast cancer cells, MCF7. As a result, presence of NP affected the expression of many genes, 145 and 111 genes were up and down-regulated, respectively. VDR (vitamin D receptor) and CDKN1A (cyclin-dependent kinase inhibitor 1A), reported as cancer prevention, were up-regulated. Moreover, antioxidant enzyme GPX2 and GPX8 (glutathione peroxidase 2 and 8) were also up-regulated. These studies provide a possibility that NP will suppress the growth of cancer cells through such a change of gene expression.

(COI: No)

P3-384

Anti-tumor p53 fagment peptides screening and treatment effect of transdermal delivery for malignant melanoma

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Intracellular delivery with Protein Transduction Domain (PTD) is rapidly evolving methodology in vitro or in vivo. We have established "protein therapy" with poly-arginine (11R) as PTD, which has been employed to transport various bioactive molecules into cells. So far, based on this method, we have applied poly-arginine (11R) to transdermal delivery system, as an application example, skin whitening and hair growth agents. Such direct and efficient "Transdermal approach" may be also effective in skin cancer (malignant melanoma). In this study, we tried to establish novel peptide therapy with transdermal approach for malignant melanoma. Recently, several paper showed p53 fragment peptide had anti-tumor effect in vitro and in vivo. In this time, we found some anti-tumor peptides from p53 fragment peptide library screening. One of them strongly induced apoptosis and inhibited human melanoma cell proliferation. Furthermore, we tried to administrate this peptide against melanoma mouse model with peptide transdermal approach.

(COI: No)

P3-385

Action of peripheral opioid to gastric emptying under peripheral acute inflammation induced by carrageenan injection on foot pad in rats

Hamamoto, Kentaro[1]; Taniguchi, Sazu[2]; Taniguchi, Hiroshi[3]; Katoh, Singo[1]; Takeshima, Chiaki[1]; Isaji, Keiyu[1]; Okoda, Misaki[1]; Taguti, Reina[2]; Itoh, Kazunori[2]; Kitakoji, Hiroshi[2]; Imai, Kenji[2] (*[1]Meiji University of Integrative Medicine Graduate school; [2]Meiji University of Integrative Medicine Department of Clinical Acupuncture and Moxibustion; [3]Meiji University of Integrative Medicine Department of Basic Acupuncture and Moxibustion*)

The aims of study were to investigate the action of peripheral opioid to gastric emptying under peripheral acute inflammation induced by carrageenan injection on foot pad in rats Thirty male Sprague-Dawley rats were divided into three groups (each of 10), that were control (as vehicle) group, subcutaneous injection of carrageenan group, and carrageenan+naloxone methiodide group, respectively. All study had been demonstrated in fasting period after 20 hours from final feeding. At 4 hours after the injection to foot pad, rats were gave the solid food with 1.5 g and they ate food within 10 minutes. The stomach was excised 90 min after feeding, to evaluate gastric emptying from the weight of the contents. Percent of gastric emptying were calculated from the dry weight of contents after 72 hours, and that were compared among three groups. Ratio of gastric emptying in the carrageenan-treated group had been significant lower than control group. Therefore, the remarkable changes of gastric emptying in the carrageenan-treated were rivaled by the administration of naloxone methiodide. From these findings suggest that gastric emptying have been delayed by the β-endorphins in the peripheral blood by the carrageenan injection.

(COI: No)

P3-386

Action of peripheral opioid to oxidative stress under peripheral acute inflammation induced by carrageenan injection on foot pad in rats

Imai, Kenji[1]; Hamamoto, Kentaro[2]; Taniguchi, Sazu[1]; Taniguchi, Hiroshi[3]; Ueda, Naoki[1]; Katoh, Singo[2]; Takeshima, Chiaki[2]; Isaji, Keiyu[2]; Okada, Misaki[2]; Taguti, Reina[1]; Itoh, Kazunori[1]; Kitakoji, Hiroshi[1] (*[1]Meiji University of Integrative Medicine Department of Clinical Acupuncture and Moxibustion; [2]Meiji University of Integrative Medicine Graduate school; [3]Meiji University of Integrative Medicine Department of Basic Acupuncture and Moxibustion*)

Aims of this study were to investigate the action of peripheral opioid to oxidative stress under peripheral acute inflammation induced by carrageenan injection on foot pad in rats Thirty male Sprague-Dawley rats were divided into three groups (each of 10), that were control (as vehicle) group, subcutaneous injection of carrageenan group, and carrageenan+naloxone methiodide group, respectively. All study had been demonstrated in fasting after 20 hours from final feeding. The blood was collected from the heart after 5.5 hours after the intervention. Using free radical analysis system, measured the redox ability and oxidative stress from the collected blood. And, pain threshold had been measured by Randall selitto at before and 4 hours after carrageenan injection. In addition, the foot circumference and body temperature had also been measured. Ratio of oxidative stress in the carrageenan-treated group had been significant higher than control group. Further, the carrageenan+naloxone methiodide administration group had shown more remarkable increase than the carrageenan-treated group. From these results suggest that the oxidative stress is elevated under the acute inflammation on limbs that are controlled by the opioid.

