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# The Journal of Physiological Sciences

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The Journal of
# Physiological Sciences

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**Aims and Scope:**

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

**Fields covered:**

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

# Plenary Lectures

# Special Lectures

# Memorial Lectures

# Plenary Lecture 1

(March 17, 11:00～12:00, Hall 1)

## PL1

### Serendipities of acquired immunity

Tasuku Honjo (*Kyoto University*)

Acquired immunity emerged sometime at the beginning of vertebrate evolution as defense mechanism against the infectious diseases caused by pathogens. During this evolution the amazing mechanism including reassembly of genetic segments was acquired probably by fortuitous events. We encountered PD-1 in 1992 and found PD-1 is the major braking system in acquired immunity. In 2002, we discovered PD-1 blockade can cure tumors in mouse models by reactivating the acquired immunity. In 2014, 22 years after PD-1 discovery, the treatment of cancer by PD-1 blockade is approved by PMDA and FDA, and considered to be revolutionary cancer treatment which is often compared to the penicillin discovery for the treatment of infectious diseases. The success of cancer treatment by PD-1 blockade owes to acquired immunity. During the long history of fight against diseases, we almost succeeded to conquer infectious diseases in the last century. Fortunately, we may be able to overcome cancer the last major life-threatening disease, by the same mechanism applied to infectious diseases.

# Plenary Lecture 2

(March 18, 11:00～12:00, Hall 1)

## PL2

### From haploid stem cells to blood vessel engineering

Josef M Penninger (*the scientific director of the Institute of Molecular Biotechnology, the head of the Life Sciences Institute of the University of British Columbia in Vancouver*)

We have previously generated murine stem cells with a single set of chromosomes, termed haploid ES cells. Using such cells we have been able to rapidly mine essential biological pathway and to use revertible mutagenesis to identify novel mediators of angiogenesis. Recently we have expanded our work to engineer human blood vessel organoids that can be transplanted into mice to establish a fully human vascular tree. We have used this system to model the pathogenesis of diabetes vasculopathies.

# Special Lecture 1

(March 17, 15:20～16:20, Hall 1)

## SL1

### Osteoimmunology and autoimmunity

Hiroshi Takayanagi (*Department of Immunology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo*)

Bone cells and immune cells share the same microenvironments in the bone marrow, communicating through various cytokines. Osteoblasts, osteoclasts and osteocytes are not only degrading or forming bone but have distinct roles in the immune regulation. Thus, much attention has been paid to the interdisciplinary filed, osteoimmunology, studying the interaction and shared molecules between bone and immune systems (Physiol Rev. 97:1295-1349, 2017). Here I summarize the recent advance in osteoimmunology and its relevance in the studies on autoimmune diseases such as rheumatoid arthritis.

Self-tolerance is primarily established by negative selection of self-reactive T cells in the thymus. We found that the transcription factor Fezf2 plays a critical role in central tolerance by directly regulating tissue-restricted antigen expression in mTECs independently of Aire (*Cell* 163, 975-87, 2015). We have made efforts to understand how Fezf2 and Aire regulate different self-antigens and explored the role of other cell types than mTECs in central tolerance. I will talk about the recent progress in understanding the mechanism of T cell tolerance in the thymus.

# Special Lecture 2

(March 18, 13:20～14:20, Hall 1)

## SL2

### Multilayer ionic mechanisms revealed by mathematical modeling of iPS derived cardiac myocyte spontaneous action potentials

Akinori Noma (*BKC Research Organization, Ritsumeikan University*)

Undifferentiated myocardial cells (hiPSC-CM) derived from Human induced pluripotent stem cell mostly shows a wide variety of spontaneous activities. The difference in the configuration and frequency of spontaneous action potential (AP) may be due to variable expression levels of the ion channels, and thereby the hiPSC-CM may embody mechanisms of various slow diastolic depolarization (SDD), which have been suggested in differentiated cardiac myocytes. To clarify mechanisms of SDD, we first created ventricular- (V-), atrial- (A-), and nodal- (N-) type hiPSC-CM mathematical models based on the human ventricular cell model (Himeno et al., 2015). Then, specific role of each ion channel in generating SDD was examined by changing the relative amplitude of $I_{Kr}$ (*KV11.1*), $I_{K1}$ (*Kir2.1*), $I_{CaL}$ (*Cav1.2* + $I_{st}$, *Cav1.3*), hyperpolarization-activated current, $I_{ha}$ (*HCN4*), and $I_{bNSC}$ to determine the range of combinations that allow generation of spontaneous action potential. The lead potential analysis of these action potential well quantified the contribution of these currents to spontaneous action potentials, and we could identify two basic mechanisms and two additional mechanisms. The primary mechanism 1 is the removal of inactivation of IKr on repolarization ($y_1$ gate) and subsequent deactivation ($y_2$ + $y_3$ gates) during 100 to 200 ms of SDD. The primary mechanism 2 is caused by positive feedback process among inward currents i.e. $I_{CaL}$ and $I_{st}$ (*Cav1.3*), $I_{CaT}$ (*Cav 3.1*), $I_N$, (*Nav1.5*), and current of $Na^+/Ca^{2+}$ exchanger, $I_{NCX}$ (*NCX1*). These primary mechanisms induce the typical sinus node low membrane potential oscillation. High membrane potential oscillation is induced by adding the secondary two channel mechanisms; activation and subsequent deactivation of $I_{ha}$, and unblocking and re-blocking of $I_{K1}$ by intracellular factors. The lead potential analysis quantitatively demonstrated the time-dependent changes in weight of these four mechanisms along the time course of SDD.

# Special Lecture 3

(March 18, 13:20～14:20, Hall 2)

## SL3

### Role of the habenula in addiction and addiction-related disease

Paul J. Kenny (*Ward-Coleman Professor and Chair, Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York.*)

The medial habenula (mHb) contains some of the densest concentrations of nicotinic acetylcholine receptors (nAChRs) in the brain and has emerged as an important region that regulates nicotine consumption. We have explored the role of the mHb in regulating the motivational properties of nicotine and tobacco-related disease and also the role for septal inputs to the mHb in these processes. We found that the transcription factor Tcf7l2 is highly enriched in mHb and, using a new line of *Tcf7l2* mutant rats, that Tcf7l2 deficiency increases nicotine self-administration behavior. CRISPR-mediated cleavage of *Tcf7l2* in the mHb similarly increased nicotine self-administration in mice. Using whole-cell electrophysiological recordings and RNA sequencing, we found that Tcf7l2 regulates the recovery of nicotinic acetylcholine receptors in the mHb from nicotine-induced desensitization through a mechanism involving local cAMP signaling. We also found that doses of nicotine that stimulate the mHb increased blood glucose levels in rodents, and that repeated exposure to this effect precipitated diabetes-like abnormalities in blood glucose homeostasis. *Tcf7l2*-deficient rats were resistant to the diabetes-related actions of nicotine. Relapse rates are remarkably high in tobacco smokers attempting to quit, particularly during early stages of withdrawal when craving is most intense. The role of the mHb in nicotine craving and relapse-related behaviors is unknown. We found that neurons in the triangular nucleus of the septum (TNS) provide excitatory input to the mHb and that nicotine profoundly decreased activity of the TNS-mHb circuit. Doses of nicotine that disrupt TNS-habenula communication triggered intense craving-like nicotine-seeking during withdrawal. Chemogenetic stimulation of the TNS-mHb circuit attenuated, whereas inhibition of this circuit enhanced, withdrawal-induced craving. These findings suggest that stimulatory actions of on a mHb-pancreas axis links the addictive properties of nicotine to its diabetes-prompting actions, and that perturbations in septal communication with the mHb contributes nicotine craving and relapse.

# Special Lecture 4

(March 19, 11:00～12:00, Hall 1)

## SL4

### Spying on dopamine modulation by developing next-generation GRAB sensors

Yulong Li (*School of Life Sciences, PKU-IDG/McGovern Institute for Brain Research, PKU-THU Center for Life Sciences, Peking University, Beijing, P.R.China*)

Dopamine (DA) is a central monoamine neurotransmitter involved in many physiological and pathological processes. A longstanding yet largely unmet goal is to measure DA changes reliably and specifically with high spatiotemporal precision, particularly in animals executing complex behaviors. We very recently reported the development of genetically encoded GPCR-activation-based-DA (GRAB-$_{DA}$) sensors that enable these measurements. In response to extracellular DA, GRAB-$_{DA}$ sensors exhibit large fluorescence increases with subcellular resolution, subsecond kinetics, nanomolar to submicromolar affinities, and excellent molecular specificity. GRAB-$_{DA}$ sensors can resolve a single-electrical-stimulus-evoked DA release in mouse brain slices and detect endogenous DA release in living flies, fish, and mice. In freely behaving mice, GRAB-$_{DA}$ sensors readily report optogenetically elicited nigrostriatal DA release and depict dynamic mesoaccumbens DA signaling during Pavlovian conditioning or during sexual behaviors. By tuning the residues in GFP and GPCRs, we now have developed a second generation of green GRAB-$_{DA}$ sensors, with over 2-4 fold larger fluorescence responses, 2-5 fold maximum brightness, up to 10-100 fold higher molecular selectivity (over norepinephrine), more affinity ranges and distinct pharmacological properties. In parallel, we also generated promising red GRAB-$_{DA}$ sensor candidates with similar affinity as green ones. The red sensors are capable of reporting DA dynamics in vivo in both flies and in rodents. The new generation dopamine sensors along with other GRAB sensors provide powerful tools to unravel the *in-vivo* dynamics of critical neuromodulators for diverse model systems in physiological and pathophysiological conditions.

# Special Lecture 5

(March 19, 11:00～12:00, Hall 2)

## SL5

### Calcium signaling: Imaging and functional analyses

Masamitsu Iino (*Division of Cellular and Molecular Pharmacology, Nihon University School of Medicine*)

Intracellular $Ca^{2+}$ signals regulate numerous physiological and pathophysiological functions throughout our body. The advent of fluorescent $Ca^{2+}$ indicators has allowed us to visualize dynamic spatiotemporal changes in the intracellular $Ca^{2+}$ concentration. Being intrigued by the spatiotemporal $Ca^{2+}$ dynamics, we looked into the basic mechanisms underlying those $Ca^{2+}$ dynamics, and clarified that regenerative $Ca^{2+}$ release mechanisms underlie such $Ca^{2+}$ signals as $Ca^{2+}$ waves and oscillations. Furthermore, $Ca^{2+}$ oscillations are found to be an efficient signaling mechanism minimizing the load to the cells. Based on our molecular understanding of the $Ca^{2+}$ signaling mechanisms, we have been searching for new cellular functions that are regulated by $Ca^{2+}$ signals, developing and utilizing powerful imaging methods both in vitro and in vivo. Our efforts have identified critical roles of $Ca^{2+}$ signaling in the central nervous system in reaction to insult to the brain. Our research strategy can be applied to various organs other than the brain.

# Special Lecture 6

(March 19, 11:00～12:00, Hall 3)

## SL6

### Recovery from Ischemic Brain Injury by Innate and Adaptive Immunity

Minako Ito, Akihiko Yoshimura (*Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo, Japan*)

Stroke including brain ischemia is one of the major causes of death and disability worldwide. Post-ischemic inflammation is an essential step not only for the progression of ischemic brain injury, but also for neurological repair. In a mouse stroke model, we have reported that IL-1$\beta$, IL-23 and IL-17 play essential roles in infarct volume growth within 1-4 days after brain ischemic stroke model [1, 2]. Extracellular DAMPs (damage-associated molecular patterns) including Peroxiredoxins (Prxs) released from dead cells activate infiltrated macrophages on day 1 through a Toll-like receptor 2 (TLR2) and TLR4 -dependent mechanism, then stimulate IL-1$\beta$ and IL-23 production [2, 3]. Then these inflammatory cytokines induce IL-17 from $\gamma\delta$ T cells, which exacerbates ischemic brain damage on day 3-4 [1]. After day 3, DAMPs are cleared by macrophages through type A scavenger receptors, which is an important process for the resolution of brain inflammation [4]. Such neuroprotective macrophages produce less inflammatory cytokines, but higher amout of tropic factors like IGF-1. At the chronic phase after a stroke (>14 days), a massive accumulation of T cells, especially Tregs, occurs in the brain [5]. Tregs regulates astroglyosis and reduce neural damages. Gene expression analysis revealed that brain Tregs related to Tregs in other tissues such as adipose tissue and muscle, however, brain Tregs are apparently different from them and express several unique genes related to the nerve system including serotonin receptor *Htr7*. Brain Our findings suggest that macrophages and Tregs, and their products may provide new therapeutic opportunities for neuronal protection against stroke.

References
1) Shichita T, et al. ***Nature Med***. 2009; 15(8): 946-950.
2) Ito M, et al. ***Nat Commun.*** 2015; 6: 7360.
3) Shichita T, et al. ***Nature Med***. 2012; 18(6): 911-917.
4) Shichita T, et al. ***Nature Med***. 2017; 23(6): 723-732.
5) Ito et al. ***Nature***. 2019; 565(7738): 246-250.

# The Sunao Tawara Memorial Lecture

(March 17, 9:30～10:30, Hall 1)

## ML1

### Heart failure as cardiac maladaptation to mechanical stress

Issei Komuro (*Department of Cardiovascular Medicine, The University of Tokyo Graduate School of Medicine*)

Pressure overload induces cardiac hypertrophy by activating many molecules such as integrin and angiotensin II type1 receptor, and at first, cardiac function is maintained normal by promoting vascular growth in the heart through hypoxia-inducible factor-1 (Hif-1)-dependent induction of angiogenic factors. Sustained pressure overload, however, induces an accumulation of p53 that inhibits Hif-1 activity and thereby impairs cardiac angiogenesis and systolic function. We have recently found that three miRNAs, which are targets of p53, are upregulated in blood of patients showing heart failure within 1 year after myocardial infarction. Through network analysis of single-cardiomyocyte transcriptomes, we have recently identified gene-modules that classify cardiomyocytes and regulate a trajectory of cardiomyocyte remodeling. Upon pressure overload, almost all cardiomyocytes activated mitochondrial ribosome/metabolism modules, whose activity was correlated with the extent of morphological hypertrophy. Sustained stimuli activated DNA damage signaling module including p53 targets in a part of hypertrophy-stage cardiomyocytes, which induced remodeling into failing cardiomyocytes and resulted in heart failure. To know how pressure overload induces expressions of p53, we examined whether pressure overload induces DNA damage in cardiomyocytes. Comet assay revealed that pressure overload on murine hearts induced single strand breaks in cardiomyocytes and enhanced accumulation of single strand breaks in Xrcc1 deficient mice caused heart failure. We have recently found that heart failure patients with severe DNA damage do not respond to drug treatment and show poor prognosis. There are many kinds of cell types in the heart such as cardiomyocytes, endothelial cells, smooth muscle cells and blood cells. We have recently found that pressure overload also induces an increase in resident macrophages and robust changes of gene expressions of macrophages in the heart. Depletion of the macrophages easily induced cardiac dysfunction and we have recently identified the critical molecules and its molecular mechanisms.

# The Makoto Arita Memorial Lecture

(March 18, 9:30～10:30, Hall 1)

## ML2

### Current status of the clinical application of regenerative medicine of the heart using iPS cells

Keiichi Fukuda (*Department of Cardiology, Keio University School of Medicine*)

Although heart transplantation can drastically improve the survival, shortage of the donor heart is a serious problem. The regenerative medicine of the failing heart had been long awaited. To address this question, we used human HLA haplotype homo-iPS cells, which matches to approximately 20% of the Japanese population, to generate ventricular cardiomyocytes. We performed transcriptome of the metabolic enzymes and fluxome analysis using $^{13}$glucose and 13lactic acid on both ES/iPS cells and cardiomyocytes, and found that their metabolic pathways were completely different. Moreover, amino acid consumption analysis and metabolome analysis revealed that glutamine is another important energy source for the iPS cells. Based on these findings, we could purify the cardiomyocytes with more than 99% purity. The transplanted cardiomyocytes did not make teratoma formation in immuno-deficient NOG mice skin and heart. We transplanted the aggregate (spheroid) cardiomyocytes using our newly developed device. The transplanted cardiomyocytes could survive in the heart for the long period, showed physiological cell hypertrophy after transplantation, and could improve cardiac function due to myocardial infarction. We are now planning to examine the first in human clinical trial to transplant the human regenerated cardiomyocytes to the patients with HLA-6 class matched dilated cardiomyopathy in the near future.

# The Susumu Hagiwara Memorial Lecture

(March 19, 9:30～10:30, Hall 1)

## ML3

### Monoamine and mechanical control of dendritic-spine synapses and psychiatric disorders

Haruo Kasai (*Graduate School of Medicine, The University of Tokyo*)

Most excitatory synapses in the cerebral cortex are formed on small appendages of dendrites called dendritic spines. The spine synapses exhibit structural plasticity in that glutamate and gamma-butyric acid competitively regulate confined spine enlargement and spreading shrinkage, respectively, resulting in synaptic competition to select the efficient synapses by cytosolic $Ca^{2+}$ signaling. We recently demonstrated that dopamine acts on the dendritic spines in the nucleus accumbens. We found that dopamine D1 receptors (D1Rs) sensitively detect the burst of dopamine neuron firing via cytosolic cAMP signaling and play a key role in reward conditioning, with marked stimulus generalization in awake mice. In contrast, D2Rs detect the short dip of dopamine firing and are involved in discrimination learning, but not in extinction. Interestingly, repeated methamphetamine treatment, in a model for psychosis, hindered the discrimination learning and a D2R antagonist restored it by enhancement of the plasticity. These findings suggest a novel hypothesis that psychotic symptoms might arise due to impairment in discrimination learning mediated by D2Rs, which results in overgeneralization. In addition to the chemical signaling, we found that the spine enlargement causes mechanical effects on the presynaptic terminals to facilitate the evoked neurotransmitter release in a presynaptic F-actin- and SNARE-dependent manner in hippocampal slice cultures. This novel mechano-sensing mechanism (actARE) is independent of cytosolic $Ca^{2+}$ and cAMP, and is likely caused by force-mediated assembly of SNAREs. Thus, spine synapses are capable of "mechanical" transmission to facilitate the electro-chemical learning processes in the brain.

# Planned Symposia

# Planned Symposium 1

## Japan-Canada Joint Symposium

## Cutting-edge approaches to the functioning mechanisms and pathophysiology of ion channels

(March 17, 9:00～10:50, Hall 2)

### PS01-01

#### Functional characterization of mutations identified in skeletal muscle channelopathy in Japan

Tomoya Kubota (*Dept Biomedical Infomatics, Grad Sch Med, Osaka Univ, Japan*)

Skeletal muscle channelopathy is one of the rare diseases which causes abnormal excitability of the sarcolemma. Most patients feel impairment of their quality of life due to myotonia and/or paralysis. In addition, some patients have a life-threatening symptom; cardiac arrythmia in Andersen-Tawil syndrome, apnea and laryngospasm in infant with myotonia, and so on. Since the discovery of the causative genes including CLCN1, SCN4A, CACNA1S and KCNJ2, functional analysis of mutant channels has revealed mechanism of the abnormal membrane excitability and provided better understanding of structural-functional relationship of protein and occasionally novel physiological role of ion channels. Since 2005, we have been conducting genetic analysis of skeletal muscle channelopathies referred from all over Japan and performed in vitro functional analysis of the mutant channels. More than 200 patients were analyzed with Sanger sequencing and 20 patients with target resequencing or exome sequencing. We have confirmed the genetic diagnosis for more than 150 patients (74 %). Among them, we have identified rare, but scientifically significant cases. In this symposium, I am reviewing skeletal muscle channelopathy cases identified in Japan and discussing the scientific relevancy. Our data of the skeletal muscle channelopathy in Japan provided important insights to the pathogenesis in both genetic and biophysical aspects of ion channels expressing in skeletal muscle. (COI:No)

### PS01-02

#### Modulation of the voltage sensor domains of KCNQ1 channels by KCNE subunits

Koichi Nakajo (*Div Integr Physiol, Sch Med, Jichi Med Univ, Japan*)

KCNQ1 channel is a voltage-gated potassium channel, which is widely expressed in the human body including heart, kidney, intestine, inner ear and pancreas. The gating behavior of the channel is uniquely regulated by a single transmembrane protein called KCNE. There are five members of KCNE proteins in the human genome. KCNE1, for example, slows gating kinetics (activation and deactivation) of KCNQ1 and thus underlies the slow $K^+$ current ($I_{Ks}$) in the heart. KCNE3 (and maybe KCNE2) make the KCNQ1 channel constitutively active (open). KCNE4 simply inhibits the channel. The properties of KCNQ1 are hugely dependent on which type of KCNE proteins are co-expressed in the tissue or organ. On the other hand, it is still an open question of why the modulations of the KCNQ1 channel by similar KCNE proteins are so diverse. Recent applications of voltage-clamp fluorometry (VCF) to KCNQ1 channels suggest the voltage-sensing domains (VSD) of KCNQ1 channel are stabilized at a certain state: KCNE1 stabilizes VSD of KCNQ1 at the intermediate state (between the down state and the up state) while KCNE3 stabilizes it at the up state to keep the channel open (Barro-Soria et al., 2014 & 2015). The next question now could be how the KCNE proteins stabilize the VSD at a certain state. We recently isolated the zebrafish orthologs of KCNQ1 (zKCNQ1) and three KCNE genes (zKNCE1, zKCNE3, and zKNCE4). While zKCNE1 and zKCNE4 modulated zKCNQ1 as well as human counterparts, zKCNE3 failed to make zKCNQ1 constitutively active. We next compared the amino acid sequences between mammalian KCNE3 and zKCNE3 and identified the second half of the transmembrane region of KCNE3 was required to make the channel constitutively active. This finding could be a good starting point to understand the molecular mechanism of how the KCNE proteins stabilize the VSD of KCNQ1. (COI:No)

### PS01-03

#### Stepwise activation of KCNQ1 and KCNQ1+KCNE1 channel complexes

David Fedida, Maartje Westhoff, Jodene Eldstrom (*Life Sciences Institute, University of British Columbia, Vancouver, B.C., Canada*)

The IKs potassium current is important in regulating the heartbeat as it activates during the late plateau phase of cardiac systole, and provides a repolarization reserve at times of higher heart rate to support increased cardiac output. The underlying KCNQ1 tetramers have four voltage-sensitive activating domains (VSs), a pore that opens to allow current to pass through, and they co-assemble with 1-4 KCNE1 accessory subunits. However, how these components together gate the IKs complex to open the pore is controversial. The number of activated VSs required to allow pore opening has been debated extensively, and currently, either a concerted movement involving all four tetramer subunits, or allosteric regulation of open probability through sequential activation of individual VSs is thought to precede opening.

By using the locking E160R mutation in KCNQ1 to prevent VSs from moving into activated conformations, and tracking VS movement, via MTSET modification and fluorescence recordings, we show that E160R-containing VS do not translocate to be detected externally upon depolarition. E160R, expressed in all four KCNQ1 subunits, produces non-conducting channel complexes, but if one, two, or three VS contain the E160R mutation, whole cell and single channel currents are still observed in both the presence and absence of KCNE1, and conductance is reduced proportionally with the number of E160R VSs. A model of independent VS movement, incorporating intermediate and activated states directly coupled to open states can simulate experimental changes in IKs current kinetics, including changes in limiting slope conductance, depolarization of the G-V, and tail current acceleration as the number of non-activatable E160R subunits is increased.

We conclude that KCNQ1 + KCNE1 channels gate like KCNQ1 alone, with movement of each VS component resulting in increasingly more current in a step-wise manner. This makes for a uniquely flexible channel complex, tunable for a broad range of responses. (COI:No)

### PS01-04

#### Molecular Dynamics Experiments of Reconstituted Potassium Channels

Shigetoshi Oiki (*Biomed. Imag. Res. Centr., Univ. Fukui, Japan*)

The KcsA channel is a prototypical potassium channel, and structure-function relationships have been examined extensively. The pore domain is shared with a superfamily of the tetrameric cation channel, and the structure of the selectivity filter is well conserved in potassium channels. Thus, elucidating molecular mechanisms of the KcsA channel would help to understand other types of potassium channels. We have studied gating and ion permeation properties of the KcsA channel using lipid bilayer techniques, by which lipid compositions and electrolyte solutions of both sides of the membrane are readily controlled. Here we examine $K^+$ permeation through the KcsA channel using lipid bilayer experiments and the molecular dynamics simulation. For ion permeation through channels, the water flux accompanying permeating ions is crucially important, which can be measured electrophysiologically by the streaming potential. The water-ion flux ratio ($J_{water}/J_{ion}$ or $CR_{w\text{-}i}$) for various potassium channels was 1 at high $K^+$ concentration, which is in accordance with the alternating array mechanism via transitions between w-i-w-i and i-w-i-w (w: water, i: ion) configurations. On the other hand, we found that the $CR_{w\text{-}i}$ value gradually increased over 2 at low $K^+$ concentrations, which cannot be explained by the conventional mechanism. Molecular dynamics simulation of the KcsA channel was performed at different $K^+$ concentrations, and the $CR_{w\text{-}i}$ value was reproduced, warranting further examination. Ion trajectories along the pore were analyzed using the event-oriented analysis method, which provides relative positional distributions of ions at a given event such as an ion entering the filter (filter-in). We found that the outermost $K^+$ in the selectivity filter escaped readily towards the extracellular space before a coming ion entered the filter, rendering only one $K^+$ ion left in the selectivity filter with more water molecules. Based on these results we provide a queueing mechanism for $K^+$ permeation through potassium channels. (COI:No)

# Planned Symposium 2

## Neuro-immune communication and homeostatic responses

(March 17, 9:00～10:50, Hall 3)

### PS02-01

#### C-type lectin Mincle mediates cell death-triggered sustained inflammation

Miyako Tanaka[1], Yoshihiro Ogawa[2], Takayoshi Suganami[1] (*[1]Dep of Mol Med and Metab, RIEM, Nagoya Univ, Japan, [2]Dep of Med and Bioregulatory Sci, Grad Sch of Med Sci, Kyushu Univ, Japan*)

Accumulating evidence has suggested that chronic sterile inflammation, which contributes to the pathogenesis of obesity and diabetes, is formed by various factors, such as cell-cell interactions, a variety of cytokines, and innervation. Since the innervation of macrophage activity had been reported, neuro-immune communication and sterile inflammation have been paid attention to. We have already elucidated that leptin, a representative hormone derived from adipocytes, regulates peripheral sterile inflammation through central nervous system. We have also showed that central leptin signaling controls the development of B lymphocytes in bone marrow. On the other hand, we have focused on the chronic sterile inflammation, especially the microenvironment in obese adipose tissue and revealed that hypertrophied adipocytes and macrophages form the inflammatory vicious cycles resulted in adipose tissue inflammation. We have screened and identified Mincle, macrophage-inducible C-type lectin, as a new inflammatory molecule on macrophages that contribute the adipose tissue inflammation. Mincle is a pathogen sensor for *Mycobacterium tuberculosis*, but Mincle also can sense cell death indicating that its involvement in sterile inflammation. Mincle expression was observed in the macrophages which infiltrated into obese adipose tissue and constructed the unique structures termed crown-like structures (CLSs). It is considered that macrophages receive danger signals from dead adipocytes and adipocyte–macrophage crosstalk may occur in close proximity in the CLSs, so CLSs are hallmarks of adipose tissue inflammation. Our data suggests that Mincle recognizes currently unknown endogenous Mincle ligands from dead adipocytes and is activated to produce cytokines and chemokines resulted in adipose tissue fibrosis and ectopic lipid accumulation. Next, we examined the role of Mincle in acute sterile inflammation using acute kidney injury model. In this symposium, we will provide our up-to-date results and discuss the dyshomeostasis caused by cell-cell interaction in the microenvironment. (COI:No)

### PS02-02

#### Neuro-immune axis on kidney disease

Tsuyoshi Inoue (*Div CKD Pathophysiol, Grad Sch Med, Univ of Tokyo, Japan*)

The kidney is a highly developed organ, and has various functions such as regulation of fluid and electrolyte, regulation of blood pressure, production of erythropoietin, and activation of vitamin D. Progression to kidney disease is caused by various causes such as diabetes and hypertension, hereditary disease, and glomerulonephritis characterized by urine protein. However, at present, there is currently no drug for chronic kidney disease other than angiotensin II receptor antagonist (ARB), which has an inhibitory effect on the progression of kidney disease. Some immune cells have receptors for neurotransmitters and the existence of immune cells that produce neurotransmitters such as acetylcholine has been clarified, and the mechanism of immune system regulation via the nervous system is being elucidated. Among them, anti-inflammatory reflex through vagus nerve are well-studied. In fact, electrical vagus nerve stimulation (VNS) has been shown in animal experiments to ameliorate various diseases such as myocardial infarction, pancreatitis and sepsis. In addition, the effectiveness of the implantable vagus nerve stimulator has been confirmed in Crohn's disease and rheumatoid arthritis patients in pilot studies.

We have so far showed the following. 1) VNS protected the kidney from acute kidney injury. 2) $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$nAChR) positive macrophages and $\beta 2$ adrenergic receptor positive CD4 T cells play an important role in exerting the renal protection effect by VNS. 3) We newly identified Hes1 (hairy and enhancer of split-1) as downstream gene of $\alpha 7$nAChR by RNA-seq and functional analysis of genes. 4) It was discovered that C1 neuron stimulation in the medulla by optogenetics had a renoprotective effect. Thus, as the elucidation of the renal protective effect through the neuro-immune system is gradually progressing, the development of further research on the neuro-immune-renal linkage is expected to lead to a new therapeutic option for inflammation-related disorders including kidney diseases. (COI:Properly Declared)

### PS02-03

#### Vagal regulation of hepatic metabolic and inflammatory response

Hiroshi Inoue[1,2], Yuka Inaba[1], Emi Hashiuchi[2] (*[1]InFIniti, Kanazawa Univ, japan, [2]Graduate School of Medical Sciences, Kanazawa Unv, Japan*)

The vagus nerve plays an essential role in the organ-crosstalk between the brain and peripheral organs, including the liver. Indeed, The hypothalamus detects whole-body energetic status and regulates hepatic glucose metabolism through the vagus nerve. Indeed, hypothalamic sensing of increases in plasma levels of nutrients and hormones results in the suppression of the vagal activity, which in turn decreases hepatic glucose production (HGP) through alpha 7 nicotinic acetylcholine receptor (A7nAchR) in Kupffer cells. In detail, activation of A7nAchR suppresses inflammatory cytokines expression in Kupffer cells, including IL-6. The acute suppression of hepatic vagal activity releases Kupffer cells from A7nAchR-dependent suppression of inflammatory cytokines, resulting in the IL-6 increase from Kupffer cells, followed by HGP suppression through hepatic STAT3 activation. These suggest that the brain and vagus nerve regulate hepatic metabolism by their control of hepatic inflammatory cells.

Obesity and insulin-resistance impairs hypothalamic sensing and reduces the fluctuation of vagus nerve activity according to energetic status. In obese mice, the failure of vagal response to energetic status results in the smoldering activation of Kupffer cells and blunting of acute activation of hepatic IL-6/STAT3 and of suppression of HGP. Furthermore, obesity-induced failure of vagal control of Kupffer cell can lead to hepatic chronic inflammation. Indeed, A7nAchR knockout mice reveal nonalcoholic steatohepatitis induced by an atherogenic-diet. These findings may indicate that autonomic nerves, inflammatory cells and metabolic cells interact with each other to maintain metabolic and inflammatory homeostasis. (COI:No)

### PS02-04

#### Sterile inflammation and neural repair after ischemic stroke

Takashi Shichita (*Stroke Renaissance Project, Tokyo Metro Inst Med*)

Inflammation is an essential step for the pathology of ischemic stroke. However, its importance in the process of neural repair after ischemic brain injury has not been clarified. Inflammation after ischemic brain injury is triggered by the extracellularly released DAMPs (damage-associated molecular patterns) from necrotic brain cells. High mobility group box 1 (HMGB1) and peroxiredoxin (PRX) have been identified as DAMPs in the ischemic brain. Additionally, we recently identified DJ-1 as previously unknown DAMPs. HMGB1 exaggerates the disruption of blood brain barrier; on the other hand, PRX and DJ-1 directly activate infiltrating immune cells through Toll-like receptor 2 (TLR2) and TLR4 to induce the production of inflammatory cytokines.

These inflammatory DAMPs are removed from ischemic brain through scavenger receptors, MSR1 and MARCO, which are expressed by microglia and infiltrating macrophages. MSR1 expression levels in these mononuclear phagocytes increase from day 1 to day 3 after stroke onset. These MSR1-high mononuclear phagocytes efficiently remove DAMPs and promote neural repair by producing IGF-1, a neurotrophic factor. Sterile inflammation after ischemic injury will be important for the induction and accumulation of reparative immune cells at the injured brain region. Novel therapeutic method for ischemic stroke will be developed by clarifying the detailed molecular mechanisms in the reparative process after ischemic stroke. (COI:No)

### PS02-05

#### Intravital imaging dissecting inflammatory cell dynamics in vivo

Masaru Ishii (*Dept Immunol Cell Biol, Grad Sch Med, Osaka Univ, Japan*)

During the last decade, intravital optical microscopy has launched a new trend in the field of biology. By using this advanced imaging technique we have established a new system for visualizing in situ behavior of a diversity of living cells within intact tissues and organs. Among them, we succeeded in visualizing the various dynamic phenomena within bones and joints, where various kinds of immune cells are produced and functioning although poorly analyzed by conventional methodology such as histological analyses with decalcified sections. We have so far identified the real modes of migration, differentiation and function of bone-destroying osteoclasts, special kind of macrophages responsible for bone and joint erosions. In this presentation I will present the recent update on intravital imaging studies on immune and other systems for clarifying in vivo behaviors of cell and tissue dynamics. (COI:No)

# Planned Symposium 3

## Integration of sensory and metabolic signals by neuropeptide in hypothalamus (navigates metabolic state.)

(March 17, 9:00～10:50, Hall 4)

### PS03-01

#### Physiological function of NPGL in feeding behavior and energy metabolism

Kenshiro Shikano (*Dept Neurophysiol, Fac Med, Oita Univ, Japan*)

Hypothalamus is the center of feeding behavior and energy metabolism, and several hypothalamic neural substrates regulate energy metabolism. However, the mechanism of feeding behavior has not yet totally elucidated. We recently found a novel secretory protein of 80 amino acids and named it neurosecretory protein GL (NPGL). *Npgl* mRNA expression was upregulated by fasting and low insulin levels, and NPGL neurons were responded to insulin. These data suggest that NPGL regulates energy homeostasis. To investigate the function of NPGL, we performed protein administration and gene overexpression using wild-type rats. These manipulations showed that NPGL increased lipid accumulation in white adipose tissue (WAT) without remarkable changes in food intake. This adiposity was associated with an induction of de novo lipogenesis in WAT but not in liver. NPGL did not change the food intake under the normal chow but increased under the high calorie diets. The food selection test was conducted because food preference for palatable food was observed by NPGL treatment. NPGL selectively induced carbohydrate intake. These results show NPGL as a novel neuronal regulator that drives feeding behavior and fat deposition through de novo lipogenesis in WAT, and acts to maintain steady fatty level. In the future, the findings of NPGL will reveal the novel mechanisms of feeding regulation related to lipid metabolism. (COI:No)

### PS03-02

#### Neural mechanisms of odor-induced feeding behavior

Koshi Murata (*Div Brain Stru Func, Faculty Med Sci, Univ Fukui*)

Smell of food can induce appetite and motivated behavior. However, neural mechanisms of odor-induced feeding behavior are still unclear. We recently found two brain regions which are supposedly involved in feeding behaviors. One region is the olfactory tubercle (OT). The OT is part of the olfactory cortex as well as the ventral striatum, which receives olfactory and dopaminergic inputs. Learned odor-induced food seeking behaviors accompanied activation of dopamine receptor D1-expressing neurons in the anteromedial domain of the OT. Manipulation of the D1 neurons in the anteromedial OT by optogenetics and chemogenetics induced attractive behavior and feeding behavior. The other region is a newly identified part of the olfactory cortex that send axons to the lateral hypothalamus. We named this area as the ventral olfactory nucleus (VON) because it is located beneath the anterior olfactory nucleus. In this talk, I will introduce our results and discuss how the OT and VON control feeding behavior. (COI:No)

### PS03-03

#### Visual and endocrine systems mediating green-light effect on fish growth

Akiyoshi Takahashi[1], Daisuke Shimizu[2], Satoshi Kasagi[1], Kanta Mizusawa[1]
([1]*Sch Marine Biosci, Kitasato Univ, Japan,* [2]*FRA Tohoku Natl Fish Res Inst, Japan*)

Biological processes of living organisms are affected by environmental photic conditions. Body color changes adapting individuals to environmental color and hue for camouflage is one of the prominent functions observed in bony fish. We recently found that green LED light (peak wavelength 518 nm) stimulated the growth of flatfishes. The potency was greater than that of blue (464 nm), blue-green (497 nm), red (635 nm), and white (447 nm and 550 nm). Green light increased total length, body weight, and food intake of flounders. It is of interest that green light improved food conversion efficiency. We investigated the roles of the visual and endocrine systems to clarify pathways evoked by green light. In the eyes of the flounder, several types of opsins were expressed. These were one ultraviolet opsin, one blue opsin, one blue-green opsin, two or three green opsins, one red opsin, and one rhodopsin, suggesting that flounders can perceive green light sensitively. In the brain, exposure to green light caused changes in the expression of melanin-concentrating hormone (MCH) and agouti-related protein (AGRP) genes. Thus, these neuropeptide hormones are suggested to be associated with food intake under green light. In the pituitary, the expression of the somatolactin (SL) gene, which belongs to the growth hormone family and is present only in bony fish, was changed with green light. SL is suggested to participate in lipid metabolism under green light. Interestingly, MCH, AGRP, and SL are also representative hormones regarding body color regulation in bony fish. Moreover, association of these hormones with food intake or appetite control is probably common in bony fish. Here, we suggest that these are key hormones under green light in flatfishes. (COI:No)

### PS03-04

#### Homeostatic coordination via alteration of hypothalamic energy sensor by feeding time

Tetsuya Shiuchi (*Dept. Integ Physiol, Tokushima Univ Grad School, Japan*)

Energy homeostasis is coordinated by hypothalamus, which is central commander for autonomic nervous and endocrine system. Neuron in hypothalamus senses blood substrate levels such as glucose and leptin, thereby regulating feeding behavior and energy metabolism. However, it had not elucidated how feeding time influence hypothalamic sensor and navigate coordination of energy homeostasis via neuropeptide function. We separated C57BL/6J mice for 3 feeding scheduled groups; given lab chows freely during dark phase (ZT12-24, Ad-lib group), first 4-hour in dark phase (ZT12-16; Morning group), and last 4-hour in dark phase (ZT20-24, Evening group). Mice in Evening group showed lower whole-body insulin sensitivity and energy expenditure despite the smaller food intake than that of Ad-lib group while these parameters in Morning group were normal. We observed that higher lipid accumulation, increased gene expression of fatty acid synthesis, decreased fatty acid oxidation and impaired insulin signals in skeletal muscle in Evening group compared to other groups. Encompassing analysis revealed that mRNA expression of agouti-related protein (AgRP) was increased in hypothalamus in Evening group. Inhibition of central AgRP expression by antisense oligo did not alter insulin sensitivity in whole body and skeletal muscle of Evening group. We further observed possibilities that glucocorticoid system is a candidate for stimulation of AgRP expression in Evening group, and that clock time-altered liver by feeding time may control hypothalamic glucocorticoid sensor by hepatic afferent nerve. These findings suggest that feeding time may influence hypothalamic neuronal sensor for glucocorticoid, thereby navigating physiological energy homeostasis mediated by hypothalamic neuropeptides such as AgRP. (COI:No)

# Planned Symposium 4

## The metabolic modulation by sensory input from peripheral organs

(March 17, 9:00～10:50, Hall 6)

### PS04-01

#### Analysis of gastro-intestinal autonomic afferents in the mouse dietary obesity

Mamoru Tanida (*Dept Physiol [2], Kanazawa Med Univ, Japan*)

The high fat diet (HFD) generates obesity which arises from hyperphagia and decreased metabolism. Recently, it has been shown that afferent vagal signals in the abdominal organs sense to nutritional and hormonal stimulations, sending the information to the brain for regulation of appetite and metabolism. For example, CCK, feeding suppressor hormone in the intestine, stimulates afferent vagal nerve signals in the anesthetized rats. In the preset study, we used HFD-induced obese mice and examined effects of CCK or leptin, hypophasic hormone in the adipose tissue on afferent vagal nerve activities in the gastro-intestinal organs in the anesthetized mice. Intravenous injection of not only CCK, but also of leptin stimulated afferent vagal nerve activities in the gastric branch, celiac branch and hepatic branch. On the other hand, in the HFD mice, afferent gastric vagal nerve responses to the CCK and leptin were attenuated, but stimulatory responses of hepatic and celiac afferents were reserved. In addition, CCK receptor A mRNA and leptin receptor (Ob-Rb) mRNA of nodose ganglion in the HFD mice did not change significantly. These data suggest that disruption of receptors function but not receptors expression in vagal afferent pathway may be involved in attenuation of responses of vagal afferents to feeding-related hormones in the HFD mice. (COI:No)

### PS04-02

#### Thermoregulation via the vestibular system

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

The vestibular system is one of the sensory systems which contributes to the sense of balance and spatial orientation. This also participates in the autonomic nervous response, which stimulation of the peripheral vestibular organs induces sympathoexcitation. This response is observed in both rodents and human beings, and we have reported that vestibular system contributes to the arterial pressure response during postural change as one of the feedforward control system. In the other autonomic nervous responses, stimulation of the otolith organs in the inner ear induces hypothermia; exposure to the hypergravity environment decreases body temperature by 8 degree Celsius in mice. This response was attenuated by vestibular lesion or genetic deletion of otolith. In order to elucidate the central mechanism of hypergravity-induced hypothermia, we examined the role of Vglut2, Vgat and ChAT neurons in vestibular nucleus complex (VNC) on thermoregulation in mice. We used Vglut2-cre, Vgat-cre, and ChAT-cre mice to manipulate each neuron in VNC. The viral vector was injected in VNC to express photo sensors (channelrhodopsin or archaerhodopsin) for optogenetics or hM3D(Gq) for chemogenetics. Unilateral photostimulation of the Vglut2 neurons in VNC induced body tilt to the ipsilateral side, while photoinhibition induced body tilt to the contralateral side. In Vgat-cre mice, opposite response was observed compared with Vglut2-cre mouse. Photostimulation of ChAT neurons did not show any responses. Chemogenetics stimulation of Vglut2 neurons showed hypothermia with increasing in activity, while Vgat stimulation increased body temperature with decreasing in activity. Deletion of Vglut2 neurons in VNC attenuated hypothermia induced by hypergravity exposure. On the other hand, hypothermia was still observed by deletion of Vgat neurons in VNC. Interestingly, chemogenetics stimulation of Vglut2 neurons in VNC 2 days before hypergravity exposure, the hypothermia was attenuated. Taken together, hypothermia induced by hypergravity exposure is due to activation of Vglut2 neurons in VNC. (COI:No)

### PS04-03

#### Molecular Mechanisms Underlying Dietary Fatty Acids and Their Metabolites-Induced Activation of Brown Adipose Tissue Function

Tsuyoshi Goto[1,2] ([1]*Grad Sch Agric, Kyoto Univ, Japan,* [2]*C-PIER, Kyoto Univ, Japan*)

Emerging evidence suggested that the enhancement of thermogenic activity in brown adipocytes or beige adipocytes is an attractive target for the management of obesity. Several environmental stimuli, such as cold exposure and exercise, activate brown adipose tissue (BAT) function. Moreover, several food factors have also been shown to be effective stimuli to activate thermogenic BAT function.

Our recent study indicated that treatment with several dietary fatty acids and their metabolites produced by gut microbiota can activates BAT function in mice. We showed fish oil intake increased oxygen consumption and rectal temperature, with concomitant upregulation of uncoupling protein 1 (UCP1), the responsible gene for the nonshivering thermogenesis in both BAT and white adipose tissue (WAT), suggesting that fish oil intake activates BAT function in mice. Mice fed fish oil diet showed the increase in urinary catecholamine levels, suggesting that fish oil intake activates sympathetic nervous system (SNS). These effects of fish oil intake were not observed in transient receptor potential vanilloid 1 (TRPV1) deficient mice. In conclusion, fish oil intake can induce UCP1 expression in classical brown and beige adipocytes via the TRPV1-mediated activation of SNS, thereby attenuating fat accumulation and ameliorating lipid metabolism. We have also showed that KetoA [10-oxo-12(Z)-octadecenoic acid], a linoleic acid metabolite produced by gut lactic acid bacteria, can activate TRPV1-SNS-BAT pathway. Actually, dietary KetoA intake ameliorated obesity and obesity-associated metabolic disorders in WT mice but not in TRPV1 deficient mice. These findings indicated that some of dietary fatty acids and their metabolites produced by gut microbiota might contribute to the body weight management via the activation of TRPV1-SNS-BAT pathway. (COI:No)

### PS04-04

#### The modulation of thyroid functions by sensory input from pharynx

Harumi Hotta, Kaori Iimura, Harue Suzuki (*Dept Auton Neurosci, Tokyo Metropol Inst Gerontol, Tokyo, Japan*)

The thyroid gland, an endocrine organ secretes hormones regulating systemic metabolisms, receives innervation from sympathetic (cervical sympathetic trunk: CST) and parasympathetic (superior laryngeal nerve: SLN) nerves. We have recently showed that electrical stimulation of the SLNs increases thyroxine (T4) and calcitonin (CT) secretion into the thyroid venous blood. Moreover, stimulation of myelinated (presumably afferent) SLN fibers had the same effect as unmyelinated efferent SLN stimulation. From these results, we predicted reflex mechanism of hormonal secretion from the thyroid via the SLN.

The SLN includes myelinated afferent nerves conveying mechanosensory information from the pharynx and larynx. Accordingly, we applied intermittent mechanical stimuli, as during swallowing food, to the pharynx in anesthetized rats and examined the effect on hormonal secretion into thyroid venous blood. During stimulation (for a period of 6-9 min), secretion rate of T4 and CT increased two-fold, whereas that of parathormone (PTH) unchanged. The increased secretion rate was returned to the pre-stimulus control level after the end of stimulation. Such responses were similar to those during electrical stimulation of the SLNs. The hormonal secretion during pharyngeal stimulation disappeared completely after cutting the SLNs bilaterally. The pharyngeal stimulation increased parasympathetic efferent nerve activities recorded from thin thyroid nerves originating in the SLN. These activities disappeared by a ganglionic blocker (hexamethonium, intravenously).

We concluded that sensory input from the pharynx promotes hormonal secretion from the thyroid gland through reflex mediated by SLN, for both afferent and efferent paths. Considering metabolic regulating actions of thyroid hormones and calcitonin, this pharyngeal-thyroid reflex may help maintain physical and mental health, whenever we intake foods from mouth. Future studies are needed to clarify whether other stimuli (e.g. vocalization), known to activate SLN myelinated afferents, can also trigger such a reflex. (COI:No)

### PS04-05

#### GLP-1 releaser D-Allulose regulates glucose metabolism via 〈vagal afferents – brain〉 axis

Yusaku Iwasaki[1], Toshihiko Yada[2,3] ([1]*Grad Sch Life Environm Sci, Kyoto Pref Univ, Japan,* [2]*Integr Physiol, Kansai Elect Power Med Res Ins,* [3]*Syst Physiol, Grad Sc Med, Kobe Univ*)

Intestinal hormone glucagon-like peptide-1 (GLP-1) is well known as incretin hormone. However, the endogenous GLP-1 is rapidly cleaved within a few minutes. It is unclear how endogenous GLP-1 acts on insulin secretion and glucose metabolism. We identified rare sugar D-allulose (Allu) as a GLP-1 releaser, which is a non-metabolizable monosaccharide. Oral administration of Allu increases GLP-1 secretion, and thereby suppresses food intake via vagal afferents expressing GLP-1 receptor (GLP-1R) in healthy and hyperphagic obese mice (Y. Iwasaki et al. Nat. Commun. 2018). In this study, we examined whether endogenous GLP-1 release by Allu regulates glucose metabolism and insulin secretion/action via [vagal afferents – brain] axis.

Endogenous GLP-1 release by Allu robustly suppressed elevation of blood glucose in ip glucose-tolerance test (GTT) in both normal and diet-induced obese (DIO) mice. The early insulin secretion at 15 min of GTT was significantly increased but only modestly, suggesting an additional mechanism underlying the glycemic control by endogenous GLP-1. Peroral Allu enhanced insulin action in insulin tolerance test in normal and DIO mice. These effects of Allu were all blunted by GLP-1R antagonist exendin 9-39, in GLP-1R deficient mice and in mice receiving the vagal afferent denervation. Therefore, GLP-1 release by Allu promotes glucose tolerance by enhancing release and action of insulin via [vagal afferents - brain] axis. Additionally, we demonstrate that endogenous GLP-1 release by Allu effectively corrects hyperglycemia via enhancement of insulin sensitivity in a glucose-dependent manner. Therefore, GLP-1 releaser Allu that acts on vagal afferents, may provide a novel category of incretin-based medicine, with high efficacy and safety to correct hyperglycemia. (COI:Properly Declared)

# Planned Symposium 5

## Brain function for physical therapy and its physiological mechanisms

(March 17, 9:00〜10:50, Hall 10)

### PS05-01

#### Central cardiovascular control during physical exercise

Ryota Asahara[1], Kei Ishii[1], Nan Liang[2], Hidehiko Komine[1], Kanji Matsukawa[3]
(*[1]AHFRC, AIST, Tsukuba, Japan, [2]Dept Human Health Sci, Grad Sch Med, Kyoto Univ, Kyoto, Japan, [3]Dept Integ Physiol, Grad Sch Biomed and Health Sci, Hiroshima Univ, Hiroshima, Japan*)

Physical exercise requires coordination of motor control with simultaneous and concomitant controls of the cardiovascular system necessary to respond to the rapid increases in metabolic demands of the exercising muscle. Accumulating evidence suggests that a feedforward signal descending from higher brain centers (termed central command) plays an important role in mediating the cardiovascular adjustments to exercise. However, little is known regarding neural circuit(s) or region(s) of central command. Understanding the central command mechanisms would be helpful to design effective rehabilitation programs for patients with cerebro- and cardio- vascular disease. We have conducted a series of healthy human studies measuring oxygenations (as index of regional tissue blood flow) of the cerebral prefrontal cortex and skeletal muscle during voluntary exercise using near-infrared spectroscopy. The oxygenated-hemoglobin concentration in the prefrontal cortex started to increase approximately 5s prior to the onset of voluntary exercise with arbitrary start. However, such increase in the prefrontal oxygenation was absent when exercise was forced to start by cue. When comparing the muscle oxygenation responses, the increase in muscle oxygenation was greater during voluntary exercise with arbitrary start than with cued start. These results suggest that the prefrontal cortex may play a role in driving neuronal circuits controlling the cardiovascular system, which may contribute to vasodilation in the skeletal muscle. In the symposium, we would like to introduce our recent updates on 1) neural circuit(s) responsible for controlling the cardiovascular system during exercise and 2) central control of muscle blood flow during exercise and discuss future directions and clinical implications. (COI:No)

### PS05-02

#### Transcutaneous spinal direct current stimulation (tsDCS) increases corticospinal transmission and facilitates ballistic movement of leg muscles in humans

Tomofumi Yamaguchi (*Dept Physical Therapy, Yamagata Pref Univ, Japan*)

Transcutaneous spinal direct current stimulation (tsDCS) is a non-invasive neuromodulation technique that can alter excitability in spinal and supraspinal circuits in animals and humans. However, it is still unclear whether the effects of tsDCS on corticospinal transmission and motor performance. Here we investigated whether tsDCS may enhance descending activation of spinal motor neurons and facilitate voluntary ballistic activation of the leg muscles in healthy adults.