(COI: No)

P3-387

Effects of press tack needle treatment in rats subjected to chronic social isolation

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Objective: The aim was to investigate the effects of press tack needle (PTN) treatment on social isolation stress and the participation of orexin A in this effect.
Methods: Male rats were divided into a non-stress group (Control), stress group (Stress) and stress plus PTN treatment group (PTN). The rats in the PTN and Stress groups were housed alone for eight days. In the PTN group, a PTN (Pyonex, Seirin Co., Japan) was fixed on the GV 20 acupuncture point (Baihui) on day 7. We measured the stress behavior based on the time the rats spent biting a wooden stick for ten minutes on days 7 and 8 and measured the plasma corticosterone levels on day 8. In addition, the plasma orexin A levels and morphology of the hypothalamic orexin neurons were investigated on day 8.
Results: On day 8, the biting time and the plasma corticosterone levels were significantly increased in the Stress group versus the Control group, although these increases were inhibited in the PTN group. Meanwhile, the plasma orexin A levels and number of hypothalamic orexin neurons were significantly increased in the Stress group versus the Control group, and these increases were also inhibited in the PTN group.
Conclusions: PTN may inhibit the response to social isolation stress. The inhibitory effects of the secretion of hypothalamic orexin are thought to be one mechanism underlying this phenomenon.

(COI: No)

P3-388

Blood glucose peak time after meal in healthy population using CGMS profiles

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Introduction: Continuous glucose monitoring system (CGMS) provides an opportunity to better understand abnormalities in glucose metabolism in both patients with diabetes. The purpose of the study is to clarify whether postprandial glucose peak time and peak value using CGMS among healthy subjects.
Methods: Thirteen healthy female volunteers were divided into two groups: under 50 year-old and over 50 year-old. The glucose levels of 13 healthy volunteers were monitored over 24 hours and required to maintain their usual life style without any limitation. We measured the postprandial glucose peak time, which was defined as the time elapsing from the start of the meal to the highest recorded glucose value. We compared the mean of the peak time between the meal periods.
Results: The peak time of postprandial interstitial glucose concentration in under50 years vs. over 50 was 31.88 ± 10.9 vs. 60.80 ± 28.6 minutes (breakfast), 50.50 ± 17.2 vs. 66.80 ± 31.5 minutes (lunch), and 60.00 ± 22.3 vs. 89.60 ± 49.4 minutes (dinner), respectively. The postprandial glucose peak time in the over-50 group was significantly longer than the under-50 group. But, no significant difference was found in the peak value of postprandial glucose between the both groups.
Discussion: The result indicates that the postprandial glucose peak time and peak value was different for the different meal period. In conclusion, this study demonstrates that the postprandial glucose peak time and peak value differs in the age bracket.

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Immunohistochemical examinations of the ganoine in regenerated scales from Lepisosteus oculatus, an actinopterygian fish

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It is necessary to compare teeth with scales during investigations of the origin of teeth in vertebrates because the dentition is considered to have arisen from skin denticles (scales). In basic actinopterygians, a well mineralized ganoine layer, which is analogous to tooth enamel, is present on the surface of ganoid scales [1]. In Polypterus, the preganoine (ganoine matrix) was found to exhibit immunoreactivity for anti-mammalian amelogenin antibodies, as has been found for collar enamel [2, 3]. However, there are no data about the preganoine in Lepisosteus (gar). In this study, the preganoine of regenerated Lepisosteus scales was immunohistochemically examined using anti-mammalian amelogenin antibodies. Positive immunoreactivity for several of the antibodies was detected in the preganoine, suggesting that the Lepisosteus preganoine contains amelogenin-like proteins that are similar to those found in tooth enamel [4, 5].

[1] Sire, J.-Y. (1994) Anat Rec, 240, 189.
[2] Zylberberg, L. et al. (1997) Anat Rec, 249, 86.
[3] Sasagawa, I et al. (2012) Cell Tissue Res, 347, 369.
[4] Ishiyama, M. et al. (1999) Arch Histol Cytol, 62, 191.
[5] Sasagawa, I et al. (2014) Connect Tissue Res, 55, 225.

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P3-390

The management of secondary infection control in gross anatomy education in Shimane University Faculty of Medicine

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For gross anatomy dissection, we embalm cadavers by formalin infusion and substitution to alcohol for preservation. However, there is no solid evidence that this method completely prevents secondary infection. Whereas the infection control manual has been established in the affiliated hospital, there has been no manual for accidental infection in the Faculty of Medicine, Shimane University. Therefore, we established procedures and the manual in the Faculty of Medicine. 1. To decline an offer of cadaver with records of active specific infectious diseases such as viral hepatitis. 2. To examine upon arrival of all cadavers for specific infections such as type B or C hepatitis, HIV infection and HTLV-1 infection. 3. To cremate cadavers without embalming when infection detected. 4. To use disposable products such as surgical gowns. 5. To antisepticise instruments using sodium hypochlorite or autoclaving. 6. We prepared the manual for accidental infection in the Faculty of Medicine. Almost all hospitals carry out infection controls these days. Infection control should be equally performed in Faculties of Medicine for workers and students. We here present the management of infection control in our Faculty of Medicine, and recommend staffs of anatomy departments in Japan to be aware of the need for secondary infection control.

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