The experiment comprised five studies designed to evaluate the immediate effects of tsDCS over thoracic-lumbar on corticospinal transmission and voluntary motor output. In experiment 1 and 2, we investigated potential dose-response relationships between tsDCS stimulus intensity (experiment 1) and duration (experiment 2) on corticospinal transmission probed by transcranial magnetic stimulation (TMS). In experiment 3, we investigated the effects of tsDCS on voluntary motor functions using a ballistic force production task involving the ankle muscles. In experiment 4 and 5, we explored the system-level neural targets of tsDCS by conditioning Soleus H-reflexes with subthreshold TMS (experiment 4), and by comparing evoked potentials from TMS over the leg primary motor cortex and from electrical stimulation of the corticospinal tract at the cervicomedullary junction (experiment 5), i.e. motor evoked potentials (MEPs) and cervicomedullary motor evoked potentials (CMEPs) respectively.

Cathodal tsDCS significantly increases voluntary EMG and force production during ballistic plantarflexion. This is accompanied by increased MEPs and short-latency facilitation of the Soleus H-reflex induced by TMS, without any changes in the H-reflex or CMEPs.

This is a strong indication that cathodal tsDCS at lumbar level enhances cortical excitability and thereby promotes motor performance. These findings suggest that cathodal tsDCS may be an effective strategy to promote rehabilitation training after spinal cord injury and stroke. (COI:No)

### PS05-03

#### Neural plasticity of the somatosensory cortex that underlies central post-stroke pain

Kazuaki Nagasaka (*Institute for Human Movement and Medical Sciences, Niigata Univ of Health and Welfare, Japan*)

Central post-stroke pain (CPSP) is an intractable type of chronic pain that may occur several weeks after the onset of stroke. This pain originates in the thalamus, specifically in the ventral posterolateral nucleus (VPL). CPSP is characterized by allodynia in which normally innocuous stimuli are perceived as painful. Developing therapeutic interventions for this abnormal pain is difficult because the pathogenic mechanisms are unclear. To address this problem, we used animal models of CPSP based on the artificial stroke in the VPL, and investigated the neural plasticity underlying the development and maintenance of CPSP. Here, we describe changes in brain activity within the somatosensory cortex in rat and macaque models of CPSP. (1) Rat model: Optical imaging using voltage-sensitive dye (VSD) is a powerful tool for visualizing stimulus-dependent changes in membrane potential and for revealing the spatiotemporal patterns of neural activity. We performed optical imaging using the VSD RH-795 to investigate changes in sensory activity evoked by forelimb stimulation in a rat CPSP model. Both the peak amplitude of the VSD signal and the activation area within the somatosensory cortex significantly correlated with pain scores, which represent the severity of allodynia. (2) Macaque model: In recent years, we have established a model of CPSP using the macaque. We applied this animal model in the functional magnetic resonance imaging (fMRI) to elucidate the changes in brain activities that underlie allodynia. The fMRI analysis demonstrated that the activation of sensory cortices, including the primary and secondary somatosensory cortices, was related to the development of allodynia. These findings support the validity of the animal models for studying the plastic changes in neural functions underlying CPSP caused by a VPL stroke. (COI:No)

### PS05-04

#### Changes in corticospinal excitability by interoceptive information

Naofumi Otsuru, Hideaki Onishi (*Dept Phys Ther, Niigata Univ health and Welfare, Japan*)

Interoception refers to a sensation that develops from within the body such as visceral feelings of vasomotor activity. Studies have shown that interoceptive information (mainly investigated using heartbeat) reaches various regions of the motor cortex including the insula, anterior cingulate cortex, prefrontal cortex, and sensorimotor cortex. These studies show that interoceptive information reaches these regions within 200–600 ms of the R wave on an electrocardiogram. Therefore, studies have reported that auditory, visual, somatosensory, and nociceptive perceptions were altered by interoceptive information within this time window. However, the influence of interoceptive information on the motor system (corticospinal pathway) has not been extensively studied. Moreover, the cortical activities evoked by heartbeat varied according to individual interoceptive accuracy as evaluated using the heartbeat perception task. We investigated whether interoceptive inputs modulate corticospinal excitability within a specific time window after the R wave. We also tested whether this modulation differs between good and poor interoceptive perceivers using transcranial magnetic stimulation (TMS).

In the first experiment, we found that at 200 ms after the R wave, there was a significantly positive correlation between corticospinal excitability and interoceptive accuracy. On the other hand, there was a significant negative correlation between corticospinal excitability and interoceptive accuracy at 400 ms after the R wave. These results indicated that interoceptive information from the heartbeat reached the motor cortex.

Based on the results from the first experiment, we hypothesized that this phenomenon is used for paired associative stimulation (PAS). PAS is used for facilitating time-dependent plasticity in the motor cortex using repetitive somatosensory inputs (exteroception) combined with TMS over the motor cortex. Therefore, we investigated whether similar plasticity can be induced by combining TMS over the motor cortex with interoceptive inputs. As a result, we found that the excitability of the motor cortex may be influenced by interoceptive PAS. (COI:No)

# Planned Symposium 6

## Recent Advancement of the Multidisciplinary Approaches to the Pathophysiology Underlying Arrhythmia

(March 17, 15:20〜17:10, Hall 2)

### PS06-01

#### Experimental visualization of rotor as a mechanism of arrhythmia using ex-vivo optical mapping

Masatoshi Yamazaki (*Dept Bioeng, The University of Tokyo, Japan*)

Atrial fibrillation (AF) is the most common sustained atrial arrhythmia and affects more than 1 million patients in Japan. It is very important to establish effective therapy for patients with AF, who are increasing explosively in number, in our highly stressed society, where population aging is accelerating under tight health economic conditions in Japan. Despite of many years of research, the precise mechanisms of underlying the initiation and maintenance of AF remain poorly understood. Since about 40 years ago, many theoretical and experimental studies have suggested that spiral-wave reentry:rotor rotating around a functional obstacle, like hurricane and tornado, is the major mechanisms of AF. Recently, an attempt to treat persistent/chronic atrial fibrillation by radiofrequency ablation of rotors was proposed in patients, however, its efficacy has not been fully established. As it is difficult to detect rotors and perform rotor ablation with low-resolution (2-3 cm) electrode mapping used in clinical electrophysiological studies, it remains difficult to accurately evaluate the efficacy of rotor ablation for reverting AF to sinus rhythm. We have developed a multiple electrode mapping system with simultaneous high-resolution optical mapping system (0.1 mm), which can reliably detect rotors. Thus, the experimental models in Langendorff-perfused rabbit heart clearly demonstrated that high frequency rotors exist in arrhythmia. (COI:Properly Declared)

### PS06-02

#### Identification of cardiac connexin syndrome using whole-exome sequence and in-vitro functional assay

Taisuke Ishikawa[1], Akiko Seki[2], Naomasa Makita[1,3] ([1]*Omics Research Center, NCVC, Osaka, Japan,* [2]*Dept Cardiovascular Medicine, Tokyo Women's Medical University,* [3]*Research Institute, NCVC*)

Progressive cardiac conduction defect (PCCD) is a rare inherited arrhythmia sometimes accompanied by sudden death. Genetic causes for PCCD include mutations in cardiac sodium channel alpha subunit *SCN5A* with affecting atrioventricular (AV) and ventricular conduction on the His-Purkinje fiber. Cardiac connexins, Cx40, Cx43, and Cx45, had been suspected as candidate genes for PCCD because connexins forming gap junctions (GJ) are responsible for the intercellular permeability of ions and small molecules to transfer the rapid signaling on the cardiac conduction system. These cardiac connexins are tissue-dependently expressed in distinctive combinations and relative quantifies. Recently we identified Cx40 and Cx45 mutations, and delineated the gene-specific clinical and electrophysiological phenotypes in the familial PCCD cases.

The first inheritable mutation of cardiac connexins is Cx40-Q58L found in a PCCD family with sudden death. Cx40 is primarily expressed at the Purkinje fiber, and each mutation carriers demonstrated the impaired AV and ventricular conduction before adolescence. In vitro study revealed Cx40-Q58L impairing Cx40 assembly to form GJ. More recently, we reported the first Cx45 mutation Cx45-R75H in two unrelated families with distinct ethnicities. Afflicted individuals had progressive AV block and atrial standstill without ventricular conduction abnormalities and sudden death. In vitro study indicated that impaired intercellular permeability of Cx45-R75H affected the automaticity and conduction of sinoatrial and AV node where Cx45 is highly expressed. Patients shared extracardiac phenotypes comprising brachyfacial pattern, finger deformity, and dental dysplasia. In summary, cardiac connexin mutations confer the isoform-specific clinical phenotypes, and the identification of Cx45-R75H suggest the novel PCCD entity of conduction defects confined to atrium. (COI:No)

### PS06-03

#### iPS cell-Based Disease Modeling and Therapeutic Approach to Inherited Arrhythmias

Takeru Makiyama (*Dept Cardiovasc Med, Grad Sch Med, Kyoto Univ, Japan*)

Human induced pluripotent stem cell (hiPSC) technology is a promising tool for regeneration therapy, drug safety testing, and the investigation of pathogenic mechanisms. The use of hiPSC-derived cardiomyocytes (CMs) for disease modeling of inherited arrhythmias provides advantages over primary cells, while, hiPSC-CMs have some limitations: CMs with mixed subtypes (atrial, ventricular and nodal), immature electrophysiological characteristics such as shallow diastolic membrane potentials, automaticity even in ventricular-type CMs, etc. To overcome these limitations, we employ dynamic clamp-based approach of IK1 injection and in-silico model of hiPSC-CM for our research. In this session, I will talk about our current challenges of hiPSC-based disease modeling and novel therapeutic approaches to inherited arrhythmias.

Long-QT syndrome (LQT) is an inherited arrhythmia characterized by delayed ventricular myocardial repolarization and an increased risk for life-threatening ventricular arrhythmias and sudden cardiac death. There is no decisive medication therapy to treat LQT and the development of new therapeutic approaches is highly needed.

A missense mutation, E1115K in CACNA1C, encoding L-type Ca2+ channels (LTCCs) is located in the crucial site of Ca2+ selectivity (Nature. 1993). Recently, the mutation was reported to be associated with overlap of LQT and Brugada syndrome. Using patient-derived hiPSC-CMs, we demonstrated impaired ion selectivity of LTCC resulting in action potential prolongation and we identified that late sodium current blockers might be candidates to rescue QT prolongation in this mutation.

Recently, calmodulin is reported to be associated with severe LQT and we generated calmodulin-related LQT15-hiPSCs carrying a CALM2 mutation. LQT15-hiPSC-CMs exhibited significant lower beating rate and prolonged action potential durations. We performed gene therapy by allele-specific knockout using the CRISPR-Cas9 system and successfully rescued the electrophysiological abnormalities in the mutant allele-specific knockout CMs.

Thus, our goal is to develop a genotype-specific treatment, i.e. "Precision Medicine" that will improve the prognosis of patients with inherited arrhythmias. (COI:No)

### PS06-04

#### Mechanisms of Pathophysiology Underlying Arrhythmia Analyzed by Computer Simulations

Yukiko Himeno[1], Yosuke Okamoto[2], Hirohiko Kojitani[3], Kyoichi Ono[2], Akinori Noma[1], Akira Amano[1] ([1]*Dept Bioinformatics, Col Life Sci, Ritsumeikan Univ, Japan,* [2]*Dept Cell Physiol, Grad Sch Med, Akita Univ,* [3]*Dept Cardiovasc Med, Grad Sch Med, Kyoto Univ*)

Computer simulation is one of the powerful tools to analyze electrical activities of the excitable cells such as cardiomyocytes. By using the simulator, it is possible to understand the mechanisms of the cellular functions quantitatively in an integrative manner and test hypotheses for consistency. It is now well known that $Ca^{2+}$, as well as ionic channels and transporters, play a role in inducing irregular rhythm to trigger arrhythmia. In order to clarify the causality of the $Ca^{2+}$-induced arrhthmogenic activities of cardiomyocytes, we have developed a mathematical model of $Ca^{2+}$ releasing unit (CaRU), which represents the function of the structure of dyad consists of ryanodine receptor channels on the membrane of sarcoplasmic reticulum and L-type $Ca^{2+}$ channels on the plasma membrane or T-tubules facing to each other. In this CaRU model, various parameters such as volumes, concentrations, diffusion constants, densities and so on, are required for each $Ca^{2+}$ diffusion compartment to reproduce its stochastic behavior. In other words, by changing those parameters, the CaRU model could be successfully adopted to various kind of cell types to reproduce various physiological and pathophysiological phenomenon exhibited by the cells in electrophysiological experiments. The importance of understanding spatio-temporal regulation of local $Ca^{2+}$ concentrations will be discussed in the presentation. (COI:No)

# Planned Symposium 7

## The emerging roles of pallidal nuclei in the basal ganglia circuitry

(March 17, 15:20〜17:10, Hall 3)

### PS07-01

#### Regulation of voluntary movements by signals through the external segment of the globus pallidus

Hiromi Sano[1,2], Indriani Dwi Wahyu[1], Satomi Chiken[1,2], Atsushi Nambu[1,2]
([1]*Div System Neurophysiol, NIPS, Japan,* [2]*Dept Physiol Sci, SOKENDAI, Japan*)

The basal ganglia receive cortical inputs and play a crucial role to execute voluntary movements. The motor cortex sends information to the input station of the basal ganglia, i.e., the striatum and subthalamic nucleus (STN). The striatum is composed of two types of projection neurons, i.e., striatonigral *direct* pathway and striatopallidal *indirect* pathway neurons. Striatonigral neurons send afferents to the substantia nigra pars reticulata (SNr), while striatopallidal neurons project to the external segment of the globus pallidus (GPe). In the classical model of the basal ganglia, striatonigral and striatopallidal neurons have opposite effects on motor activity. Striatonigral neurons suppress the activity of the SNr and increase motor activity by disinhibiting thalamic and cortical activity, on the contrary, striatopallidal neurons increase the activity of the SNr through the striato-GPe-STN-SNr pathway and suppress motor activity. To elucidate the mechanism of regulation of voluntary movements, it is essential to investigate information flow along the basal ganglia circuit. We performed electrophysiological recordings to understand relation between neuronal activity of the basal ganglia and motor activity under awake state. In this symposium, we will show the relation between neuronal activities in the GPe and motor activity in the following three groups and discuss the role of the GPe on regulation of voluntary movements.
1) We selectively ablated striatopallidal neurons by applying immunotoxin-mediated cell targeting method to transgenic mice. These mice showed increased motor activity.
2) We investigated pathophysiology of L-DOPA-induced dyskinesia (LID) by applying L-DOPA to Parkinson's disease (PD) model mice. PD and LID model mice showed decreased motor activity and involuntary movements, respectively. We also examined physiological effects of an anti-parkinsonian drug on LID.
3) We investigated electrophysiology of catalepsy model mice induced by dopamine D2 receptor antagonist haloperidol. (COI:No)

### PS07-02

#### Ventral Pallidum mediates acquired aversive tastes

Tadashi Inui (*Department of Psychology and Program in Neuroscience, Florida State University*)

The ventral pallidum (VP) plays a critical role in the consumption of palatable substances. We investigated its involvement in the decrease of a palatability of a solution caused by conditioned taste aversion (CTA). The rats received a pairing of either of "sweet" saccharin sodium or "bitter" quinine hydrochloride solution as a conditioned stimulus (CS) with an intraperitoneal injection of lithium chloride as an unconditioned stimulus. They were microinjected with bicuculline (a $GABA_A$ receptor antagonist) into the VP immediately before the CS test. The drug treatment increased the intake of the saccharin CS but not quinine. Bicuculline alters the palatability of the saccharin CS from aversive to ingestive. Our study addressed the involvement and impact of endogenous GABA in the VP on CTA. We measured the extracellular GABA level before and after presenting saccharin or quinine in conditioned and non-conditioned animals. Only the conditioned rats that were given saccharin showed an elevated GABA level, suggesting that the VP GABAergic transmission signals decreased the palatability of a normally preferred sweet, but not aversive bitter, solution. We next investigated the nucleus accumbens (NAc) that densely innervates the VP, by using a manganese-enhanced magnetic resonance imaging (MRI) technique. Conditioned or non-conditioned rats were presented with either saccharin (CS) or water and microinjected with manganese chloride as an MRI enhancer and activity-dependent anterograde tracer into the NAc. The aversive CS increased manganese movements toward the VP, suggesting an augmented activity of the NAc-VP projection. Another study demonstrated that the stimulation of mu-opioid receptors in the VP attenuated aversion to the saccharin CS. Therefore, we conclude that the GABAergic and opioidergic transmissions in the VP, which may derive from the NAc, mediates learned aversive tastes and suppresses their consumption. (COI:No)

### PS07-03

#### A role for Enkephalin-expressing ventral pallidal neurons in controlling aversive Pavlovian Conditioning

Tom Macpherson[1], Hiroyuki Mizoguchi[2], Akihiro Yamanaka[2], Takatoshi Hikida[1]
([1]*Lab for Advanced Brain Functions, IPR, Osaka Univ,* [2]*RIEM, Nagoya Univ*)

The ventral pallidum (VP) is a critical component of the limbic loop of the basal ganglia, and has been implicated in the regulation of incentive motivation. However, the precise role of the VP in controlling Pavlovian conditioning of cues paired with appetitive or aversive outcomes is still unclear.
Here we used a Tet-Tag AAV virus system, in which designer receptors exclusively activated by designer drugs (DREADDs) were expressed in a population of VP neurons containing the peptide enkephalin, known to be highly expressed within the VP, to investigate the possible role of the VP in appetitive and aversive Pavlovian associative learning. During acquisition of an appetitive autoshaping task, hM3Dq DREADDs were activated by administration of CNO, leading to increased activity in enkephalin-expressing VP neurons. Mice treated with CNO did not significantly differ from saline-treated controls in their ability to acquire the task, as indicated by an increase in Pavlovian approach behavior to a reward-associated cue over the course of 6 daily sessions. However, in a passive avoidance task measuring aversive Pavlovian conditioning, administration of CNO during conditioning of an aversive foot-shock upon entering a dark chamber resulted in a decreased latency, in comparison to saline-treated controls, to enter the shock-associated chamber when tested 24 hours later.
Here, we demonstrate that activity in enkephalin-expressing VP neurons disrupts aversive, but not appetitive, Pavlovian conditioning. Thus, decreased activity in enkephalin-expressing VP neurons is necessary for aversive learning, supporting previous evidence from our group that neurotransmission blocking in nucleus accumbens D2-neurons, likely leading to disinhibition of downstream VP neurons, similarly inhibits aversive Pavlovian conditioning in a passive avoidance task. (COI:No)

### PS07-04

#### Is the external globus pallidus a simple relay of indirect pathway in the basal ganglia circuitry?

Yoshihisa Tachibana (*Div Syst Neurosci, Grad Sch Med, Kobe Univ, Japan*)

The external globus pallidum (GPe) is located at a central position of the basal ganglia (BG) circuitry and plays important roles in the physiology and pathophysiology of the BG. Classically, the GPe has been thought to be a relay station of striatal indirect pathway: the nucleus receives direct inputs from the striatum and sends outputs to the subthalamic nucleus (STN), and finally to the internal globus pallidus (GPi). However, anatomical studies demonstrate that the GPe receives reciprocal inputs from the STN. To support this idea, we have shown that electrical stimulation of the primary motor cortex induces the triphasic response (early excitation, inhibition, late excitation) in the GPe as well as the internal globus pallidum (GPi), and that the early excitation in the GPe is blocked by the muscimol inactivation of the STN or intrapallidal injection of antagonists of ionotropic glutamate receptors. This reciprocal STN-GPe transmission is also important for the generation of abnormal oscillations observed in BG neurons in the parkinsonian primate model. To investigate the GPe firing in the behavioral paradigm, we have recorded single unit activity in the GPe using a reward-biased saccade task. A half of GPe neurons show inhibitory response during saccadic behavior, which may reflect striatal excitatory response through the striato-GPe GABAergic transmission. On the other hand, the remaining GPe neurons show excitatory response, which may reflect STN excitatory response through the STN-GPe transmission. Additionally, our recent anatomical data indicate that a population of GPe neurons project back to the striatum. Taken together, the GPe may balance the cortico-STN-GPi 'braking system' and the cortico-striato-GPi 'accelerator system' through the dual reciprocal connections with the STN and striatum. (COI:No)

# Planned Symposium 8

## Systems biology of hearing: from the inner ear to the brain

(March 17, 15:20〜17:10, Hall 4)

### PS08-01

#### In vivo detection of sub-nanoscale motion of outer hair cells in guinea pig cochlea by a high performance optical coherence tomography

Fumiaki Nin (*Dept Mol Physiol, Grad Sch Med Dent, Niigata Univ, Japan*)

Sound evokes sub-nanoscale vibrations within the sensory epithelium in the mammalian cochlea. The epithelium includes not only immotile cells but also contractile outer hair cells that actively shrink and elongate in synchronization with sound frequency. Vibrometrical studies of cochlear mechanics have revealed the active vibrations in these cells. However, the real motion of outer hair cells remains uncertain due to low spatial resolution of imaging systems and low reflectivity of the cells. In this study, we dramatically improved performance of a commercial spectral-domain optical coherence tomography that can carry out vibrometry and examined the sensory epithelium of live guinea pigs. With the tomography of this system we visualized outer hair cells and separated them from other cellular components. When the animals were exposed to stimuli at moderate sound pressure, we detected that, in the hair cells, the amplitude of the vibrations on the apical edge exceeded that on the basal edge. In addition, the phase of the motion in the former region led that in the latter. These characteristics may contribute to high sensitivity and broad intensity range of normal hearing. (COI:No)

### PS08-02

#### Regulation of glutamate release by Bassoon and Otoferlin at the inner hair cell ribbon synapse

Hideki Takago (*Dept Rehab for Sensory Functions, Research Inst, National Rehab Center for Persons with Disabilities, Saitama, Japan*)

Sound encoding relies on $Ca^{2+}$-triggered exocytosis at the afferent synapse between the inner hair cell (IHC) and type I spiral ganglion neurons (SGNs). This auditory first synapse exhibits wide variations in excitatory postsynaptic current (EPSC) size and shape. The IHC contains a specialized structure called synaptic ribbon, which tethers plenty of synaptic vesicles for securely transferring continuous auditory signals. In the IHC active zones, the scaffold protein Bassoon and the hair cell-specific putative $Ca^{2+}$ sensor Otoferlin regulate synaptic ribbon formation and synaptic vesicle cycle, respectively. Here we aimed to elucidate structural and functional roles of Bassoon and Otoferlin in IHC neurotransmission. Immunohistochemical analysis revealed that most of, but not all of, Bassoon immunopuncta are coupled to synaptic ribbons, whereas those of Otoferlin are widely distributed in IHCs. Postsynaptic patch-clamp recordings showed that disruption of Bassoon or Otoferlin drastically reduced EPSC frequency during IHC depolarization. Moreover, large amplitudes of EPSCs persisted in both mutants despite lowered EPSC frequency. These results support the hair cell-specific hypothetical release mechanism (i.e. univesicular release) that a single vesicle fusion might produce EPSC size and shape diversity, and suggest that Bassoon and Otoferlin are involved in this mechanism. (COI:No)

### PS08-03

#### Regulation of ion channel expression in brainstem auditory circuit

Hiroshi Kuba (*Dept Cell Physiology, Grad Sch Med, Nagoya Univ, Japan*)

Avian nucleus magnocellularis is a homologue of mammalian anteroventral cochlear nucleus, and represents temporal information of sound with great precision for a wide frequency range. This precision is accomplished in part because the expression of voltage-gated $K^+$ channel, Kv1.1, is differentiated tonotopically within the nucleus; it increases toward higher-characteristic-frequency regions. However, the mechanisms underlying this differentiation remained elusive. We examined the development of tonotopic differentiation of Kv1.1 channel expression, using in-vivo and in-vitro preparations of chickens. Our findings were; (1) the dependence of Kv1.1 expression on auditory input got strengthened specifically in neurons tuned to higher-frequency sound at a late period of maturation, creating the difference in Kv1.1 expression among tuning frequencies; (2) attenuation of auditory input suppressed the differentiation in a level-dependent manner; (3) elevation of auditory input during earlier periods could not reproduce the differentiation; (4) the differentiation of Kv1.1 completely disappeared in the organotypic culture, in which peripheral input was totally absent; (5) treating neurons in the culture with a high K+ media augmented the expression of Kv1.1 only in those from higher-characteristic-frequency regions. Our results indicated that the capability of neurons to express Kv1.1 via auditory input develops in a cell-specific manner and directs differentiation, highlighting the importance of neuronal capability as well as the level of input in the frequency tuning of auditory circuits. (COI:No)

### PS08-04

#### Neural correlates of tinnitus in the auditory cortex of rat

Hirokazu Takahashi, Naoki Wake (*Dept Mechano-Informatics, Univ Tokyo, Japan*)

Tinnitus is the phantom perception of sound without an external source. The auditory cortex is a putative neural origin of tinnitus because it plays critical roles in subjective perception of sound. Specifically, a number of past studies demonstrated that the subjective percepts are associated with neural synchrony in the sensory cortex. Furthermore, the auditory cortex exhibits rich map plasticity depending on auditory learning and environments. Because tinnitus is usually associated with hearing loss, we hypothesize that partial hearing loss induces distortion of the tonotopic map in the auditory cortex, and this distorted map causes aberrant neural synchrony of spontaneous activity, i.e., in silence. To address our hypothesis, we attempted to quantify tinnitus percepts in rats and explore the neural correlates of tinnitus in the auditory cortex. Following unilateral exposure of 10-kHz, 125 dB-SPL tone for 60 min, behavioral tests of prepulse and gap inhibition showed that rats exhibited a sign of tinnitus of 32 kHz tones without hearing loss around 32 kHz. In these exposed rats, electrophysiological characterization in the auditory cortex demonstrated that the tonotopic map gained the areal proportion of exposed frequency of 10 kHz and that neural synchrony increased between 10 kHz and 32 kHz regions, i.e., the exposed and tinnitus frequency regions. These measures of hearing-loss-induced plasticity were significantly correlated with the behavioral index of tinnitus. Thus, the present results support our hypothesis that partial hearing loss induces the distorted tonotopic map and aberrant neural synchrony, which are possible neural correlates of tinnitus. (COI:No)

# Planned Symposium 9

## Scientific basis of the oriental medicine: mechanisms improving muscle blood flow at rest

(March 17, 15:20～17:10, Hall 9)

### PS09-01

#### Improvement of the blood flow following acupuncture and Kampo herbal medicine treatments

Masataka Sunagawa (*Dept Physiol, Sch Med, Showa Univ, Japan*)

The blood flow depends on the cardiovascular system condition and blood characteristics. For instance, the cardiac contractile force and elasticity and the inner diameter of blood vessels influence the flow as cardiovascular system conditions, and the blood coagulation ability, platelet aggregation activity, fibrinolytic activity, erythrocyte deformability and blood viscosity affect it as blood characteristics.

In Eastern medicine, the pathophysiologic concept of blood stasis is called *Oketsu*. Some Kampo medicines (Japanese traditional herbal) and acupuncture treatment are empirically known to be effective in ameliorating *Oketsu*. Crude drugs such as Peach kernel (Latin=*Persicae* Semen/Japanese=Tonin), Moutan bark (*Moutan* Cortex/Botanpi), and Rhubarb (*Rhei* Rhizoma/Daio) have the strong effect of improving *Oketsu*, while Japanese angelica root (*Angelicae Acutilobae* Radix/Toki), Cnidium rhizome (*Cnidii* Rhizoma/Senkyu), and Peony root (*Paeoniae* Radix/Shakuyaku) have a mild effect. Kampo medicines including these agents, such as Tokakujokito, Keishibukuryogan, Tokishakuyakusan, Tsudosan, Jidabokuippo, and Daiobotanpito, have been used for patients with *Oketsu*. We focused on the blood fluidity influenced by changes in blood characteristics and the platelet aggregation activity. In rats administered Tokakujokito, Keishibukuryogan, or Tokishakuyakusan, the blood fluidity improved, and the platelet aggregation activity decreased. Furthermore, in patients diagnosed with *Oketsu*-type disease, the decreased blood fluidity and exacerbated platelet aggregation activities improved following the administration of these medicines.

We next performed acupuncture treatment at relevant acupoints in rats, such as LI-14 (Chinese=Hegu/Japanese=Gokoku), SP-6 (Sanyinjiao/Saninko), and ST-36 (Zusanli/Ashi-sanri). As the result, the blood fluidity improved, and the platelet aggregation activity decreased. However, the acupoints PC-6 (Nei Guan/Naikan) and BL-23 (Shenshu/Jin-yu) interestingly showed no influence on the condition.

These findings suggest that acupuncture and Kampo medicine treatments are effective for improving the blood flow. (COI:No)

### PS09-02

#### Mechanosensing and regulation of blood flow in vascular endothelial cells

Kimiko Yamamoto[1], Joji Ando[2] ([1]*Lab System Physiol, Grad Sch Med, U Tokyo, Japan,* [2]*Lab Biomed Eng, Sch Med, Dokkyo Med U*)

The endothelial cells (ECs) that line the lumens of blood vessels are constantly exposed to a fluid dynamic force, the shear stress generated by the blood flow. The ECs sense shear stress and transduce it into intracellular biochemical signals, resulting in changes in the morphology, functions, and gene expression profiles of the cells. These EC mechanoresponses are critical for the homeostasis of the circulatory system, and their impairment can lead to the development of various vascular diseases such as hypertension, aneurysm, thrombosis, and atherosclerosis. $Ca^{2+}$ signaling via a P2 purinoceptor P2X4 plays a significant role in this mechanotransduction which leads to the regulation of vascular functions, including the control of blood pressure, blood flow-induced vasodilation, and flow-dependent vascular remodeling. The ECs release ATP in response to shear stress and then the released ATP activates P2X4, which results in flow-dependent intracellular $Ca^{2+}$ responses; however, the mechanism by which the shear stress evokes ATP release remains unclear. Here we report that the cellular mitochondria play a critical role in this process. Cultured human pulmonary artery ECs were exposed to controlled levels of shear stress in a flow-loading device, and the changes in the mitochondrial ATP levels were examined by real-time imaging using a fluorescence resonance energy transfer-based ATP biosensor. Immediately upon the exposure of the cells to flow, the mitochondrial ATP levels increased, which was both reversible and dependent on the shear stress intensity. Inhibitors of the mitochondrial electron transport chain and ATP synthase abolished the shear-stress-induced mitochondrial ATP generation, resulting in the loss of the ATP release and intracellular $Ca^{2+}$ responses. These results indicate the novel role of endothelial mitochondria as mechanosignaling organelles that can transduce shear stress information into ATP generation, triggering ATP release and purinoceptor-mediated $Ca^{2+}$ signaling which results in regulation of vascular functions. (COI:No)

### PS09-03

#### Contribution of vasodilators to the increase in muscle blood flow after manual acupuncture

Hisashi Shinbara (*Dept Acp Mox Ther, Fac Hlth Promo Sci, Tokoha Univ, Japan*)

Increase in muscle blood flow (MBF) induced by acupuncture is considered to contribute to muscle recovery by washing out waste and algesic substances. However, the underlying mechanism is unclear. Thus, we studied this mechanism using radiolabeled or fluorescent microspheres, which are able to quantitatively and noninvasively estimate MBF. Additionally, we used receptor antagonists and enzyme inhibitors to clarify the involvement of vasodilators. Manual acupuncture (MA) with the sparrow pecking technique significantly increased MBF of the hindlimb in rats. The increase was observed only in the muscles where the acupuncture needle was inserted. The increase was not affected by acute denervation (cutting of the sciatic nerve). However, the increase was significantly but incompletely suppressed by topical injection of hCGRP, a CGRP receptor antagonist. Moreover, the increase was not observed in capsaicin-treated rats. These results suggested the involvement of the axon reflex. Nevertheless, the increase was also observed in chronically denervated rats.

The increase was significantly but incompletely suppressed by theophylline, a non-selective adenosine receptor antagonist; suramin, a purine 2 receptor antagonist; or L-NAME, a nitric oxide (NO) synthase inhibitor. In contrast, the increase was not affected by indomethacin, a cyclooxygenase inhibitor.

Our studies suggest that the increase in MBF after MA is caused by the following vasodilators: 1) CGRP, which is released from afferent nerve endings via the axon reflex, 2) ATP which is leaked from muscle cells damaged by acupuncture needles, and 3) ADP and adenosine which are the degradation products of ATP. As a result, the binding of these vasodilators to their respective receptors facilitates the production of NO in the vascular endothelium, which relaxes the vascular smooth muscle. The contribution of other vasodilators is suggested, but vasodilatory prostaglandins do not seem to be involved. (COI:No)

### PS09-04

#### Neural mechanism of the improvement of muscle blood flow elicited by acupuncture or local heat application

Kenichi Kimura (*Dept Health Sci, Kansai Univ Health Sci, Japan*)

Acupuncture improves local muscle blood flow (MBF), which may flush out algesic or sensitizing substances to relieve pain. Therefore, acupuncture has been used to treat musculoskeletal impairments such as shoulder stiffness and muscle pain. The calcitonin gene-related peptide (CGRP) and substance P (SP) released from sensory nerve terminals may be partially responsible for the mechanism underlying the improvement in local MBF elicited by acupuncture. Recent studies have indicated that nitric oxide (NO) and adenosine are also involved in the increased MBF induced by acupuncture. In addition to local blood flow, acupuncture may influence distant blood flow through the vasomotor nerve. Muscle sympathetic nerve activity (MSNA) is thought to primarily represent vasoconstrictor fiber activity, which innervates the skeletal blood vessels, thereby playing an important role in regulating the MBF. We previously reported that locally applying heat induced an increase in MBF at the heated area by inhibiting MSNA. The increased MBF following acupuncture may also help suppress MSNA. However, whether acupuncture confers sympatho-inhibitory effects on MSNA in humans remains unknown. We examined whether MSNA plays a role in the increased MBF following acupuncture and found that acupuncture did not alter the MSNA at rest. Thus, suppressing MSNA did not mediate the increased MBF. Therefore, the increased MBF following acupuncture may be mainly due to vasodilators such as CGRP, NO, and adenosine rather than MSNA suppression. (COI:No)

# Planned Symposium 10

## Japan-China Joint Symposium

## Cutting edge of cell fate determination and migration in the developing cerebral cortex

(March 18, 9:00～10:50, Hall 2)

### PS10-01

#### Chromatin regulation during neural development

Yusuke Kishi, Naohiro Kuwayama, Yoshikuni Wada, Yukiko Gotoh (*Grad Sch Pharm, Univ of Tokyo, Japan*)

Neurons, an essential cell type for brain function, are derived from neural stem/progenitor cells (NPCs). During the early stage of mouse neocortical development, NPCs proliferate symmetrically to increase their pool size (the expansion phase). They then acquire neurogenic competence and generate neurons (the neurogenic phase). The transition of the expansion-to-neurogenic phase is critical for determining the number of NPCs and neurons and thus should be strictly regulated. However, it remains unclear how the timing of this transition is regulated, partly due to the difficulty of genetic manipulation of NPCs during the expansion phase. We therefore developed a new method to express gene-of-interest in NPCs at E8 during the expansion phase. In addition, we examined the changes in chromatin accessibility and transcriptional profile during the transition of the expansion-to-neurogenic phase to list up candidate molecules regulating the transition. (COI:No)

### PS10-02

#### Regulation of neural differentiation and cell migration during neocortex development

Qin Shen (*School of Life Sciences and Technology, Tongji Hospital, Tongji University, China*)

Neural progenitor cells (NPC) in the developing neocortex undergo precisely controlled proliferation and differentiation to ensure proper generation and settlement of neurons for correct cortical architecture. Genes expressed in NPCs but downregulated upon differentiation are often studied for their roles in promoting proliferation and inhibiting differentiation, but it is less clear whether mis-regulated expression would also disrupt the behavior of their differentiated progeny. Zinc finger E-box binding homeobox 1 (Zeb1) is well known as a key regulator of the epithelial-mesenchymal transition and cancer metastasis. Mutation of Zeb1 is associated with human genetic eye diseases and defective brain development. However, its role in neural development is less clear. We found that Zeb1 is expressed in NPCs but turned off during neuronal differentiation in the developing neocortex. Altering Zeb1's expression in cortical cells in vivo by in utero electroporation affected neuronal differentiation and migration. Surprisingly, overexpression of Zeb1 did not increase proliferation of NPCs in the germinal zone, but disrupted the correct positioning of NPCs and differentiating neurons. This was accompanied by suppression of NeuroD1, a transcription factor important for neuronal differentiation, and delayed multipolar-to-bipolar transition. Consequently, long-term overexpression of Zeb1 caused severe migration defects and heterotopia bands in the white matter at postnatal stage. We found that Zeb1 regulated a cohort of genes involved in cell differentiation and migration and identified CTBP2 as the functional partner of ZEB1 in the embryonic cortex. Similarly, overexpression of CTBP2 also repressed the neuronal migration. Binding to CTBP2 is required for ZEB1 to elicit the effect on multipolar-to-bipolar transition but not suppression of NeuroD1. This study demonstrates that a gene specifically expressed in NPCs can affect aspects of neuronal behavior if its expression is mis-regulated, providing an example of requirement for dynamic control of gene expression during cortical development. (COI:No)

### PS10-03

#### Mechanisms of neocortical organization by neuronal activity of subplate neurons

Chiaki Ohtaka-Maruyama[1], Noe Kaneko[1, 2], Ai Fujii[1, 2], Hldeya Kawaji[3], Kei Yura[2, 4]
(*[1]Tokyo Metropol. Inst. Med. Sci., Neural Network, [2]Ochanomizu Univ. Dept. Science, [3]Tokyo Metropol. Inst. Med. sci., Genomic Med., [4]Waseda Univ. Sch. Adv. Sci. Engineering*)

In the mammalian neocortex, an enormous number of neurons are precisely arranged in an ordered 6-layered structure in an inside-out manner. This structure is formed by the sequential generation of neurons and their migration toward the brain surface, termed radial neuronal migration. In order to complete the neocortical layer structure within the limited time period of embryogenesis, the radial migration process must be controlled precisely and efficiently. We previously reported that subplate neurons (SpNs), which are one of the earliest born and matured types of neurons in the developing neocortex, play an important role in the regulation of radial migration. We revealed that SpNs exhibit spontaneous calcium oscillations at E15, and actively extend processes to contact newly born multipolar migrating neurons. Electron microscopic observation demonstrated that synapse-like structures were formed at these contact sites. This synaptic communication leads to the switch from multipolar migration to locomotion. It is known that SpNs are the first cortical neurons to receive sensory input from thalamic axons. SpNs then project axons toward layer IV neurons to establish a temporal link between thalamic axons and their final target in layer IV. This initial wiring also depends on SpN activity, suggesting that they function as a conductor for mammalian neocortical formation by organizing multiple processes. To elucidate the function of SpNs, we are now characterizing their subpopulations using an SpN-specific transgenic mouse lines (Lpar1-EGFP or Cre-ERT2 line;D1B-Cre). We found that they have different characteristics in terms of molecular marker expression. This can be a piece of evidence for SpNs consisting of heterogeneous cell populations, and supports the idea that each subpopulation has distinct roles in neocortical formation. We are currently analyzing single cell RNA-seq data obtained from Lpar1-GFP positive cells using C1 single cell prep system to clarify gene expression profiling of SpNs. (COI:No)

### PS10-04

#### Novel migration mode in the developing cerebral cortex

Kazunori Nakajima (*Dept Anat, Keio Univ Sch Med, Japan*)

Neurons and astrocytes are generated directly or indirectly from the progenitors in the ventricular zone (VZ). When we observed mouse cortical slices at embryonic day (E) 17.5 that had been transfected into the VZ with a green fluorescent protein (GFP) expression vector 2 days earlier using in utero electroporation, we found that some GFP-positive cells migrated rapidly and almost randomly within the IZ and CP. These cells divided frequently, and the daughter cells continued to exhibit the same irregular movement. Since this migration pattern was different from that of well-known radial migration, by which neurons migrate from the IZ to CP, we named this novel migration mode "erratic migration". Cells undergoing erratic migration (hereafter referred to as "erratic migration cells") moved more quickly than those undergoing radial migration. Erratic migration cells frequently changed direction, although they ultimately tended to move toward the brain surface. To determine the final fate of the erratic migration cells, we performed electroporation at E15 to label them with a photo-convertible protein, kikGR. After identifying the cells undergoing erratic or radial migration in slices prepared at E16, we irradiated these cells with a 405-nm laser, dissociated the slices, and cultured the cells on cover slips. Most of the erratic migration cells differentiated into astrocytes, whereas the cells that had moved in a radial migration mode differentiated into neurons. These results indicated that erratic migration cells derived from the cortical VZ were astrocyte progenitors. Further analyses of these cells will be presented. (COI:No)

# Planned Symposium 11

## Women scientists in the optogenetic field – the new fact has come to light using optical approaches

(March 18, 9:00～10:50, Hall 4)

### PS11-01

#### Cell-type-specific patterned activities specify gene expression patterns for olfactory circuit formation

Ai Nakashima (*Grad. Sch. of Pharm. Sci., Univ. of Tokyo*)

The development of precise neural circuits is initially directed by genetic programming and subsequently refined by electrical neural activity. The most prevailing model for the activity-dependent development of neural circuits postulates the interaction between pre- and post-synaptic neurons. In Hebbian plasticity, the correlated activity of pre- and post-synaptic neurons strengthens synaptic connections, whereas uncorrelated or lack of activity weakens them. However, the olfactory map develops even in mutant mice lacking synaptic partners, suggesting another activity-dependent mechanism for the olfactory map formation. During development, axons from various olfactory neurons expressing the same olfactory receptor (OR) segregate into specific glomeruli in an activity-dependent manner. We found that OSNs exhibited OR-specific temporal patterns of spontaneous activities. Moreover, differing patterns of neuronal activity induced different expression patterns of axon-sorting molecules that regulate glomerular segregation. We propose an activity-dependent mechanism that is fundamentally different from the Hebbian plasticity theory in which cell-type-specific patterned activity contributes to generating the combinatorial code of axon-sorting molecules for the olfactory map refinement. (COI:No)

### PS11-02

#### Glutamatergic input into the dorsal raphe nucleus and aggressive arousal

Aki Takahashi (*Lab of Behav Neuroendocrinol, Univ Tsukuba, Japan*)

Escalated aggression, or violence, has been huge problem in public health. In many cases, violence incidents are triggered by social instigation or frustration. Social instigation-heightened aggression has been observed in several animal models from fish to rodents in which short encounter with a potential rival escalates aggressive behavior in the subsequent encounter with an opponent due to increases of aggressive arousal of individuals. We have shown an involvement of excitatory input into the dorsal raphe nucleus (DRN) in the instigation-heightened aggression in male mice. In vivo microdialysis showed that glutamate release increased in the DRN during an aggressive encounter, and the level of glutamate was further increased when the animal was engaged in escalated aggressive behavior after social instigation. Importantly, the increase of glutamate was observed during the period of social instigation without direct physical interaction, suggesting that the glutamate input into the DRN may represent a status of arousal which increases aggressive behavior in the following encounter. We also found that microinjection of L-glutamate into the DRN escalated aggressive behavior of male mice. The DRN receives glutamatergic projections from many brain areas including the prefrontal cortex, lateral habenula, and hypothalamus. In this talk, I will talk about our approach to identify the origin of glutamate input into the DRN which is involved in aggressive arousal and causes an escalation of aggressive behavior by using optogenetics and DREADD techniques. (COI:No)

### PS11-03

#### The role of thalamic matrix cells in wake/sleep cycle regulation

Sakiko Honjoh (*IIIS, Univ of Tuskuba*)

The neocortex and the thalamus are reciprocally connected and are believed to be critical for arousal and cognitive function. Thalamic neurons that project to the cerebral cortex are divided into two neural subpopulations, core and matrix cells, based on their projection patterns. Core cells are enriched in sensory and motor relay nuclei and project mainly to layer 4 of specific cortical areas. Matrix cells abound in some intralaminar and medial thalamic nuclei project diffusely to cortex, primarily to superficial layers. In particular, multiareal type matrix cells may constitute a veritable thalamic activating system that facilitates effective interactions among many cortical areas and thereby sustains arousal and consciousness. Here we focused on ventromedial nucleus (VM), which is comprised of only multiareal type of matrix cells. We found that VM matrix cell activity is highly correlated with activated EEG, with sustained high firing during wake and REM sleep and low firing during NREM sleep in freely behaving mice. VM matrix cells increased firing before cortical activation in both transitions from NREM sleep to wake or REM sleep, suggesting its role in cortical activation. To investigate its causal role, we employed optogenetic stimulation and found that high frequency stimulation of matrix cells elicits cortical activation and behavioral arousal from NREM sleep. However, interestingly, the identical optogenetic stimulation failed to promote arousal from REM sleep showing the vigilance state-dependent thalamo-cortical interaction. Our analyses revealed that Grangier causality from thalamus to cortex is the highest during wake and the lowest during REM sleep. The low causality from thalamus to cortex during sleep could be a potential mechanism for the vigilance state-dependent fluctuation of consciousness and cognition. (COI:No)

### PS11-04

#### Optical measurements of brain energy dynamics during sleep

Tomomi Tsunematsu[1,2] ([1]*Grad Sch Life Sci, Tohoku Univ, Japan,* [2]*JST, PRESTO*)

Mammalian sleep can be classified into two categories, i.e., rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. However, we still do not know why we have to sleep despite repeating the behavior every day. One attractive hypothesis is the energy conservation theory. It is reasonable to conserve energy by sleeping since the brain is surely an organ that consumes so much energy. Here, we challenged to clarify the energy dynamics in brain cells during sleep in mice using optical imaging. First, we focused on the change of intracellular calcium concentration in astrocyte which receives energy from blood vessels and passes it to neurons. Next, we revealed the dynamics of intracellular ATP, which is a metabolic molecule and used as energy source, concentration in neurons. Our in vivo optical recording with genetically-encoded calcium and ATP fluorescent sensor demonstrated that intracellular calcium level of astrocyte and ATP level of neuron globally fluctuated across sleep/wakefulness state, especially in REM sleep. These results help us to understand physiological significance of sleep from an energy perspective. (COI:No)

# Planned Symposium 12

## New frontiers of locomotion research using zebrafish as a model system

(March 18, 9:00～10:50, Hall 5)

### PS12-01

#### Regulation of locomotor speed and selection of active sets of neurons by V1 neurons

Shin-ichi Higashijima (*NINS, ExCELLS/NIBB, Japan*)

During fast movements in vertebrates, slow motor units are thought to be deactivated due to the mechanical demands of muscle contraction, but the associated neuronal mechanisms for this are unknown. Here, we performed functional analyses of spinal V1 neurons by selectively killing them in larval zebrafish, revealing two functions of V1 neurons. The first is the long-proposed role of V1 neurons: they play an important role in shortening the cycle period during swimming by providing in-phase inhibition. The second is that V1 neurons play an important role in the selection of active sets of neurons. We showed that strong inhibitory inputs coming from V1 neurons play a crucial role in suppressing the activities of slow-type V2a and motor neurons, and, consequently, of slow muscles during fast swimming. Our results thus highlight the critical role of spinal inhibitory neurons for silencing slow-component neurons during fast movements. (COI:No)

### PS12-02

#### Swimming characteristics of different zebrafish strains assayed by a swimmill

Hiromi Hirata, Yuma Wakamatsu (*Aoyama Gakuin University, Sagamihara, Japan*)

Several zebrafish strains such as AB, Tübingen (TU), Wild India Kolkata (WIK) and Tupfel long fin (TL) have been established for genetic study. Each strain has its morphological and behavioral traits. Motor traits, however, have not been explored in zebrafish strains. We here applied a swimmill, which is a treadmill for fish species, and measured swimming capability of adult zebrafish by critical swimming speed, which is the maximum water velocity in which fish can keep swimming. First, we confirmed that swimming capability does not vary between female and male. Second, we found that the appropriate water temperature for swimming was between 16 and 30 oC. Third, our fin clip experiments using long-finned zebrafish revealed that they can exhibit high swimming capability when the caudal fin length was set between 3 and 10 mm, implying that long-finned zebrafish are unfavorable for fast swimming. Finally, we compared swimming capability of several zebrafish strains and demonstrated that WIK fish was significantly less capable of swimming despite that they have short caudal fin (~9 mm). The offspring of WIK fish were less capable of swimming, while hybrids of WIK and TU showed high swimming performance. Thus, lower swimming capability of WIK strain is inheritable as a motor trait. (COI:No)

### PS12-03

#### Drug screening using zebrafish models of muscle diseases

Genri Kawahara, Yukiko Hayashi K (*Dept PathoPhysiol, Tokyo Med, Univ, Japan*)

Zebrafish are useful to analyze the pathomechanism of various human diseases. There are some excellent zebrafish models of muscle diseases including muscular dystrophies, which show severe abnormalities in skeletal muscle easily detected by birefringence analysis. Moreover zebrafish are very powerful tools for therapeutic drug discovery because their characteristics (small size, fast development, clear muscle structures and muscle phenotypes in mutant fish) are ideal for drug screening. Zebrafish muscle disease models can be used in high-throughput drug screens to identify candidate drugs that improve the muscle disease phenotypes. In this presentation, we will show characteristics of model fish of muscular dystrophies and results of drug screening to discover future therapy for muscle diseases. (COI:No)

### PS12-04

#### Synaptic silencing of fast muscle is compensated by rewired innervation of slow muscle

Buntaro Zempo[1], Yasuhiro Yamamoto[1], Tory Williams[2], Fumihito Ono[1,2] ([1]*Dept Physiol, Div Life Sci, Faculty of Med, Osaka Medical College,* [2]*Laboratory of Molecular Physiology, NIAAA, NIH*)

It is generally accepted that the skeletal muscle in vertebrates consists of two types of muscle fibers: slow and fast. For decades, numerous studies have proposed that fast muscles contribute to quick movement, while slow muscles underlie locomotion requiring endurance. By generating mutant zebrafish whose fast muscles are synaptically silenced, we examined the contribution of fast muscles in both larval and adult zebrafish. Zebrafish have various advantages in studying synapses in slow and fast muscles. Slow and fast muscle cells in zebrafish are spatially segregated and can easily be distinguished by their anatomical and histological characteristics. In addition, recent studies suggested distinctive compositions of nicotinic acetylcholine receptors (AChR) in slow and fast muscles of zebrafish. In zebrafish as well as in mammals, nicotinic AChR expressed in the neuromuscular junction of fast muscles are pentamers, composed of $\alpha$, $\beta$, $\delta$, and $\varepsilon$ (or $\gamma$) subunits. $\gamma$ subunits are expressed in larvae and change to $\varepsilon$ as the animal matures. However, recent studies showed that AChRs in slow muscles of zebrafish lack $\gamma/\varepsilon$ subunits, and are composed of only $\alpha$, $\beta$ and $\delta$ subunits. In the present study, we established knockout zebrafish lines that lacked $\varepsilon$ and/or $\gamma$ subunits, and analyzed their locomotion and synaptic traits. In the larval stage, mutants lacked the characteristic startle response to tactile stimuli: bending of the trunk (C-bend) followed by robust forward propulsion. Surprisingly, adult mutants with silenced fast muscles showed robust C-bends and forward propulsion upon stimulation. Retrograde labeling revealed that motor neurons genetically programmed to form synapses on fast muscles are instead re-routed and innervate slow muscles, which led to partial conversion of slow muscles to fast muscles. Thus, extended silencing of fast muscle synapses changed motor neuron innervation and caused muscle cell type conversion, revealing an unexpected mechanism of locomotary adaptation. (COI:No)

# Planned Symposium 13

## Combined approaches of cutting-edge techniques and structural information to reveal the elaborate dynamics of membrane proteins

(March 18, 9:00～10:50, Hall 9)

### PS13-01
#### Cryo-EM analysis of the volume-regulated anion channel LRRC8

Go Kasuya (*Dept Physiol, Jichi Medical Univ, Japam*)

Cell volume regulation against intracellular and extracellular osmotic alterations is a fundamental homeostatic mechanism for all organisms. Various ion channels are involved in cell volume regulation. The leucine-rich repeat-containing 8 (LRRC8) protein family, composed of the five isoforms named LRRC8A-E, are considered to serve as a pore-forming component of the volume-regulated anion channel (VRAC), which is activated by cell-swelling and permeates anions and other organic osmolytes to the extracellular side, thereby facilitating water efflux to counteract cell-swelling. As the overall sequence of LRRC8 has little similarity to any other known classes of ion channels, the detailed structural information has been awaited to clarify the molecular mechanisms of the LRRC8 protein family. Here, I present the present two homomeric structures of human LRRC8A and LRRC8D isoforms, determined by single-particle cryo-electron microscopy. These two structures show a hexameric assembly, and the transmembrane region features a topology similar to gap junction channels. The LRR region, containing 15 leucine-rich repeats, forms a long, twisted arc, and is flexible and mobile, with rigid-body motions, which might be implicated in structural transitions on pore opening and closing. The channel pore is located along the central axis and constricted on the extracellular side, where the residues at the tip of the extracellular helix contribute to permeability to substrates. The structural comparisons with the LRRC8A and LRRC8D isoforms, together with the structure-based electrophysiological analysis, revealed the two key features involved in substrate permeability: the wider pore constriction on the extracellular side of the LRRC8D isoform than that of the LRRC8A isoform, and an additional helix facing to the channel pore formed by the N-terminal residues observed only in the LRRC8D isoform. Overall, this work provides a framework for understanding the mechanisms of this unique class of ion channels.
(COI:No)

### PS13-02 (AP-3)
#### PI(3, 4)$P_2$- and voltage-dependent gating of two-pore $Na^+$ channel 3

Takushi Shimomura[1,2], Ki-ichi Hirazawa[1,2], Yoshihiro Kubo[1,2] ([1]*Div Biophys and Neurobiol, Natl Inst Physiol Sci,* [2]*Dept Physiol Sci, SOKENDAI*)

Two-Pore $Na^+$ Channels (TPCs) contain two domains (DI and DII) of a functional unit of voltage-dependent cation channels. Each domain has its own voltage sensor domain that possesses three positively charged arginine residues in helix S4. Characteristically, TPC3 shows the shift of the voltage dependence by long depolarization stimulus, so called as "induction", in *Xenopus* oocyte expression system.
The structural basis of this "induction" mechanism was investigated using multiple approaches based on two-electrode voltage-clamp technique and the structural model of TPC3. We found the correlation between $PIP_2$ level and "induction" currents. Simultaneous recordings of TPC3 current and the fluorescence from specific $PIP_2$ sensors showed that PI(3, 4)$P_2$ concentration is increased by long depolarization stimulus, possibly through any endogenous system in *Xenopus* oocytes. The "induction" kinetics of TPC3 is well correlated with the fluorescent change of PI(3, 4)$P_2$ sensors, but not with that of PI(4, 5)$P_2$. The PI(3, 4)$P_2$ sensitivity of TPC3 was confirmed using direct phosphoinositide injection method and the excised-patch membrane. These results reveal that "induction" is PI(3, 4)$P_2$-induced modulation of voltage dependence of TPC3. We also found that a cluster of basic amino acid residues in the cytosolic side of DI is critical for PI(3, 4)$P_2$ sensitivity. Interpretation of the mutational effects based on the TPC3 structural model explained how TPC3 selectively recognizes PI(3, 4)$P_2$ in DI region. While DI recognizes PI(3, 4)$P_2$, DII is considered to be mainly responsible for voltage-sensing. The voltage-dependent movement of DII-S4 was verified using the voltage clamp fluorometry, in which the voltage-dependent fluorescent change was detected from the fluorophore incorporated into some residues in DII-S4. These electrophysiological data of various approaches, combined with the structural model, revealed the detailed voltage-dependent gating mechanism of TPC3.
(COI:No)

### PS13-03
#### The conformational change of the cytoplasmic region of voltage-sensing phosphatase

Akira Kawanabe[1,2], Manami Nishizawa[3], Kazuhisa Nishizawa[3], Hirotaka Narita[4], Tomoko Yonezawa[2], Yuka Jinno[2], Souhei Sakata[5], Atsushi Nakagawa[4], Yasushi Okamura[2] ([1]*Fac. Med., Kagawa. Univ., Kagawa, Japan,* [2]*Grad. Sch. Med., Osaka Univ., Osaka, Japan,* [3]*Fac. Med. Tech., Teikyo Univ., Tokyo, Japan,* [4]*IPR, Osaka Univ., Osaka, Japan,* [5]*Fac. Med., Osaka Med. Coll., Osaka, Japan*)

Conformational changes of cytoplasmic regions of membrane proteins play fundamental roles both in membrane electrical signals and signal transduction. However, detailed analysis of such conformational change in living cells has been difficult because of the lack of efficient molecular tools. To overcome this issue, we utilized a cytoplasmic fluorescent-label technique and detected its stimulation-dependent fluorescent changes. In this presentation, we introduce our recent studies on voltage-induced conformational changes of cytoplasmic region of VSP (Sakata et al. 2016, Kawanabe et al. 2018).
Voltage sensing phosphatase (VSP) consists of a voltage sensor domain (VSD) and a cytoplasmic catalytic region (CCR) which has phosphatase activity toward PI(4, 5)$P_2$ regulated by membrane potential change. Although the X-ray crystal structure of each domain in Ciona intesinalis VSP (Ci-VSP) has been individually resolved, the coupling mechanism from the voltage sensor to enzymatic activity has not been fully understood.
From our dynamics simulations of phospholipid bilayer/CCR systems, we found the characteristic hydrophobic region on membrane-protein interface in Ci-VSP (Leu284 and Phe285, called "hydrophobic spine") which may play an important role in the coupling. Systematic mutation screening in the hydrophobic spine showed the voltage-dependent phosphatase activity depends on the hydrophobicity and presence of aromatic ring in the amino acid side chain.To gain more mechanical insights, we attempted to analyze the conformational changes of the cytoplasmic region of Ci-VSP with unnatural fluorescent amino acid (Anap) introduced at Lys555. Two components of voltage-dependent fluorescence changes were detected in K555Anap (smaller fast decrease and larger slow increase). In contrast, a larger component was enhanced in a higher-activity mutant (L284F/K555Anap), whereas it disappeared in a lower-activity mutant (L284Q/K555Anap). These findings indicate that VSP has two-step activation (resting to partially-activated and, then to fully-activated state) and the latter transition depends on the nature of the hydrophobic spine.
(COI:No)

### PS13-04
#### Unveiling the lipid bilayer effects on the ion channel function using a cutting-edge artificial lipid bilayer technique

Masayuki Iwamoto (*Dept Molec Neurosci, Univ Fukui Fac Med Sci*)

All the membrane proteins are under the effects of the lipid bilayer properties such as chemical composition of phospholipids and physical force from the lipid bilayer structure. Taking these factors into consideration would be essential for understanding the functional mechanism of membrane proteins at molecular level. To date, numerous information concerning such effects has been accumulated and the specific lipid bilayer environment, which is essential for the activity, has been revealed for various membrane proteins. However, the quantitative aspect and the molecular mechanism of the lipid bilayer effects remain unclear for many cases because of difficulties in the functional assay under the strictly regulated lipid bilayer environment. To overcome such problems, we have developed an artificial lipid bilayer technique, the contact bubble bilayer (CBB) method. The CBB method enabled us the single-channel current recording of ion channels under the completely regulated lipid bilayer composition and tension. Using the CBB method, we have revealed the molecular mechanism of the anionic lipid effect on the open probability of the KcsA potassium channel, a canonical ion channel protein. Furthermore, we have showed quantitatively that the lipid bilayer tension was necessary for full activity in addition to the ligand binding. The lipid bilayer-dependent property found in the KcsA channel might be a common feature of ion channel proteins.
(COI:No)

# Planned Symposium 14

## Past, present and future of JPS

(March 18, 15:20〜17:10, Hall 1)

### PS14-01

#### About The Journal of Physiology

Yasushi Okamura (*Dept Physiol, Grad Sch Med, Osaka Univ, Japan*)

The Journal of Physiology has been run by the Physiological Society in UK as one of the traditional international journals in the field of physiology since its foundation in 1878. Many important works in the fields of physiology were published in JP. This includes Sherrington's concept of neural networks and reflex, Adrian's first electrical recording of action potentials, Erlanger and Gasser's conduction velocity measurement of peripheral nerves and their classification of nerve fibers, Hodgkin and Huxley's works of voltage-gated ion channel and Katz's works of mechanisms of synaptic transmission including quantum vesicle release. I will talk about how policies of the Journal have been changed with progress of science and the style and environments of research activities in the academics. I will also briefly explain current reviewing processes and scope of papers which the Journal now covers. (COI:No)

### PS14-02

#### About Pflügers Archiv European Journal of Physiology

Makoto Tominaga (*Div Cell Signaling, Natl Inst Physiol Sci*)

Pflügers Archiv European Journal of Physiology is a peer-reviewed scientific journal in the field of physiology. A continuation of a journal founded in 1868 by the German physiologist, Eduard Friedrich Wilhelm Pflüger, Pflügers Archiv is the oldest physiological journal, and currently published by Springer with 11 issues per year. Editor-in-Chief is Dr. Armin Kurtz. Average dates from submission to first decision are 23 days, and average dates from acceptance to publication are 15 days. Pflügers Archiv European Journal of Physiology publishes the results of original research considered likely to further the physiological sciences in their broadest sense. Topics include pathophysiological or methodological issues when these can be used as a tool for further investigation of physiological mechanisms. Papers should give mechanistic insights into physiological functions at the molecular and cellular level. Priority will be given to manuscripts that provide conceptual novelty. Pflügers specifically aims at publishing work on ion channels, transporters, cardiac electrophysiology, and sensory physiology on a molecular and cellular level. It should be noted that Drs. Erwin Neher and Bert Sakmann, Nobel laureates for Physiology or Medicine in 1991, published the first description of the patch-clamp technique in this journal. (COI:No)

### PS14-03

#### Past, present and future of JPS

Yoshihiro Ishikawa (*CVRI, Grad Sch Med, Yokohama City Univ, Japan*)

The Journal of Physiological Sciences is the only English Journal issued by the Physiological Society of Japan and has a history of more than sixty years. The quality and reputation of the Journal has been improved significantly in the past several years due to the major contributions by our Society members. Its impact factor is now more than 3, which is equivalent to that of American Journal of Physiology. Thus, our Journal is growing from a major Asian physiological Journal to a world-class Physiological Journal. In order to achieve the next goal for us, the Journal will become an open access journal from 2020. The Journal already started to accept manuscript submission to a new open access Journal. In the Symposium, we will explain our progress to our young researchers. In particular, we will explain the history of our Journal as well as how to contribute their work to our Journal successfully. We will also explain the status of other major physiological Journals such as Journal of Physiology in England. (COI:No)

# Planned Symposium 15

## Leading-edge approach for regulated exocytosis in neural system

(March 18, 15:20〜17:10, Hall 2)

### PS15-01

#### Quantal glutamate release organized by supramolecular assembly at presynaptic terminals

Kenzo Hirose (*Dept Pharmacol, Grad Sch Med, Univ Tokyo*)

Synaptic transmission is described by the quantal release mechanism which was proposed by Bernard Katz more than a half century ago. According to the quantal release mechanism, the synaptic transmission should be stochastic and discrete rather than deterministic and continuous. These features of quantal release should have great impact on overall brain computation. However the actual features of synaptic transmission and the regulatory mechanism at the single synapse level remain unclear level. We have addressed the problem by combining two imaging techniques: a glutamate imaging technique and a superresolution imaging technique. We developed a fluorescent glutamate probe named eEOS [enhanced E (glutamate) Optical Sensor] and used it to visualize glutamate in cultured hippocampal neurons. The glutamate imaging revealed stochastic nature of glutamate release at individual synaptic terminals which is predicted by the quantal release mechanism. Mathematical analysis of the imaging data provided detailed features of the quantal release mechanism at the single synapse level. We found that each synaptic terminal has distinct multiple release sites for vesicular exocytosis and the number of the release sites per synapse varies from only a few to more than ten. These variability in the number is intriguing because the number provides unique synaptic weights on presynaptic side. Next we used STORM, a superresolution imaging technique, to explore molecular and structural correlates of the release sites. We found that Munc13-1, a presynaptic protein essential for vesicular exocytosis, forms nanosized molecular assemblies at the presynaptic terminal and the Munc13-1 assemblies serve as the release sites. These findings provide new insight into the synaptic weighting mechanism which might afford unique features important for brain computation. (COI:No)

### PS15-02

#### Ca-Transmitter release coupling at hippocampal mossy fiber synapses

Takeshi Sakaba (*Doshisha University*)

Hippocampal mossy fiber (hMF) –CA3 synapses exhibit presynaptic forms of short- and long-term presynaptic plasticity. The presynaptic mechanisms of transmitter release and synaptic plasticity still remain to be studied. In order to determine basic properties of hMF-CA3 synapses, we have used simultaneous recordings of pre- and postsynaptic compartments, and used presynaptic capacitance and EPSCs for measuring the kinetics of transmitter release quantitatively. We have obtained basic information such as release probability, the number of readily releasable synaptic vesicles, and the rate of synaptic vesicle replenishment. Compared to other model synapses such as the calyx of Held and the synapses in the cerebellum, a large pool of readily releasable vesicles is a unique feature of this synapse type, which allows flexibility of synaptic strength at this synapse. Implications in short-term and long-term synaptic plasticity will be discussed. (COI:No)

### PS15-03

#### Mechanosensing of the enlargement of dendritic spines by presynaptic terminals in rat hippocampus

Hasan Ucar[1], Satoshi Watanabe[3], Jun Noguchi[3], Sho Yagishita[2], Yuicihi Morimoto[2], Noriko Takahashi[4], Haruo Kasai[1,2] (*[1]International Research Center for Neurointelligence (WPI-IRCN), UTIAS, The University of Tokyo, Japan, [2]Laboratory of Structural Physiology, Graduate School of Medicine, The University of Tokyo, [3]National Center of Neurology and Psychiatry, Tokyo, Japan, [4]Department of Physiology, Kitasato Univ. School of Medicine, Tokyo, Japan*)

In this work, we have identified clues for a novel phenomenon where presynaptic activity is enhanced by sensing the mechanical pressure emerging from spine enlargement. We used the Schaffer collateral (SC) innervating CA1 pyramidal neurons in hippocampal slice cultures. First, we measured SNARE assembly in presynaptic boutons of SC by using a probe which measured Förster resonance energy transfer (FRET) between t-SNARE (Syntaxin1A) and v-SNARE (VAMP2) by utilizing fluorescence lifetime imaging (FLIM) and time-correlated single photon counting (TCSPC) method. When we pushed single presynaptic boutons by a fine glass pipette FRET value was increased, indicating that the mechanical pushing of the plasma membrane towards the vesicle membrane can induce the trans-SNARE formation. Second, we measured glutamate release in responses to single action potentials from individual boutons which expressed iGluSnFR. We found the pushing augmented the glutamate release in a good correlation with the FRET increase. The presynaptic FRET increase was independent of cytosolic Ca2+, but dependent on the assembly of SNARE proteins and actin scaffolds in the presynaptic terminals. Similar enhancement of SNARE assembly and glutamate release was observed by a low hypertonic sucrose solution. Lastly, to test the physiological relevance of the mechanical effect, we induced spine enlargement by spike-timing-dependent plasticity (STDP) at the dendritic spine using 2-photon glutamate uncaging. We found that spine enlargement caused an increase in the trans-SNARE formation and glutamate release when spines pushed the presynaptic bouton. However when spines twitched and did not exert pushing, even though spines enlarged, FRET or glutamate release enhancements were not observed. Thus, we have found a new mechanosensing mechanism, involving SNARE proteins and actin fibers in the presynaptic terminals, which can respond to fine alterations in the postsynaptic structures. (COI:No)

### PS15-04

#### Lysosomal exocytosis in neurons: mechanisms and its possible roles

Michisuke Yuzaki (*Dept Physiol, Keio Univ Sch Med*)

Lysosome is a membrane-bound organelle containing hydrolytic enzymes, such as cathepsin B (CatB), which can degrade a variety of biomacromolecules inside animal cells. $Ca^{2+}$-dependent exocytosis of lysosomes occurs in hematopoietic lineage cells and some specialized cells. However, physiological roles of lysosomal exocytosis and its underlying mechanisms have remained largely unclear in neurons. We found that a synaptic organizer Cbln1, which plays crucial roles in formation of synapses between cerebellar granule cells (GCs) and Purkinje cells, was released from lysosomes located at axons, but not dendrites, of developing GCs. Cbln1 exocytosis required a physiological range of repetitive GC action potentials (300 pulses at 2.5-40 Hz), indicating that it serves as a leaky integrator of neuronal activities over time. Exocytosed Cbln1 was mostly retained in partially fused vesicles or recovered by vesicles on a time scale of minutes, leaving a small fraction of Cbln1 retained on GC axons by binding to its cell-surface receptor neurexin. Cbln1 was co-released with CatB from GC axons and inhibited by lysosomal inhibitors. Cbln1 release did not involve VAMP7, as well as other TeNT-insensitive R-SNAREs, but was sensitive to Q-SNAREs SNAP29 and Stx4, indicating involvement of unique SNARE complexes. Inhibition of release of Cbln1 and CatB reduced axonal bouton formation of developing GC axons *in vivo*, implying that lysosomal exocytosis of Cbln1 and CatB serve as a new mechanism of activity-dependent coordinated synapse modification. Recently, we have found that activity-dependent lysosomal exocytosis also occurs from dendrites of hippocampal neurons via distinct mechanisms and plays crucial roles for synaptic plasticity. In this talk, I would like to discuss mechanisms and possible physiological roles of neuronal lysosomal exocytosis in dendrites and axons. (COI:No)

# Planned Symposium 16

## New Insights into Vascular Aging Mechanisms and Therapeutic Targets

(March 18, 15:20～17:10, Hall 4)

### PS16-01

#### Early loss of coronary regulation by endothelium-derived hyperpolarization factors in diabetes

James Pearson[1,2], Jennifer Ngo[1], Hirotsugu Tsuchimochi[1], Takashi Sonobe[1], Mark Waddingham[3], Huiling Jin[1] (*[1]Dept Cardiac Physiol, Res Inst, NCVC, Japan, [2]Dept Physiol, Monash Univ, [3]Dept Adv Medical Res Pulm Hypertension, NCVC*)

States of chronic vascular inflammation and excess oxidative stress develop early in prediabetes, metabolic syndrome and hypertension and contribute to accelerated vascular aging and a progressive decline in coronary function. However, the time course of changes in coronary vasodilators, vasoconstrictors, transcription factors and markers of senescence is poorly understood. Moreover, the potential influences of the cardiomyocytes and the extracellular matrix on vessel aging is rarely considered. Utilising synchrotron based microangiography on rodents we are examining coronary macro- and microcirculation function changes in young and aging rodents. Gene and protein expression changes are examined with rtPCR and Western Blotting. To date, our findings suggest that there is a decline in production of endothelium derived hyperpolarization factors (EDHF) in small coronary arterial vessels ahead of impaired nitric oxide (NO) signaling associated with insulin resistance. Rho-kinase is upregulated in various vascular disease states, in particular by hyperglycemia and hyperlipidemia associated with insulin resistance. Oxidative stress and upregulation of Rho-kinase activity have been shown to increase expression of TRPC6 channels leading to increased vasoconstriction and permeability and diminished NO signaling. Moreover, an important EDHF, the large conductance $Ca^{2+}$-activated potassium channels ($BK_{Ca}$) in coronary vessels are negatively regulated by Rho-kinase. Here, we examine the relations between NO signaling, $BK_{Ca}$ expression, Rho-kinase activation, senescence and vasomotor regulation in vivo in rodent models of diabetes, hypertension and aging. (COI:No)

### PS16-02

#### The functional role of vascular microRNAs in diabetes-induced microangiopathy of the heart

Shruti Rawal[1], Pujika Emani Munasinghe[1], Isabelle van-Hout[1], Sean Coffey[2], Michael J Williams[2], Patrick Manning[2], Philip Davis[3], Costanza Emanueli[4], Rajesh Katare[1] (*[1]Department of Physiology, HeartOtago, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand, [2]Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand, [3]Department of Cardiothoracic surgery, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand, [4]National Heart and Lung Institute, Imperial College, London, United Kingdom*)

Diabetes adversely affects the function and number of endothelial cells in the microvessels of the heart, resulting in a condition called diabetic microangiopathy. Compelling evidence from clinical and pre-clinical animal studies confirm the regression of microvasculature in the diabetic heart. We, along with others, have shown that molecular dysregulation starts much earlier in the diabetic heart leading to functional and structural deterioration in the later stage. MicroRNAs (miRNAs) are key molecular regulators playing an important role in regulating a wide range of physiological and pathological processes. In the current study, we tested the role of miRNAs in diabetes-induced microangiopathy of the heart. miRNA microarray studies in human type-2 diabetic heart showed significant dysregulation of pro- (miR-126 and -132) and anti-angiogenic (miR-92a and -206) miRNAs. Using the type-2 diabetic mouse model, we identified that downregulation of miR-126 and -132 (angiomiRs) start from the early stages (8wks of age) in the diabetic heart while the changes in the vascular profile were not observed until 20wks of age. Downregulation of angiomiRs was associated with the upregulation of anti-angiogenic target protein P120RasGAP and SPRED-1. Overexpression of miR-126 and -132 in in vitro cultured diabetic endothelial cells improved the migration and survival and enhanced the angiogenic ability of the diabetic endothelial cells. Finally, to determine if in vivo overexpression of angiomiRs prevent diabetes-induced microangiopathy in the diabetic heart, we overexpressed miR-126 expression via intramyocardial injection of miR-126 mimic in the type-2 diabetic mouse heart at 10wks of age. Results showed preserved capillary and arteriole density and reduced endothelial cells apoptosis in the diabetic heart. This eventually preserved cardiac contractility. Altogether, our study showed that diabetes induces dysregulation of vascular miRNAs and that therapeutic restoration of these miRNAs could be a novel tool to prevent/treat diabetes-induced microangiopathy. (COI:No)

### PS16-03

#### An updated overview on TRP channels involved in cardiovascular inflammatory/fibroproliferative diseases

Ryuji Inoue[1], Lin Kurahara H.[2], Keizo Hiraishi[1,2], Yuanyuan Cui[1] (*[1]Dept Physiol, Fukuoka Univ Sch Med, [2]Dept Cardiovasc Physiol, Kagawa Univ Sch Med*)

Inflammatory/fibroproliferative disorders are a major cause of morbidity and mortality in the modern world. The pathogenic mechanisms underlying these disorders involve very complex and interwoven networks of numerous biochemical mediators and signaling pathways including cytokines/growth hormones and their receptors, proteolytic enzymes, kinases, phosphatases, transcription factors, ion channels, and other regulatory molecules. Recently, considerable evidence has accumulated for the essential significance of stress-responsive $Ca^{2+}$/$Na^{+}$-permeable cation channel superfamily, the transient receptor potential (TRP) protein, in these processes. Notable examples include several TRP channels such as TRPC3, TRPC6, TRPV1, TRPA1 and TRPM7, which likely contribute to a diverse array of fibroproliferative disorders in the brain, lung, heart, liver, kidney, bowel and blood vessels. This symposium talk attempts to provide an updated view on this topic, with particular emphasis on cardiovascular diseases such as atrial fibrillation and pulmonary arterial hypertension. (COI:No)

### PS16-04

#### Changes in small artery tone regulation and structural remodeling in aging, hypertension and obesity/diabetes. Do they have a common underlying cause?

Lars Jørn Jensen (*Dept. of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen*)

We investigate the interplay between myogenic tone (MT), flow-mediated vasodilatation (FMVD), and structural remodeling in small arteries and arterioles controlling mean arterial blood pressure (MAP) and organ blood flow. Low voltage-activated T-type calcium channels are abundantly expressed in the microcirculation, but their physiological role is still debated. In mice deficient in the $Ca_V3.1$ T-type channel we found that MT was abolished at low arterial pressures (40-80 mm Hg) but strongly activated at higher pressures (80-120 mmHg). In contrast, young mice deficient in the $Ca_V3.2$ T-type channel isoform had increased MT and decreased FMVD. In old mice these effects of $Ca_V3.2$ deletion were abolished, and the expression of $Ca_V3.1$ T-type channels was dramatically reduced. In the same study, we found that MT and noradrenaline-induced constriction was increased in old mice. We later showed that the arterial expression of Rho-kinase 2 isoform (ROCK2) was increased in middle-aged mice, coinciding with an increased MT and increased effect of the ROCK2-specific inhibitor KD025. The mechanisms responsible for the age-dependent contributions of T-type channels and ROCK2 will be pursued in future studies.

In 32-week old rats fed a high-fat/high-fructose diet for 28 weeks (DIO rats), we found that a decreased expression of arterial $SK_{Ca}$, $IK_{Ca}$, and Kir2.1 $K^{+}$ channels was associated with a decreased FMVD, acetylcholine- and $K^{+}$-induced vasodilatation and an increase in MAP. This was interpreted as the loss of EDH-type vasodilatation due to the metabolic syndrome. In humans, obesity and diabetes leads to hypertrophic remodeling of resistance arteries. We found that outward hypertrophic remodeling in small arteries from obese and diabetic Göttingen minipigs was correlated with increased fasting plasma levels of glucose and cholesterol, and with the mRNA expression levels of Rho-kinase 1 and Transglutaminase 2. The data are discussed in relation to the metabolic syndrome and vascular aging in general. (COI:No)

# Planned Symposium 17

## Cardiac mechano-physiology: physiology and pathophysiology of the mechano-electrical coupling

(March 18, 15:20～17:10, Hall 6)

### PS17-01
#### Role of mechanically induced crosstalk between organelles in myocardial mechanics

Gentaro Iribe (*Dept Physiol, Asahikawa Med Univ, Japan*)

Cardiac muscle adapts to acutely altered mechanical load by a rapid change in contractile force. Therefore, it is not surprising if the heart is regulated by feedback from its mechanical state to functions of excitation contraction coupling (ECC) modules. Recent observations demonstrated that myocardial stretch hyperpolarizes mitochondrial membrane potential ($\Delta\psi$m) and increases NADPH oxidase (NOX)2-derived reactive oxygen species (ROS) production, which causes an immediate increase in $Ca^{2+}$ sparks. However, physiological roles and underlying mechanisms of these responses remain unknown. In the present study, we hypothesized that stretch-induced nucleotide signaling (extracellular nucleotide release via pannexin hemichannels followed by an activation of purinergic P2 receptors) is involved with these responses to adapt increased preload. To probe the possibilities of our hypothesis, enzymatically isolated mouse ventricular myocytes were subject to stretch using carbon fiber technique to study stretch-induced changes in $\Delta\psi$m and ROS production, and single cell mechanics. Applying stretch significantly hyperpolarised $\Delta\psi$m, while the response was abolished in the presence of carbenoxolone (CBX), an inhibitor of pannexin hemichannels. Stretch-induced increase in ROS production was abolished in the presence of either Pyridoxalphosphate-6-azophenyl-2', 4'-disulfonic acid (PPADS), a P2 purinergic antagonist, or CBX. Stretch did not affect any morphological parameters of $Ca^{2+}$ transient in C57BL/6 wild type (WT) mice, while the stretch significantly delayed the time to maximal rising rate of $[Ca^{2+}]_i$ in NOX2 knock out (KO) mice. The resultant cellular contractility in NOX2 KO group was significantly lower than WT group. The present results suggest that stretch-induced extracellular ATP release via pannexin hemichannels may reduce intracellular ATP, therefore, stimulates electron transport chain to hyperpolarize $\Delta\psi$m. And then the released ATP activates P2 to NOX2 pathway for stretch-induced ROS production, which facilitates $Ca^{2+}$ release from the sarcoplasmic reticulum to maintain cellular contractility against increased preload. (COI:No)

### PS17-02 (AP-6)
#### The role of TRPC3 and TRPC6 in a stretch-induced slow force response in cardiomyocytes

Yohei Yamaguchi[1], Gentaro Iribe[1], Keiji Naruse[2], Akira Takai[1] ([1]*Dept Physiol, Med, Asahikawa Med Univ, Japan,* [2]*Dept Cardio Physiol, Grad Sch Med, Okayama Univ, Japan*)

An increase in preload induces a biphasic active force enhancement in the heart. The short-term increase in preload rapidly augments contractile force due to the Frank-Starling mechanism, which accelerates $Ca^{2+}$ sensitivity of contraction proteins. A further long-term increase in preload for several minutes to hours causes the increase in $[Ca^{2+}]_i$, leading to a slow force response to stretch (SFR), and a further increase in the contractile force. The stretch-induced release of angiotensin II has been implicated in the SFR, to raise intracellular $Na^+$ levels, followed by an increase in intracellular $Ca^{2+}$ levels via the $Na^+/Ca^{2+}$ exchanger. However, the extracellular cation influx pathway is poorly understood. To better understand the cation influx pathway, we focused on TRPC3 and TRPC6, receptor-operated, mechanosensitive non-selective cation channels. In our studies, cardiomyocytes were enzymatically isolated from mouse hearts, and both cell ends were held by two carbon fibers to apply stretch to the cells. Using this stretch device, we found that TRPC3 and TRPC6, regulated by the angiotensin II type 1 (AT1) receptor via diacylglycerol produced by phospholipase C, played a pivotal role in the SFR. Our recent data also showed that stretch-induced activation of TRPC3 increased intracellular $Ca^{2+}$ influx, causing the accumulation of $Ca^{2+}$ in the sarcoplasmic reticulum, which in turn increased $Ca^{2+}$ release and, consequently, the twitch force, suggesting that a functional sarcoplasmic reticulum (SR) is necessary for the SFR. Other data indicate that a myocardial stretch stimulated the AT1 receptor, followed by endothelin release to increase nicotinamide adenine dinucleotide phosphate oxidase (NOX)-derived reactive oxygen species, regulating TRPC3 and TRPC6 to increase $Na^+$ influx, leading to the SFR. These data suggest the possibility that two AT1 receptor-operated cation influx pathways via TRPC3 and TRPC6, causing the enhancement of $Ca^{2+}$ release from the SR, may cooperate in the SFR. (COI:No)

### PS17-03
#### Pannexin-1 Contributes to Maintenance of Cardiac Function against Acute Pressure-overload and Rapid Ventricular Pacing

Kensuke Ihara[1], Tetsuo Sasano[2], Kentaro Takahashi[1], Masahiro Yamazoe[1], Tetsushi Furukawa[1] ([1]*Dept Bio-inform Pharm, MRI, TMDU, Japan,* [2]*Dept Cardiovasc, TMDU, Japan*)

Pannexin is a member of gap-junction channels and consists of 3 isoforms, pannexin-1, -2, and -3. Ventricles of hearts mainly express pannexin-1, while atrium express pannexin-2. Unlike connexin, pannexin expresses only on the surface of membrane, not between a cell and a cell, and transports small molecules including ATP between inside and outside of the cell. Mechanical stimuli change the conformation of the C-terminus of pannexin-1, thereby inducing release of ATP. For example, it was shown that ATP release from erythrocytes when they travel through narrowed vessels can dilate vessels and compensate disturbed blood circulation, as a protective mechanism against ischemia. Here, we examined the roles of pannexin-1 in hearts using global pannexin-1 knock-out (Panx1-/-) mice. After transverse aortic constriction (TAC) surgery, survival rate was significantly lower in Panx1-/- than in WT mice. Movement of ventricular walls was severely deteriorated in Panx1-/- mice, not in WT mice, immediately after TAC operation. We also examined the effects of rapid pacing in WT and Panx1-/- mice. Rapid pacing at 900 bpm caused circulatory collapse in Panx1-/- mice immediately after start of pacing, WT mice could tolerate it for at least 30 minutes. After pacing with slower rate (750bpm), left ventricular fractional shortening (%FS) was significantly reduced in Panx1-/- mice, but not in WT mice. During pacing, coronary flow velocity was significantly less in Panx1-/- mice than in WT mice. The average diameter in both left and right coronary arteries, measured after hearts had been chemically made transparent, was significantly smaller in Panx1-/- mice than in WT mice. Capillary density was also significantly lower in Panx1-/- mice than WT mice. From these data, we conclude that pannexin-1 acts as a protective mechanism against pressure overload and tachycardia in hearts via maintaining coronary circulation. (COI:No)

### PS17-04
#### Electropharmacological analysis of precordial percussion pacing in a cardiac standstill animal model: Clinical efficacy of knocking the chest as a "bridge to pacemaker"

Atsushi Sugiyama[1,2], Mihoko Hagiwara-Nagasawa[1], Ryuichi Kambayashi[1], Ai Goto[1], Koki Chiba[1], Yoshio Nunoi[1], Hiroko Izumi-Nakaseko[1], Akio Matsumoto[2] ([1]*Dept Pharmacol, Faculty Med, Toho Univ, Japan,* [2]*Dept Aging Pharmacol, Faculty Med, Toho Univ, Japan*)

Benefits of precordial percussion pacing (PPP) during cardiac standstill are unknown. We created a cardiac standstill model with microminipig by inducing complete atrioventricular block with a catheter ablation technique. The efficacy of cardiopulmonary resuscitation by standard chest compressions (S-CPR), PPP and ventricular electrical pacing in this model were analyzed in series. To assess the mechanism of PPP, a non-selective stretch-activated channel blocker amiloride was administered during PPP. Peak systolic and diastolic arterial pressures during S-CPR, PPP and ventricular electrical pacing were statistically similar. However, the duration of developed arterial pressure with PPP was comparable to that with ventricular electrical pacing, and significantly greater than that with S-CPR. Amiloride decreased the induction rate of ventricular electrical activity by PPP in a dose-related manner. Each animal survived without any neurological deficit at 24, 48 h and 1 week, even with up to 2 h of continuous PPP. In microminipig with cardiac standstill, PPP can become a novel means to significantly improve physiological outcomes after cardiac standstill or symptomatic bradyarrhythmias even without cardiac pacing. Activation of the non-selective stretch-activated ion-channels may be involved in the mechanophysiological effects of PPP. Thus, PPP was shown to effectively provide a stable heart rate and blood pressure without inducing lethal ventricular arrhythmias, and to prevent neurological deficits during cardiac standstill. The efficacy and safety of PPP as shown in pigs have been recently demonstrated in a patient with a cardiac standstill, indicating that PPP may also have significant utility for patients with life-threatening bradycardia until more definitive therapies are available. (COI:No)

# Planned Symposium 18

## Inter-organ communication: molecular mechanism and pathophysiology induced by its disruption

(March 18, 15:20〜17:10, Hall 8)

### PS18-01

#### A Sarcoplasmic Reticulum Localized Protein Phosphatase Regulates Phospholamban Phosphorylation and Promotes Ischemia Reperfusion Injury in Heart

Toru Akaike[1], Susumu Minamisawa[1], Yibin Wang[2] (*[1]Dept Cell Physiol, The Jikei Univ, Japan, [2]Dept Anesthesiology, Univ of California, Los Angeles, USA*)

Phospholamban is a key regulator of sarcoplasmic reticulum calcium uptake in cardiomyocyte, its inhibitory activity to SERCA is regulated by phosphorylation. Phospholamban hypophosphorylation is a common molecular feature in failing heart. We have discovered that protein phosphatase 2Ce (PP2Ce) is a novel serine/threonine protein phosphatase specifically targeted to sarcoplasmic reticulum membrane, is a specific and potent phospholamban phosphatase in cardiomyocytes. However, its function has not unknown yet. Here we assessed the hypothesis that PP2Ce is an important player in calcium cycling and cardiac remodeling in heart. PP2Ce expression was elevated in failing human heart and induced acutely at protein level by $\beta$-adrenergic stimulation or oxidative stress in cardiomyocytes. PP2Ce protein stronger dephosphorylated $\beta$-adrenergic stimulated increase of phospholamban phosphorylation at threonine 17 site than at serine 16 site in heart. PP2Ce expression reduced $\beta$-adrenergic stimulated intracellular calcium transient in isolated adult rat ventricular myocytes, and promoted hydrogen peroxide and ionomycin induced cell death in cultured neonatal rat ventricular myocytes. Transgenic mice with cardiac specific expression of PP2Ce showed no significant basal phenotype. However, in isolated perfusion heart preparation, we observed significantly larger infarct sizes and more impaired functional recovery following global ischemia/reperfusion injury in the transgenic hearts comparing to wild type controls. Therefore, PP2Ce is a novel component of sarcoplasmic reticulum calcium regulatory network that has a potentially important role in cell death regulation and cardiac contractility. (COI:No)

### PS18-02

#### TAK-242, an antagonist of toll-like receptor 4, attenuates cardiac remodeling induced by infusion of *Porphyromonas gingivalis* lipopolysaccharide in mice

Yoshiki Ohnuki, Kenji Suita, Satoshi Okumura (*Dept Physiol, Tsurumi Univ Sch Dent Med, Japan*)

*Porphylomonas gingivalis* (PG) is one of the most frequent pathogens in periodontal disease (PD) and its endotoxin, lipopolysaccharide (LPS), circulates systemically in over 50% of PD patients. Circulating low systemic PG-LPS has been reported to induce cardiac dysfunction; however, the underlying mechanisms remain elusive. In order to elucidate the contribution of toll-like receptor 4 (TLR4) to the PG-LPS-induced cardiac dysfunction, we investigated the effects of co-administration of TAK-242, an antagonist of TLR4, with PG-LPS on cardiac function in mice. Eight-week-old male mice (C57BL/6) were divided into four groups, control (vehicle), LPS (0.8 mg/kg/day, i.p.), TAK (3 mg/kg/day, i.p.) and LPS+TAK groups. After the completion of each treatment for 4 weeks, echocardiographic measurements were performed under anesthesia, and then the hearts were excised and used for later analyses. Compared to control group, left ventricular ejection fraction was significantly decreased in LPS group, but the decrease was blunted in LPS+TAK group. On the other hand, the number of apoptotic myocytes and the area of fibrosis were significantly increased in LPS group, but not in LPS+TAK group, compared to control group. In addition, phosphorylation levels of calmodulin kinase II (CaMKII, Thr-286) and $Ca^{2+}$-handling proteins, phospholamban (PLB, Thr-17) and ryanodine receptor 2 (RyR2, Ser-2814) were also increased in LPS group, but not in LPS+TAK group, compared to control group. These results suggest that low systemic PG-LPS induces cardiac dysfunction in association with apoptosis and fibrosis by $Ca^{2+}$ leakage from sarcoplasmic reticulum through CaMKII-mediated phosphorylation of PLB and RyR2 in TLR4-dependent manner. (COI:No)

### PS18-03

#### Pathogenic factors of diabetic neuropathy: how pancreatic ß cell dysfunction affects peripheral nervous system?

Kazunori Sango, Hideji Yako, Naoko Niimi, Shizuka Takaku (*Diabetic Neuropathy PJ, Tokyo Met Inst Med Sci, Tokyo*)

Peripheral neuropathy is one of the major chronic complications of diabetes mellitus, and its prevalence correlates closely with the degree and duration of hyperglycemia caused by pancreatic ß cell dysfunction and impaired insulin secretion. Although its precise pathogenesis remains unclear, metabolic disorders of neurons and Schwann cells (e.g. augmentation of the polyol and other collateral glucose-utilizing pathways, formation of advanced glycation endproducts, oxidative and endoplasmic reticulum stress) and microvascular abnormalities are assumed to play key roles in progressive neural dysfunction and irreversible nerve fiber damage. Streptozotocin (STZ), a compound that has a preferential toxicity toward pancreatic $\beta$ cells, is widely utilized to induce diabetes in rodents, and the STZ-diabetic animals exhibit peripheral neuropathy that partially mimics human disorders. However, recent studies have indicated that several factors other than continuous hyperglycemia (e.g. recurrent short-term hypoglycemia and hyperglycemia, altered lipid metabolism, and impaired insulin actions on neurons and Schwann cells) may also contribute to the development of diabetic neuropathy. In this presentation, we introduce up-to-date studies regarding the neurological disorders in close association with the disruption of metabolic regulatory mechanisms. (COI:No)

### PS18-04

#### Towards Molecular Pathology Control for Lifestyle-Molecular Dissection of Hypertension and Atherosclerosis focusing on Eating habit

Tomoaki Ishigami (*Dept MS and CR, Grad Sch Med, Yokohama City Univ, Japan*)

Both hypertension and atherosclerosis are global health burden for healthy longevity especially among developed countries. Life with daily dietary excess of salt, fat and carbohydrate is characteristics for modern developed countries, and definite contributions to cardiovascular diseases and impairment of healthy longevity. However it is well known that there is farm relationship between the quality of daily meal and cardiovascular diseases, underlying molecular mechanisms are not known well. Detailed molecular pathophysiological analyses for these diseases should be essential for handling the subjects appropriately. For hypertension, it was shown that primary molecular abnormalities existed in tubular sodium transports by molecular genetical analyses for human hereditary and familial hypertension. We have analyzed molecular pathophysiological dissections for sodium sensitivity and hypertension focusing on Nedd4-2 which is ubiquitin ligase for epithelial sodium channels located in aldosterone sensitive distal nephron. Using Nedd4-2 C2 domain knock-out mice, we discovered oral sodium sensitive enhancements of sodium reabsorption via ENaC in the distal nephron. In addition, the commensal microbes-derived atherosclerosis via the metabolism-independent mechanisms have received increasing attention. However, whether the effect of the intestinal microbiota on atherosclerosis is mediated by immune mechanisms remain uncertain. Our reports provided evidence for a pathway of immune activation in commensal microbes-derived atherosclerosis, which driven by high fat and high carbohydrate diet with enhancement of substantial population of splenic B2 cell activation. (COI:No)

# Planned Symposium 19

## Progress and perspectives of neuroscience with data-driven intelligence

(March 18, 15:20〜17:10, Hall 10)

### PS19-01

#### Decoding animal behavior from cellular-resolution neural data

Yasuhiro Tanaka R.[1,2], Yoshito Masamizu[2], Yasuyo Tanaka H.[2], Takanori Shinotsuka[2], Masanori Matsuzaki[2] (*[1]Brain Science Institute, Tamagawa University, Japan, [2]Dept Physiol, Grad Sch Med, the University of Tokyo, Japan*)

Recently, more and more cellular-resolution neural data have been recorded with the advancement of technology, such as two-photon microscopy or silicon probes. At the same time, the extraction of animal behavior from movies has been automatized with deep learning techniques. With such technical progress, neural decoding, a machine-learning method to infer animal sensory input and motor output from neural data, is becoming essential in neuroscience study. We will first clarify what the neural decoding is like and why we use it both from machine-learning and neuroscience perspectives. Secondary, we will share our knowledge about evolving neural coding in the primary motor cortex (M1) of mice during motor learning as a practice of neural decoding. We recorded neuronal ensembles with two-photon microscopy in M1 of the mice that were trained to get water reward by pulling the lever with their right forelimb. We found layer-specific changes in the stability of the neural representation. In layer 2/3, the accuracy of neuronal ensemble prediction of lever trajectory remained unchanged globally, with a subset of individual neurons retaining high prediction accuracy throughout the training period. By contrast, in layer 5a, the ensemble prediction accuracy steadily improved, and one-third of neurons evolved to contribute substantially to ensemble prediction in the late stage of learning. Thirdly, we will show the application of a decoding-related technique to estimate neuronal dynamics. We recorded the neuronal activities of thalamic axons with two-photon microscopy in M1 of the mice that were trained similarly as above. In layer 1 axons, we found the sequence of the neuronal activity during lever-pull movements. Finally, we would like to discuss the recent study and future direction. (COI:No)

### PS19-02

#### Automated 3D reconstruction from a 2D stack of neuronal EM images

Hidetoshi Urakubo (*Grad Info, Kyoto Univ, Japan*)

Recently, there has been a rapid expansion in the field of micro-connectomics, which targets the three-dimensional (3D) reconstruction of neuronal networks from a stack of two-dimensional (2D) electron microscopic (EM) images. The spatial scale of the 3D reconstruction grows rapidly owing to deep neural networks (DNNs) that enable automated image segmentation. Several research teams have developed their own software pipelines for DNN-based segmentation. However, the complexity of such pipelines makes their use difficult even for computer experts and impossible for non-experts. We here introduce a new software program, called UNI-EM, that enables DNN-based EM image segmentation, including ground truth generation, training, inference, postprocessing, proofreading, and visualization. Non-computer experts can conduct EM image segmentation based on 2D/3D DNNs, such as 2D U-Net, 2D ResNet, 2D HighwayNet, 2D DenseNet, and 3D flood-filling networks (FFNs). We would like to show how we can obtain benefits from such automated segmentation on the software UNI-EM, together with the principles of DNN-based segmentation algorithms. We would also report the current status of a collaborative study with Prof. Yoshiyuki Kubota for 3D segmentation of a large-scale neuronal EM images. (COI:No)

### PS19-03

#### Acquisition of Large Volume EM Data Set and 3D Reconstruction with Automated Segmentation Application

Yoshiyuki Kubota[1,2] (*[1]Natl. Inst. Physiol Sci., Okazaki, Japan, [2]SOKENDAI*)

An electron microscopy (EM)-based reconstruction of neuronal circuits from serial ultrathin sections method was introduced about 30 years ago. It had been achieved all the steps manually: cutting the serial ultrathin sections using ultramicrotome, image capturing with transmission electron microscope (TEM), reconstruction using cardboard of the selected profiles of neural structures to provide impression of depth. A computer software for the reconstruction was introduced to make it more efficient in 1990s. This technology had not been popular because of a high skill demand in electron microscopy, however, the reconstruction analysis method offered significantly valuable results. Then, we started to adapt new EM technologies using scanning electron microscopy (SEM) for the neural network analysis using the reconstruction method in early 2000. It has been modified and improved them vigorously and a lot of noteworthy results were published in this decade. EM volume data sets were getting larger year by year, and the volume size could be huge in size. The success of large volume EM acquisition using these new EM systems has created an issue, i.e., how to process large image data sets thus obtained. Soon it became obvious that it was difficult to handle large EM volume data sets using conventional 3D reconstruction image processing computer applications. To achieve segmentation easily and efficiently, automated segmentation computer applications have been developed and used. Segmentation performance has increasingly been improved and reached to account for more than 90% of the volume. I would like to introduce our recent improvement for the segmentation of the large volume EM data set acquired with ATUM-SEM using the automated segmentation applications Flood Filling Network (FFNs). We found it works better for the EM data set with 30 nm thick sections than the one with 50 nm thick sections. (COI:No)

### PS19-04

#### Utilization of animal markerless motion capture in neuroscience

Jumpei Matsumoto[1], Hisao Nishijo[1], Koki Mimura[2], Kenichi Inoue[3], Yasuhiro Go[4], Tomohiro Shibata[5] (*[1]System Emotional Sci, Grad Sch Med, Univ of Toyama, Japan, [2]National Institutes for Quantum and Radiological Science and Technology, [3]Primate Research Institute, Kyoto Univ, [4]Exploratory Research Center on Life and Living Systems, National Institutes of Natural Sciences, [5]Grad Sch of Life Science and Systems Engineering, Kyushu Institute of Techonlogy*)

Animal behavior analyses are fundamental in neuroscience. The video-based analyses are important to measure animal's complex interactions with the environment and/or the other animals (e.g., social interactions), and to estimate animal's internal states based on the careful inspection of its motion and posture (e.g., freezing behavior). In the conventional video analyses, human observers visually inspect images or simple computer-based systems track binary images of animals. Visual inspection methods have often problems in reproducibility and objectivity, while it is difficult for the latter method to analyze complex behaviors. On the other hand, motion capture systems are useful to analyze detailed motions and postures quantitatively. However, this method has also problems; tracking markers must be attached to the animal's body in the conventional systems and the markers often disturb natural behavioral expression. Recent advances in 3D depth camera and deep learning have allowed image-based markerless motion capture, overcoming those limitations of the conventional approaches. In this paper, those new markerless motion capture tools and their applications are introduced. We will also show our plan to further improve those tools for a wider range of applications. Finally, we will discuss how to further utilize the "motion big data" obtained by motion capture. Markerless motion capture can be of great help in understanding the brain functions that underlie the adaptive behaviors of animals in complex real-world situations, as well as in understanding various neurological and neuropsychiatric disorders. (COI:No)

### PS19-05

#### Optical and computational dissection of neural circuit for fear memory

Masakazu Agetsuma[1,2,3], Issei Sato[4], Yasuhiro Tanaka R.[5], Atsushi Kasai[6], Yoshiyuki Arai[3], Miki Yoshitomo[1], Hitoshi Hashimoto[6], Junichi Nabekura[1], Takeharu Nagai[3] (*[1]Div Homeostatic Development, NIPS, Aichi, Japan, [2]Japan Science and Technology Agency, PRESTO, Kawaguchi, Japan, [3]The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Japan, [4]Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan, [5]Brain Science Institute, Tamagawa University, Machida, Japan, [6]Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Japan*)

For efficient and accurate information processing in cerebral cortex, neural circuit dynamics must be spatially and temporally regulated with great precision. Medial prefrontal cortex (mPFC) of rodents has been shown important for various types of learning and memory, including fear memory, and related to various psychiatric diseases. However, it has been challenging to understand the computational mechanism in the mPFC, of which major problems are the complexity and heterogeneity of the prefrontal networks. Here we investigate this by chronic two-photon Ca2+ imaging from populations of neurons in mouse mPFC in vivo, which allows us to 1) record activities simultaneously from large number of neurons at the single cell resolution with high temporal resolution, and 2) investigate changes of neuronal responses over the learning process. We investigated the change in responses of mPFC neurons during Pavlovian fear conditioning and memory retrieval using a new device to perform them under the microscope with a head fixed mouse. We used two types of tones: one of them (CS+) was associated with the aversive foot shock while the other (CS-) was not. Tone responsive cells were observed even from the early stage of learning process, and the ratio of different types (CS+ specific, CS- specific, responsive to both, non-responsive) was not changed over the learning process, suggesting that emergence of specific function in individual neurons was unlikely the basis to encode fear memory. In contrast, population responses were significantly changed during the learning process and maintained enhanced during memory retrieval, suggesting possible emergence of novel population codes. By further analyses based on machine learning, we detected neural ensembles crucial to decode the conditioned response (i.e. freezing behavior during CS+ after the learning). These results suggest that population coding may be a critical basis for the fear memory in mPFC. (COI:No)

# Planned Symposium 20

## Neural Mechanism of Psychological Stress: Molecules, Circuits and Disorders

(March 19, 9:00～10:50, Hall 2)

### PS20-01

#### Central circuit mechanisms of sympathetic and behavioral responses to psychological stress

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

Psychological stress elicits a variety of sympathetic and behavioral responses in mammals, which help animals cope with stressors. Sympathetic stress responses include increases in body temperature, heart rate and blood pressure. However, the central circuit mechanisms for psychological stress responses are unknown. The initial purpose of our stress research project was to elucidate the brain network that evokes stress-induced sympathetic responses. We have reported that psychological stress induces thermogenesis in brown adipose tissue (BAT), hyperthermia and tachycardia by activating a monosynaptic neural pathway from the dorsomedial hypothalamus (DMH) to the rostral medullary raphe. Recently, we discovered that the DMH receives glutamatergic excitatory stress inputs from the dorsal peduncular cortex and dorsal tenia tecta (DP/DTT), located at the ventral limit of the medial prefrontal cortex. To determine how important the DP/DTT–DMH neural pathway is for the drive of sympathetic stress responses, we selectively lesioned DP/DTT–DMH projection neurons in rats by Cre-dependent expression of a genetically engineered caspase-3 with a combination of anterograde and retrograde adeno-associated viruses injected into the DP/DTT and DMH, respectively. Lesions of DP/DTT–DMH neurons abolished BAT thermogenesis and hyperthermia induced by social defeat stress, a psychosocial stress model. We further tested the effect of optogenetic inhibition of the DP/DTT–DMH pathway on stress-induced social avoidance. Wistar rats that underwent social defeat stress avoided interaction with the dominant Long-Evans rats that defeated the Wistar rats. Intriguingly, optogenetic inhibition of DP/DTT–DMH projection neurons in the stressed Wistar rats, which expressed iChloC-mCherry, a chloride-conducting channelrhodopsin, restored social interaction, and also eliminated sympathetic stress responses including hyperthermia and tachycardia. These results demonstrate that DP/DTT neurons transmit a glutamatergic excitatory stress input to the DMH, which is a master signal to drive a variety of autonomic and behavioral stress responses. (COI:No)

### PS20-02

#### Multiple roles of social stress-induced inflammatory responses in the brain

Tomoyuki Furuyashiki (*Div Pharmacol, Grad Sch Med, Kobe Univ, Japan*)

Stress due to adverse and demanding conditions may alter mental and physical functions, and is thought to precipitate various diseases including depression. Depressive subjects show elevated levels of proinflammatory molecules in the blood and an inflammatory marker in the brain. However, the causal relationship between inflammation and stress-related pathology of depression remained elusive. Using social defeat stress, a mouse model for depression research, we have revealed roles of inflammatory responses in the brain for stress-induced depressive-like behaviors. Repeated social defeat stress induces microglial activation in the medial prefrontal cortex (mPFC) through innate immune receptors TLR2/4, thereby leading to neural and behavioral changes through proinflammatory cytokines IL1$\alpha$ and TNF$\alpha$. HMGB1, a non-histone nuclear protein that can act as an endogenous TLR2/4 ligand upon its extracellular release, is translocated from the nuclei of mPFC neurons upon repeated social defeat stress, and promotes depressive-like behaviors. In parallel, repeated social defeat stress attenuates mPFC dopaminergic response to increase stress susceptibility through prostaglandin (PG) $E_2$ and its receptor EP1. Social defeat stress upregulates $PGE_2$ synthesis in subcortical regions in a manner dependent on monoacylglycerol lipase that catalyzes 2-arachidonoylglycerol to free arachidonic acid and cyclooxygenase 1, a PG synthase expressed in microglia. Notably, this $PGE_2$ synthesis also depends on TLR2/4 integrity. Collectively, we propose that multiple microglia-derived inflammatory responses upon social stress are coordinated by innate immune receptors and promote depressive behaviors through distinct, but cooperative, mechanisms. (COI:Properly Declared)

### PS20-03

#### Neuronal hypertrophy dampens stress responsiveness during habituation

Wataru Inoue (*Robarts Research Institute, Western University, Canada*)

Encountering a stressor immediately activates the hypothalamic-pituitary-adrenal (HPA) axis, but this stereotypic stress response also undergoes experience-dependent adaptation. How the brain adjusts stress responsiveness in the long-term remains poorly understood. We studied hypothalamic corticotropin-releasing hormone neurons that form the apex of the HPA axis in a mouse model of repeated restraint. Using patch-clamp electrophysiology, we found that the intrinsic excitability of these neurons substantially decreased after daily repeated stress in a time course that coincided with their loss of stress responsiveness in vivo. This intrinsic excitability plasticity co-developed with an expansion of surface membrane area, which increased a passive electric load, and dampened membrane depolarization in response to the influx of positive charge. Multiphoton imaging and electron microscopy revealed that repeated stress augmented ruffling of the plasma membrane, suggesting an ultrastructural plasticity that may efficiently accommodate the membrane area expansion. Overall, we report a novel structure-function relationship for intrinsic excitability plasticity as a neural correlate for habituation of the neuroendocrine stress response. (COI:No)

### PS20-04

#### Mechanisms for regulation of fear memory after retrieval

Satoshi Kida (*Grad Sch Agricul Life Sci, Univ Tokyo, Japan*)

Memory retrieval is not a passive process. Retrieval of contextual fear memory by short or long reminders initiates reconsolidation and extinction, respectively; reconsolidation maintains or enhances fear memory, while extinction weakens it. We have tried to understand mechanism for regulation of fear memory after retrieval at the molecular, cellular and circuits levels using contextual fear conditioning and inhibitory avoidance tasks. Our previous studies showed that reconsolidation of fear memory requires the activation of gene expression in the hippocampus, mPFC and amygdala, while long-term extinction requires gene expression activation in the mPFC and amygdala. Based on these findings, we are trying to identify and characterize fear and extinction engram neurons in the hippocampus, mPFC and amygdala.

We identified fear engram neurons in the hippocampus and examined the function of these neurons using c-fos-tag system. Fear engram neurons were labeled by ChR2 or ArchT and effects of optogenetic activation and inactivation of them were examined during memory retrieval. Inactivation of fear engram neurons during retrieval erased contextual fear memory, perhaps, by blocking memory reconsolidation, whereas activation of these engrams during retrieval blocked acquisition and consolidation of fear memory extinction. These results suggest that modulation of fear memory engram cells are crucial processes to determine the fate of memory; reconsolidation or extinction.

We also identified fear and extinction engram neurons in the amygdala and mPFC. Interestingly, distinct fear and extinction engram neurons were observed in the amygdala, while single neuronal fear/extinction engram neurons was observed in the mPFC. We are now characterizing these engram neurons using optogenetics. (COI:No)

# Planned Symposium 21

## The ethics, laws, and guidelines for human and animal researches

(March 19, 9:00～10:50, Hall 4)

### PS21-01

#### Law for the Humane Treatment and Management of Animals - past, present and future perspectives -

Naoko Kagiyama (*Central Institute for Experimental Animals*)

The Law for the Humane Treatment and Management of Animals (Law) was enacted in 1973. The law protects mammals, birds and reptiles, and emphasizes respect for life and well-being of animals. It specifies the responsibility of the owner of the animal, and calls for the alleviation of pain and distress as well as the humane death of animals used for scientific purposes. Based on the law, the Standards Relating to the Care and Management of Experimental Animals (Standards) were specified in 1980. In the same year, the Science Council of Japan (SCJ) advised the government to prepare administrative guidance for the use of animals for scientific purposes.

The law specifies the 3R-Principle (Replacement, Reduction and Refinement) of animal experimentation and adopts the institutional self-regulation system because life science should not be regulated only for the aspect of humane treatment of animals, and "Replacement" and "Reduction" were considered as the items to be discussed the animal experiment protocols prepared. In addition, appropriate animal experimentation could be better accomplished based on guidelines and not by stringent legislation.

Since 2006, animal experimentation has been conducted based on the institutional regulation system following the Law, Standards, and basic policies on animal experimentation (MEXT, MHLW, MAFF) as quasi-regulations. The items to be investigated when drafting an animal experiment protocol are listed in the detailed guidelines formulated by SCJ. As proposed by SCJ, adequate self-regulation should be validated by an outside organization and such validation improves the social transparency of animal experimentation. However, there is still disagreement over whether we should aim toward more stringent regulations, or continue the current self-regulation system. The author feels some limitations in the Law for balancing scientific justification of procedures and animal well-being. It is time to discuss more appropriate guiding systems not only for scientists but for the public. (COI:No)

### PS21-02

#### Guidelines for the Care and Use of Laboratory Primates in Neuroscience and Behavioral Research

Katsuki Nakamura (*Primate Res Inst, Kyoto Univ*)

In Japan, the Ministry of the Environment published Act on Welfare and Management of Animals and Standards related to the Care and Keeping and Reducing Pain of Laboratory Animals for *Appropriate Care and Keeping of Animals*. The Ministry of Education, Culture, Sports, Science and Technology published Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities on Academic Research Institutions for *Appropriate Use of Laboratory Animals*. In addition to these, the Science Council of Japan published Guidelines for Proper Conduct of Animal Experiment. Then each university or Institution should have its own guidelines for animal experiments. When we conduct animal research, the research should adhere to these laws and guidelines. In a university, the president is the responsible person for all animal experiments. The university should have the animal care and use committee and education and training program. Information disclosure about animal experiments is demanded. Our system is, so-called Institution or University Management System. We think this system works very well.

However, once we think international collaboration or data sharing, our system is very complicated. Two years ago, the Japan Neuroscience Society set up an ad-hoc committee to publish Guidelines for the Care and Use of Laboratory Primates in Neuroscience and Behavioral Research. We will publish these guidelines both in Japanese and English. In this talk, I am going to introduce the guidelines. (COI:No)

### PS21-03

#### Rules for medical research on human subjects in Japan

Tsunakuni Ikka (*National Cancer Center, Japan*)

I am going to speak on the following points.
1) The basic idea of Reserch Ethics; Protection of human subjects.
2) Ethical guideline for medical research on human subjects.
3) Clinical trial act. (COI:No)

### PS21-04

#### Recent trends in global neuroethics: an overview

Norihiro Sadato (*Nat'l Inst Physiol Sci*)

The purpose of neuroscience research on humans is to clarify how the brains of healthy people work. Obtaining new knowledge about brain function is essential scientifically, and it also leads to the elucidation of the pathological conditions of various brain diseases and the development of treatments. As the number of patients with dementia and movement disorders will increase in an aging society, such research has social significance. On the other hand, accurately communicating the new knowledge gained about the work of the brain to the general public is essential to gain trust from society and to recognize the social usefulness and significance of the research. Care should be taken not to cause human rights violations as a result of non-invasive brain function studies. With recent advances in artificial intelligence (AI) and robots, and the development of neuro-intervention technology, international discussions on the above ethical aspects in neuroscience are increasing. In particular, there are increasing concerns about individual identity as basic human rights (i.e., physical and mental integrity), behavioral independence (ability to choose behavior), and the possibility of infringing on privacy. The importance of explanation and consent in participating in experiments as a procedure for protecting human rights is emphasized. Also, sufficient attention must be paid to the changes in social norms and the possibility of new forms of discrimination caused by specific cognitive enhancement using neural intervention techniques. As neuroscience is now a global effort, neuroethics must be equally ready to address global values. The group of internationally recognized brain projects (the International Brain Initiative, IBI) forms the Neuroethics Workgroup that organized The Global Neuroethics Summit in 2017 through 2019. The trends of global neuroethics will be overviewed by introducing the series of summit meetings. (COI:No)

# Planned Symposium 22

## Challenge for the unsolved mechanism of arrhythmias; from the view of physiologists and cardiologists

(March 19, 9:00～10:50, Hall 5)

### PS22-01

#### There remained issues to be solved in infants, children, and adolescents with long QT syndrome

Masao Yoshinaga (*National Hospital Organization Kagoshima Medical Center*)

Huge progress has been achieved in understanding the pathogenesis, diagnosis, and treatment of inherited arrhythmia syndrome, particularly of long QT syndrome (LQTS) after discovery of LQTS-causative genes and rapid advances in DNA sequencing technologies. Knowledge gained from the progress includes genotype-phenotype relationships and gene-specific risk-management strategies; however, exercise-related cardiac events occur not only in patients with LQT1 but in those with LQT2 and LQT3. Cardiac events by emotion, during rest or sleep without arousal occur in both patients with LQT2 and LQT3, although the underlying causative genes for ion channels are different. Additionally, a Japanese study also showed circadian distribution of cardiac events, suggesting the effect of autonomic nervous activity. Details of genetic and physiological backgrounds in terms of circadian distribution and autonomic effects remain unclear.

In Japan, a school-based ECG screening is mandatory for all first, seventh, and tenth graders from 1995. Our data showed that the cumulative risk of symptom in patients with definite LQTS considerably decreased from the 2005-2011 to 2012-2018 periods, suggesting that the program had a profound impact on the improvement in the outcome. At the same time, the program uncovered many patients with a definite LQTS without symptoms. Pediatric cardiologists in Japan should consider an additional guideline for asymptomatic patients who are diagnosed through this program.

Finally, one of the most important issues in pediatric fields is prevention of sudden infant death syndrome (SIDS). SIDS is multi-factorial in origin, but genetic analysis showed that approximately 10% of SIDS victims carry functionally significant mutations in LQTS genes. Up to 83% of SIDS occur during night-time sleep. Number of SIDS was 109 in 2016 in Japan; however, number of out-of-hospital cardiac arrest with unknown etiology in infancy was 428 in 2016. Association between QT dynamics and sleep should be thoroughly investigated.

(COI:No)

### PS22-02 (AP-4)

#### Molecular characterization of the arrhythmogenic trigger unique to pulmonary vein cardiomyocytes

Yosuke Okamoto[1], Yoshinobu Nagasawa[2], Aung Naing Ye[3], Yukiko Himeno[4], Akinori Noma[4], Akira Amano[4], Daichi Takagi[1], Kuniaki Ishii[5], Kyoichi Ono[1] (*[1]Dept Cell Physiol, Grad Sch Med, Akita Univ, Japan, [2]Dept Pharmacol Ther, Fac Pharm Sci, Toho Univ, Japan, [3]Dept Pathol Diagn, Fac Med, Yamagata Univ, Japan, [4]Dept Bioinfomat, Coll Life Sci, Ritsumeikan Univ, Japan, [5]Dept Pharmacol, Fac Med, Yamagata Univ, Japan*)

Pulmonary veins (PVs) are the major origin of atrial fibrillation. We have reported that $IP_3R_2$ in rat PV cardiomyocytes cooperates with $Na^+$-$Ca^{2+}$ exchanger (NCX) on T-tubule in triggering the norepinephrine (NE)-induced automaticity, and identified a unique hyperpolarization activated $Cl^-$ current, $I_{Cl,h}$, that potentially facilitates the automaticity. The mathematical model which incorporated the interaction between $IP_3R_2$ and NCX, and electrophysiological characteristics of $I_{Cl,h}$ successfully reproduced the NE-induced automaticity. Here, we show two further topics for the properties of rat PV cardiomyocytes in relation to its arrhythmogenicity. The first one is that a $Ca^{2+}$-stimulable adenylyl cyclase (AC) is involved in the NE-induced automaticity. Microarray, RT-PCR and immunohistochemistry uncovered that one of $Ca^{2+}$-stimulable AC was expressed regionally in the supraventricular area including PV. In particular, enriched expression of the AC was detected along T-tubule of PV myocytes, while atrial myocytes hardly displayed T-tubules. HEK293 cells exhibited sustained $Ca^{2+}$ oscillation in response to UTP under isoproterenol pre-application. Gene-knockout of our interest in the cells impaired the ability to keep the $Ca^{2+}$ oscillation. The NE-induce automaticity in PV cardiomyocytes was reversibly arrested by AC inhibitor. The second topic is related to the structural basis of $I_{Cl,h}$. Mass spectrometry identified HSPA8 as the CLCN2 interacting protein from rat PV. The auxiliary subunit of CLCN2 was subcloned and introduced into PC12 cell. With co-expression of the HSPA8, CLCN2 current exhibited the unique voltage-dependency similar to $I_{Cl,h}$. All these findings suggest that unique molecular interaction among NCX, $IP_3R_2$, and the AC along T-tubule potentiates the arrhythmogenicity of rat PV, and that rat heart possessed HSPA8 as the auxiliary subunit of the hyperpolarization activated $Cl^-$ channel.

(COI:Properly Declared)

### PS22-03

#### Possibility of recent balloon technologies for persistent atrial fibrillation ablation

Shiro Nakahara (*Dept Cardiology, Dokkyo Medical University Saitama Medical Center*)

Ablation of persistent and long-standing persistent atrial fibrillation (AF) is a potentially complex procedure that carries a lower success rate than ablation of paroxysmal AF. Atrial substrate modification is required for a successful outcome in most patients with persistent AF. Substrate modification is considered when AF persists despite effective elimination of the pulmonary vein (PV) arrhythmogenicity by an extra-ostial PV isolation (PVI), antral PVI, or wide area circumferential ablation. Currently, we favor a posterior wall isolation, careful attention to low-voltage areas, and non-pulmonary vein trigger mapping for the persistent and long-standing persistent AF population. The balloon ablation system is a safe and predictable toolset to generate a reliable large-area pulmonary vein antral modification for the treatment AF. Further, hot balloon ablation of AF makes use of a compliant balloon that optimizes the PV contact. This feature provides for both a wide antral ablation and ablation of the left atrial (LA) posterior wall, which is implicated in the perpetuation of AF. To achieve this effect, we developed a hot balloon-based wide antral ablation (HBWA) protocol and have since applied it in patients with persistent and longstanding persistent AF. In this session, the techniques and outcomes of the current substrate modification in patients with persistent AF will be reviewed and discussed.

(COI:No)

### PS22-04

#### Development of fatal arrhythmias mediating subcellular $Na^+$ channel expression changes: *in silico* study

Kunichika Tsumoto[1], Takashi Ashihara[2], Narumi Naito[3], Takao Shimamoto[3], Akira Amano[3], Yoshihisa Kurachi[4], Yuichi Kuda[1], Mamoru Tanida[1], Yasutaka Kurata[1] (*[1]Dept Physiol, Kanazawa Med Univ, Japan, [2]Dept Med Info Biomed Eng, Shiga Univ Med Sci, Japan, [3]Dept Bioinfo, Coll Life Scis, Ritsumeikan Univ, Japan, [4]Global Center Med Eng Info, Osaka Univ, Japan*)

Cardiac voltage-gated sodium ($Na^+$) channels play key roles in the action potential (AP) initiation and propagation. Numerous experimental studies have reported that the $Na^+$ channel expression was changed within each of the myocytes in congenital and acquired heart diseases such as Brugada syndrome and myocardial infarction. We hypothesized that an alteration in subcellular $Na^+$ channel expression caused fatal arrhythmias. To test this hypothesis, we have proposed physiologically relevant *in silico* human ventricular myocardial strand and ring models where myocytes were electrically connected by both gap junctions and an electric field mechanism, the latter of which involves an interference effect between intercalated discs (IDs), in which the electrical communication between myocytes is mediated by the extracellular potential changes elicited in the intercellular cleft space facing the IDs. We investigated the relationship between the altered subcellular $Na^+$ channel expression and arrhythmogenicity through computer simulations of AP propagation in the myocardial strand and ring models. In this symposium, we will present our recent simulation results and would like to discuss the proarrhythmic effects of alteration in the subcellular $Na^+$ channel expressions.

(COI:No)

### PS22-05

#### Inherited arrhythmia in the era of next generation sequencer

Seiko Ohno (*Dept.Bioscience and Genetics, NCVC. Japan*)

Inherited primary arrhythmia syndromes (IPAS) are diseases mainly caused by mutations in genes encoding ion channel and related proteins. Long QT syndrome (LQTS) is the most notable disease in IPAS. The main cause of LQTS is mutations in genes encoding potassium channel (KCNQ1 and KCNH2) or sodium channel (SCN5A). The genotypes caused by these genes are classified into LQT1, 2 and 3. Mutations in KCNQ1 and KCNH2 causes decrease of IKs and IKr, in contrast, mutations in SCN5A increase the late INa. These genotype and electrophysiological relationships have been progressed complementary. Since the early 2000s, next generation sequencer (NGS) system has been developed, and we can now access to the huge genetic data in short time. Until now, 17 genes have been reported in LQTS, and NGS enabled us to screen all the genes at once. We recently identified that the frequency of LQT8, caused by CACNA1C mutations was higher than we expected. The identification of novel mutations in CACNA1C encoding alpha subunit of L type calcium channel will help us understand calcium handling in the cardiomyocytes. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is one of the IPAS, and it was caused by mutations in RYR2 encoding cardiac ryanodine receptor. Although RYR2 is a huge gene, we can now screen RYR2 easily and new disease entity caused by RYR2 mutations has been shown. KCND3 encode KV4.3, an alpha subunit of Ito. We recently identified a novel KCND3 mutation in a patient with early repolarization syndrome. The mutation increased the Ito, and simulation study proved the mechanism of the early repolarization. Thus, new genetic discovery in IPAS using NGS lead us to find a new mechanism of cardiac electrophysiology. These progress in both fields will be indispensable to explore the effective treatment for IPAS.

(COI:No)

# Planned Symposium 23

## Angiology evolving into new research fields

(March 19, 9:00～10:50, Hall 6)

### PS23-01

#### Sequential phosphoinositide conversion is required for TGF$\beta$-induced receptor endocytosis and endosomal receptor signaling in endothelial cells

Sho Aki[1], Kazuaki Yoshioka[1], Noriko Takuwa[1,2], Yoh Takuwa[1] (*[1]Department of Physiology Kanazawa University School of Medicine, Japan, [2]Department of Health Science, Ishikawa Prefectural University, Japan*)

Phosphatidylinositol (PI) 3-kinases (PI3Ks) are a family of enzymes that phosphorylate membrane inositol phospholipids at the 3'-position of the inositol ring and comprise three classes. Class I PI3Ks, which mainly generate PI(3, 4, 5)$P_3$ are activated by RTK and GPCR to mediate activation of Akt and Rac and stimulation of cell proliferation and migration. Class III PI3K, Vps34, generates PI(3)P to regulate autophagy. In contrast to the well characterized class I and III PI3Ks, physiological roles of class II PI3Ks were not well understood. We previously demonstrated that class II $\alpha$ isoform (PI3K-C2$\alpha$), which generates PI(3, 4)$P_2$ by phosphorylating PI(4)P, is required for endocytosis of angiogenic receptors for TGF$\beta$, VEGF and S1P and endosomal signaling of these receptors, by which PI3K-C2$\alpha$ contributes to angiogenesis. However, it is unknown how the PI3K-C2$\alpha$ substrate PI(4)P is derived and the PI3K-C2$\alpha$ product PI(3, 4)$P_2$ is catabolized, and which enzymes are involved in the phosphoinositide conversions. TGF$\beta$ induced rapid decreases in PI(4, 5)$P_2$ at the plasma membrane (PM) with increases in PI(4)P, followed by increases in PI(3, 4)$P_2$, in a TGF$\beta$ receptor ALK5－dependent manner. siRNA－mediated knockdown studies showed that PI3K-C2$\alpha$, the 5'-phosphatase synaptojanin1 (Synj1), and the 4'-phosphatase INPP4B were required for TGF$\beta$－induced receptor endocytosis. TGF$\beta$ induced the recruitment of both Synj1 and PI3K-C2$\alpha$ to the PM with their substantial colocalization. Interestingly, PI3K-C2$\alpha$ knockdown abolished not only TGF$\beta$–induced PI(3, 4)$P_2$ increases, but also Synj1 recruitment to the PM, PI(4, 5)$P_2$ decreases. Finally, the phosphoinositide conversions were necessary for Smad2/3 signaling, participating in angiogenic actions of TGF$\beta$. These observations demonstrate that the sequential phosphoinositide conversions mediated by Synj1, PI3K-C2$\alpha$, and INPP4B are essential for TGF$\beta$ receptor endocytosis and its signaling. (COI:No)

### PS23-02

#### Intravital imaging analysis of the substance discharge through a vascular wall in microcirculation using noninvasive and nonlinear optical microscopy

Naoki Honkura[1,2] (*[1]Dept Med Physiol, Hamamatsu Univ, Japan, [2]PRESTO, JST*)

The functional blood circuit always provides oxygen, nutrients and endogenous hormonal molecules to our organs and tissues from blood vessels. These are essential to keep biological homeostasis of multicellular organisms. The capillary vessels, constructed mainly by endothelial cells, are known to undergo changes in blood flow, blood pressure, diameter, permeability in response to various stimuli on the local environment. The underlying mechanisms and interdependences of these responses in different vessel types are largely unknown. Here I report a non-invasive technique to study dynamic events in different vessel categories using nonlinear optical microscopy and an image analysis tool, RVDM (relative velocity, direction and morphology), which allows the classification of vessel categories by the calculated distortion-parameters of flowing red blood cell (RBC) captured with slow frame rate. Additionally, oxygen release from RBCs in blood vessels could be visualized by high spatio-temporal resolution images with multiphoton excitation of fluorescent molecules and harmonic generation that originated from biological molecules, which enabled us to analyze the location as well as the mechanism of oxygen release. Moreover, the mouse who expresses Claudin5 promoter-driven green fluorescent protein (GFP) is used to distinguish capillary subtypes by its fluorescence signal in blood vessels in in vivo. Intradermal injection of vascular endothelial growth factor A (VEGFA) into ear skin appeared to induce leakage of circulating fluorescent dye conjugated dextrans, with vessel-type dependent different kinetics, from only capillaries and venules devoid of GFP expression. VEGFA-induced leakage in capillaries coincides with vessel dilation and reduced flow velocity. Thus, intravital imaging of non-invasive stimulation combined with nonlinear optical effects and RVDM analysis appeared beneficial for recording and quantification of very rapid events taking place in the blood circuit. (COI:No)

### PS23-03

#### Lymphatic vascular development and embryonic edema in mice

Masanori Hirashima (*Div Pharmacol, Grad Sch Med Dent Sci, Niigata Univ, Japan*)

Lymphatic vessels comprise a secondary vascular system in mammals and play important roles in tissue fluid homeostasis, immune response, and fat absorption. We have been studying lymphatic vascular patterning in mouse embryonic skin and found that Aspp1 plays an important role in lymphatic vessel assembly. Aspp1$^{-/-}$ mice show embryonic edema with delayed lymphatic network formation and are growth-retarded during early postnatal periods. However, Aspp1$^{-/-}$ mice survive mostly to the adulthood without any overt abnormalities including lymphedema. We also found a similar transient embryonic edema with enhanced vascular permeability in haploinsufficient mice for Flt1/VEGFR1, a decoy receptor for VEGF-A. To address whether regulation of fluid homeostasis is crucial for embryonic development, we crossed these two mutant mice exhibiting a transient embryonic edema. Flt1$^{+/-}$; Aspp1$^{-/-}$ double mutant mice showed greater degree of embryonic edema, compared to either single mutant, and turned out to be lethal in large part. These results indicate that embryos are very sensitive to a change of fluid homeostasis. Embryonic edema in mice is very similar to the increased nuchal translucency, a sign of embryonic edema found by ultrasonography during human pregnancy. Here I would also like to discuss our novel genetic screen for gene mutations causing embryonic edema in mice to investigate the pathogenesis, risk, and prognosis of dysregulated fluid homeostasis. (COI:No)

### PS23-04

#### Light and dark sides of aPKC in endothelial cells

Masanori Nakayama (*MPI HLR, Germany*)

Cell polarity is critical for tissue and organ architecture and its loss is frequently associated with overproliferation and tissue expansion. The polarity protein atypical protein kinase C lambda/iota (aPKCl) is an oncogene and is associated with cell proliferation through unknown mechanisms. In endothelial cells, suppression of aPKCl impairs proliferation despite hyperactivated vascular endothelial growth factor (VEGF) signaling. Here we show that aPKCl phosphorylates the DNA binding domain of forkhead box O1 (FoxO1) transcription factor, a gatekeeper of endothelial growth. Although VEGF signaling is known to exclude FoxO1 from the nucleus, consequently increasing c-Myc abundance and proliferation, aPKCl controls c-Myc expression without affecting its localization. We find this pathway is strongly activated in the malignant vascular sarcoma, angiosarcoma, and aPKC inhibition reduces c-Myc expression and proliferation of angiosarcoma cells. Moreover, FoxO1 phosphorylation by aPKC and aPKC expression correlates with poor patient prognosis. Our findings may provide a new therapeutic strategy for treatment of malignant cancers, such as angiosarcoma. (COI:No)

# Planned Symposium 24

## What is 'data-driven' science

(March 19, 14:10〜16:00, Hall 1)

### PS24-01

#### Data-driven drug discovery and healthcare by artificial intelligence

Yoshihiro Yamanishi (*Dept Biosci Bioinf, Fac Comp Sci Syst, Kyushu Inst Tech, Japan*)

Recent developments in biotechnology have contributed to the increase in the amounts of high-throughput data in the genome, transcriptome, proteome, metabolome, phenome and diseasome. These biomedical big data can be useful resources for drug discovery and healthcare. Drug repositioning, or the identification of new drug indications (new applicable diseases of existing drugs), is an efficient strategy for drug development. The drug repositioning approach has received remarkable attention in pharmaceutical industry, because it can increase the success rate of drug development and to reduce the cost in terms of time, risk, and expenditure.
In this study, we developed novel machine learning methods (basic technologies of artificial intelligence) for predicting potential target proteins and new indications of drug candidate compounds toward automatic drug discovery and repositioning. We performed the prediction of unknown therapeutic effects based on various large-scale omics data of drugs, compounds, genes, proteins, and diseases in a framework of supervised network inference. Our results show that the proposed method outperforms previous methods in terms of accuracy and applicability. We performed a comprehensive prediction of new indications of all approved drugs and bioactive compounds for a wide range of diseases defined in the International Classification of Diseases. We show several biologically meaningful examples of newly predicted drug indications for cancers and neurodegenerative diseases. The proposed methods are expected to be useful for various applications in drug discovery and healthcare. (COI:No)

### PS24-02

#### A data-driven approach to identify drugs against ischemia-reperfusion injury

Yuhei Nishimura (*Dept Integrative Pharmacol, Mie Univ Grad Sch Med, Japan*)

Recent technological advances have enabled us to obtain large amounts of multi-level omics data. The increasing volume of multilevel omics data continues to create larger and more complex datasets that are publicly available and can be used to generate disease-associated biological networks and to identify potential therapeutic targets within the networks. Further progress in computational methodology combined with improved disease models will facilitate the prioritization of therapeutic targets in the networks. Data-driven approaches, such as the integration of public omics database, bioinformatics tools, and disease models, can provide a strong foundation for deciphering the complex mechanism of various diseases and for the identification of novel therapeutics. In this symposium, I would like to demonstrate an example of our data-driven approach to identify potential therapeutic drugs against ischemia-reperfusion injury. (COI:No)

### PS24-03

#### Pharmacological research development based on medical big data (disease name, Lab tests, medication, etc.) of 2.4 million patients at Nihon University Hospital for 15 years

Satoshi Asai (*Divi Pharma, Sch Med, Nihon Univ, Japan*)

Nihon University, having been striving for the further improvement of medical progress and medical services, is engaged in research concerning the utilization of information from daily clinical practice, and in 2004 has constructed the clinical data warehouse (CDW) known as "Nihon University School of Medicine's Clinical Data Management System" (NUSM's CDMS) As of March 2019 for 15years, this system stores over 2.43 million patient profiles, medical history data for 1.27 mil. patients (24 mil. records), drug prescription data of 690,000 patients (40 mil. records), injection medicine prescription data of 270,000 patients (17.5 mil. records), and clinical test data of 800,000 patients (440 mil. records). This system possesses a sufficient amount of information for highly reliable statistical analysis. We have announced to international journals our research thus far that utilizes this abundance of information, which includes studies of the additional effects in hypertensive drugs (add-on effects), and of the side-effects that occur in the combined use of antithrombotic drugs. NUSM's CDMS is a healthcare database based on information from daily clinical practice and furthermore constructed on the premise of use in research, already at a sufficiently practical level. In observational studies using NUSM'S CDMS, the drug effects, or so-called "effectiveness", in actual clinical settings, which cannot be understood through randomized clinical trials (RCTs) carried out in limited environments, are able to be verified and helpful evidence provided. (COI:Properly Declared)

### PS24-04

#### Drug repositioning and target finding based on clinical evidence

Shuji Kaneko (*Dept Mol Pharmacol, Grad Sch Pharm Sci, Kyoto Univ*)

Adverse drug reactions have been utilized to make animal models of human disease since there are some similarity between the human symptoms and adverse drug reactions. In this context, accumulating case reports of human adverse events would be attractive clinical evidence, if we could statistically identify a concomitant medication that reduces the occurrence of an adverse event. This simply enables 'drug repositioning' that proposes a practical relief of adverse reaction. Moreover, the drug-drug interaction may provide important clues to the discovery of new molecular mechanism of adverse effects, and hopefully, of human diseases. The FDA Adverse Event Reporting System (FAERS) is a public database that accumulates huge self-reports of adverse events. In nearly half of the cases, multiple drugs are prescribed, so that potential drug-drug interactions are to be analyzed. Focusing on adverse reactions relating to diabetes mellitus (DM) caused by an atypical anti-schizophrenic drug, quetiapine, we found that concomitant use of vitamin D analogs significantly suppresses the occurrence of the quetiapine-induced DM in FAERS. Experimental validation using mice revealed that quetiapine acutely caused insulin resistance, which was mitigated by dietary supplementation with cholecalciferol. In a gene expression database, several genes downstream of insulin receptor were downregulated by quetiapine in the liver. Further experiments clarified that a PI3K regulatory protein gene, pik3r1, was downregulated by quetiapine, which was reversed by cholecalciferol in mouse skeletal muscle. In addition, the insulin-stimulated glucose uptake into cultured myotubes was inhibited in the presence of quetiapine, which was reversed by pretreatment with calcitriol in a PI3K-dependent manner. These results suggest that vitamin D prevents the atypical antipsychotic-induced hyperglycemia and insulin resistance by up-regulation of PI3K function. This new strategy will pave the way for drug repositioning and clarifying unknown disease mechanisms. (COI:No)

# Planned Symposium 25

## Mechanisms of experience- or metabolism-dependent behavioral changes

(March 19, 14:10〜16:00, Hall 2)

### PS25-01

#### The role of the amygdalohippocampal area neurons that projects to the medial preoptic area

Taiju Amano (*Dept Pharmacol, Grad Sch Pharm, Hokkaido Univ, Sapporo, Japan*)

The medial preoptic area (MPOA) is thought to be the most important region for pup directed behavior. Previous study revealed that lesion of the MPOA blocked parental behavior and also initiated the aggressive behavior toward pups. However, the potent input sources into the MPOA to control the behavioral pattern is not well addressed. In this study, we explored the neural nucleus that projects to the MPOA and addressed the function and modulatory mechanisms. We infused retrograde tracer into the MPOA and checked the expression pattern of neural activity marker c-Fos in the mice experienced the exposure of pups. Among the sources of the input into the MPOA, amygdalohippocampal area (AHi) showed double positive neuron of retrograde tracer and immunoreactive to c-Fos. It is reported that AHi is one of the areas expressing oxytocin receptor (OXTR), which is important for affiliative behavior. Therefore, we performed whole-cell patch-clamp recording from the AHi neurons projecting to MPOA. Application of oxytocin increased the frequency of the spontaneous inhibitory post synaptic currents (sIPSC). Next, to address the role of the AHi neurons projecting to MPOA on the behavior toward pups, we infused adeno-associated virus to express hM3Dq. Activation of the AHi neurons projecting to MPOA resulted in the disruption of parental behavior and promoted the aggressive behavior toward pups. These evidences contribute to understand the circuit-based mechanism to control the parenting behavior. (COI:Properly Declared)

### PS25-02

#### The role of oxytocin in behavioral changes induced by social defeat stress

Ayumu Inutsuka, Masahide Yoshida, Yuki Takayanagi, Tatsushi Onaka (*Dept Physiol, Med, Jichi Med Univ, Japan*)

Stress affects various behaviors including social interactions. It has been reported that oxytocin modulates anxiety- and stress-related behaviors. The prefrontal cortex (PFC) is innervated by oxytocin neurons and densely expresses the oxytocin receptor (OXTR). In this study, we investigated the physiological role of the OXTR of the PFC in behavioral changes induced by social defeat stress. After social defeat stress, expression of c-Fos protein was increased in oxytocin neurons of the bed nucleus of the stria terminalis, supraoptic nucleus, and paraventricular hypothalamic nucleus. Using virus vectors employing GFP-dependent Cre, we genetically labeled and traced Venus-expressing neurons in the PFC of *Oxtr-Venus knock-in* mice. We found that OXTR-expressing neurons in the PFC consist of two populations: one is somatostatin-positive interneurons and the other is projection neurons in the layer 2/3 that send their axons to the amygdala. Chronic social defeat stress increased anxiety-related behaviors and induces social avoidance in C57BL/6J mice. We performed stereotaxic injections of Cre expressing adeno-associated virus vectors into the PFC of *Oxtr-floxed* mice and investigated the behavioral changes after chronic social defeat stress. We will discuss the behavioral differences between the test mice and the control mice. (COI:No)

### PS25-03

#### Molecular and Neuronal substrates that regulate nutrient appetite

Tsutomu Sasaki (*Nutrition Chemistry Lab, Div Food Science Biotechnol, Grad Sch Agr, Kyoto Univ, Japan*)

Nutrition is defined as the process of ingesting food for the purpose of growth, metabolism, and repair. In other words, food is ingested to obtain nutrients that are necessary for maintaining homeostasis. However, the studies on the homeostatic regulation of appetite have focused mostly on energy balance. Macronutrients (carbohydrate, fat, and protein) generates energy, but they cannot be distinguished among each other if we only think about calorie. It is empirically known that animals have appetite for nutrient (called "nutritional wisdom"), yet the molecular and neuronal substrates that regulate nutrient appetite remain largely elusive.

Our works on the homeostatic regulation of appetite by $NAD^+$-dependent protein deacetylase SIRT1 unexpectedly revealed a mechanism that regulates simple sugar appetite. Simple sugar ingestion induces secretion of the hepatokine FGF21, which activates hypothalamic oxytocin neuron, and suppresses simple sugar appetite. Therefore, FGF21-oxytocin axis is a negative feedback system that regulates simple sugar appetite.

My lab is currently working on fat appetite and protein appetite, and the updates on macronutrient appetite will be presented at the talk. (COI:No)

### PS25-04

#### Effects of copulatory experience on neural circuits controlling male sexual activity via the oxytocin-oxytocin receptor system in rats

Hirotaka Sakamoto (*Ushimado Marine Inst (UMI), Okayama Univ, Japan*)

Male sexual activity is activated by copulatory experience. However, it remains unclear that effects of copulatory experience on neural circuits in the central nervous system. The purpose of this study is to reveal effects of copulatory experience on neural circuits controlling male sexual activity in rats, focusing on the oxytocin-oxytocin receptor (OTR) system. To visualize OTR-expressing neurons, we generated an OTR promoter-human diphtheria toxin receptor-2A-channelrhodopsin-2-enhanced yellow fluorescent protein (ChR2-EYFP) BAC transgenic (Tg) rat line and studied effects of copulatory experience on neural circuits controlling male sexual activity by examining the EYFP fluorescence. OTR-expressing neurons were first localized by using these Tg rats, and the medial preoptic area, bed nucleus of the stria terminalis and ventrolateral part of the ventromedial nucleus (VMHvl) were clearly labeled with EYFP fluorescence. These nuclei are reported to express OTR in rats. Subsequently, effects of copulatory experience in males on OTR expression were examined, and copulatory experience increased in the expression levels of OTR in these 3 brain areas. Because the increase in the expression level of OTR (EYFP) in the VMHvl was remarkable, the toxin receptor cell-knockout (TRECK) and optogenetic analyses were performed in the VMHvl of the copulatory experienced males. By microinjecting diphtheria toxin into the VMHvl, the local lesion of OTR-expressing neurons significantly decreased the number of mounts during male sexual behavior. In contrast, the local optogenetic stimulation of OTR-expressing neurons in the VMHvl significantly attenuated ejaculation reflexes, but did not affect any pre-ejaculatory behaviors during normal sexual activity. In conclusion, these results suggest that copulatory experience reorganizes neural circuits expressing OTR, and consequently, the enhanced oxytocin-OTR system in the brain may facilitate male sexual activity. (COI:No)

# Planned Symposium 26

## New insights of sensory and brain functions affecting feeding behaviors: circadian, swallowing, taste and pain

(March 19, 14:10〜16:00, Hall 3)

### PS26-01

#### Meal Timing, Aging, and Circadian Rhythm

Wataru Nakamura (*Dept Oral Chrono-Physiol, Grad Sch Bio-Med, Nagasaki Univ, Japan*)

Some people can quite accurately time the end of their night's sleep without using an alarm clock, demonstrating that it might be controlled by biological clock. The regulation of daily activity onset has been thought to be embedded in circadian rhythms controlling the hormone release, body temperature, and resulting consciousness. Normally, the release of ACTH and cortisol increases during late stages of sleeping, reaching a daily maximum at the time of behavioral activity onset. In some rodents, schedules of restricted timed daily feeding can substantially modify the temporal distribution of an animal's behavioral and physiological activities. When food availability is restricted to a short temporal window in the day, animals display pre-feeding locomotor activity and feeding-associated physiological changes known as food anticipatory activity (FAA). In the present study, we clarified the property of food anticipatory activity during temporally restricted feeding (RF) and investigated the effects of aging on the associated behavioral characteristics. Molecular clock–deficient mice provide crucial evidence for the involvement of molecular clock genes in circadian regulation of behavioral FAA, and they may be important models for furthering our understanding of behavioral regulation in aging by interaction between time measurements and circadian rhythms. (COI:No)

### PS26-02

#### Peripheral mechanisms of mechanically evoked swallows

Takanori Tsujimura, Makoto Inoue (*Div Dysp Reha, Niigata Univ Grad Sch Med Dent Sci, Japan*)

Swallowing has a vital function for airway protection and is one of the initial steps in food ingestion. Various non-noxious stimuli, including mechanical, taste, and thermal stimuli, may be involved in natural swallowing. Because impaired laryngeal mechanical sensation is associated with food bolus aspiration, it is important to know how the laryngeal mechanosensory system regulates swallowing initiation. This study was performed to clarify the neuronal mechanism of mechanically evoked swallows. Urethane-anaesthetized Sprague–Dawley male rats were used. A swallow was identified by electromyographic burst of suprahyoid and thyrohyoid muscles. The swallowing threshold was measured by von Frey filament or electrical stimulation of the larynx. The number of swallows induced by upper airway distension or capsaicin application (0.03 nmol, 3 μl) to the laryngeal mucosa was counted. The effects of topical application of epithelial sodium channel (ENaC) and acid-sensing ion channel (ASIC) blockers (amiloride, benzamil, and dimethylamiloride), ASIC inhibitors (mambalgine-1 and diminazene), and gadolinium (0.3–30 nmol, 3 $\mu$l) to the laryngeal mucosa on swallowing initiation were evaluated. A nerve transection study indicated that afferents carried by the superior laryngeal nerve play a primary role in the initiation of laryngeal mechanically evoked swallows. The mechanical threshold of swallowing was increased in a dose-dependent manner of amiloride analogues and gadolinium, but not ASIC inhibitors. The increased swallowing threshold by amiloride analogues or gadolinium was diminished following saline washout. The number of swallows by upper airway distension was significantly decreased by benzamil application. However, the initiation of swallows evoked by capsaicin or electrical stimulation was not affected by benzamil application. We speculate that ENaC is involved in the initiation of mechanically evoked swallows in the larynx. (COI:No)

### PS26-03

#### Molecular mechanism for the suppressing effect of low pH on the sweet receptor sensitivity

Keisuke Sanematsu[1,2], Yuzo Ninoimya[2,3], Noriatsu Shigemura[1,2] ([1]*Sect of Oral Neurosci, Grad Sch of Dental Sci, Kyushu Univ, Japan,* [2]*R and D Ctr for Five-Sense Devices, Kyushu Univ, Japan,* [3]*Monell Chemical Senses Ctr, USA*)

Taste information influences on feeding behaviors. The sweet taste is involved in maintaining homeostasis and mediated by the taste G-protein-coupled receptors (GPCR), TAS1R2 + TAS1R3. It is well known that a glycoprotein, miraculin induces sweet taste by acidification. Our previous study suggests that the protonation of the sweet receptor was also required for the sweet-inducing effect of miraculin, praising a possibility that pH affects the sweet receptor sensitivity. Therefore, we examined responses to sweet compounds under low pH conditions by using the sweet receptor assay. We found that responses to saccharin were reduced when its solutions were acidified. This effect was species-specific to humans but not to mice. Using mixed-species pairings of human and mouse sweet receptor subunits and chimeras, we determined that the suppressing effect of low pH on saccharin responses was mediated by the transmembrane domain of human TAS1R3. Point mutation analysis revealed the key residues for this effect. These were consistent with the data from molecular dynamics simulation, indicating that saccharin strongly binds to the negative allosteric moderator site by acidification and suppresses its agonistic activity. Our results suggest that the function of the sweet taste receptor is regulated by the environmental pH and provide new insights into the functional characterization of other GPCR. (COI:No)

### PS26-04

#### Tongue pain hypersensitivity hampering feeding behaviors

Masamichi Shinoda (*Dept Physiol, Sch Dent, Nihon Univ, Japan*)

Feeding behavior is known to be not only essential for life support but also important for maintaining quality of life. Once orofacial pain such as tooth, tongue, periodontal tissue or temporomandibular joint pain develops, feeding disorders are immediately caused, resulting in lower quality of life. Despite burning mouth syndrome (BMS) is more common among orofacial pain inducing feeding disorders, its pathophysiologic mechanisms remain incompletely understood. We have found that the Artemin (Artn) mRNA expression in the tongue mucosa of BMS patients was enhanced compared with nomal subjects. Therefore, we developed the BMS mouse model by 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) application onto the tongue dorsum to assess the involvement of Artn in the tongue mucosa in tongue pain hypersensitivity. Persistent, week-long tongue heat hypersensitivity with no signs of tongue histological changes and Artn hyperexpression in the tongue mucosa were induced by the tongue TNBS application. The consecutive transient receptor potential vanilloid 1 (TRPV1) antagonist or anti-Artn neutralizing antibody into the tongue mucosa suppressed the persistent tongue heat hypersensitivity by the tongue TNBS application. Moreover, the TRPV1 expression in the glial cell line-derived neurotrophic factor family receptor $\alpha$3 (GFR$\alpha$3)-positive trigeminal ganglion (TG) neurons terminating the tongue was potentiated via p38 signaling cascades. Its TRPV1 hyperexpression was depressed by successive anti-Artn neutralizing antibody into the tongue mucosa. The tongue TNBS application enhanced the TRPV1 agonist-induced inward current in acute dissociated TG neurons terminating the tongue, which was inhibited by Artn neutralization. These findings indicate that Artn overexpression in tongue mucosa in BMS patients potentiates the excitability TG neurons terminating the tongue due to TRPV1 overexpression attributable to the enhancement of GFR$\alpha$3 signaling via p38 signaling cascades, resulting tongue heat hypersensitivity. Together with recent studies, we would like to discuss pathophysiologic mechanisms associated with BMS hampering feeding behaviors. (COI:No)

# Planned Symposium 27

## Pathophysiology of ion channels and transporters in tumor growth

(March 19, 14:10〜16:00, Hall 5)

### PS27-01

#### Patho-physiological significance of $Ca^{2+}$-activated $K^+$ channels in tumor microenvironment

Susumu Ohya, Junko Kajikuri, Hiroaki Kito (*Dept Pharmacol, Grad Sch Med Sci, Nagoya City Univ, Japan*)

$Ca^{2+}$-activated $K^+$ channels (especially, $K_{Ca}1.1$ and $K_{Ca}3.1$) overexpressing in several solid cancers promote cancer cell proliferation and migration. Hypoxic tumor microenvironment is a common characteristic of solid cancers and elevated hypoxia-inducible factor (HIF) under hypoxic condition is associated with tumor metastasis and poor patient prognosis. HIF signaling pathways also promote cancer cell stemness and chemoresistance. Our studies revealed the patho-physiological significance of $K_{Ca}$ channel up-regulation in hypoxic solid-tumor microenvironment in vitro using ultra-low attachment cultureware making three-dimensional cell spheroid formation. The anti-inflammatory cytokine, interleukin-10 (IL-10) is an immunosuppressive factor involved in tumorigenesis, and plays a crucial role in escape from tumor immune surveillance. We also describe that $K_{Ca}3.1$ activators are a possible therapeutic option to suppress the tumor-promoting activities of IL-10. (COI:No)

### PS27-02

#### Overexpression of constitutively active $K^+$ channel (KCNK9) and cell survival

Yoshihiro Konno[1,2], Makoto Sato[1], Ikuo Norota[1], Yutaro Obara[1], Kuniaki Ishii[1] ([1]*Dept Pharmacol, Faculty Med, Yamagata Univ, japan,* [2]*Dept. Radiology, Faculty Med, Yamagata Univ, Japan*)

KCNK9, a member of the two-pore $K^+$ channel family, is responsible for background $K^+$ currents that regulate cell resting membrane potential and excitability. KCNK9 channel is overexpressed in several types of human carcinomas, such as breast cancers and lung cancers, and is generally thought to be an oncogenic channel. However, little is known about the mechanisms by which KCNK9 promotes oncogenesis. Here we assessed how ectopic overexpression of KCNK9 affects cell survival in human pancreatic cancer (PANC1) cell. Stable KCNK9 expression led to hyperpolarization of the resting membrane potential, which did not alter cell proliferation and cell cycle. However, the stable KCNK9 cells acquired resistance to cell apoptosis induced by hyperosmotic stress with enhanced expression of Bcl-2 gene. On the other hand, PANC1 cells transiently transfected with KCNK9 died by caspase-3/7-dependent apoptosis, which was accompanied by activation of p38 MAPK. One possible explanation for these paradoxical results is the difference in KCNK9 expression level: stable low expression is anti-apoptotic and transient high expression is pro-apoptotic. Our data suggest that p38 MAPK pathway is involved in KCNK9-induced apoptosis, but it is not known whether p38 MAPK is activated by KCNK9 stable expression. Although further study is necessary to reveal the mechanisms underlying the oncogenic property of this channel, p38 MAPK might be a candidate key molecule. In this presentation, I would like to talk about the effects of overexpression of KCNK9 on cell survival and the possible mechanisms involved. (COI:No)

### PS27-03

#### Novel pathophysiological properties of $Na^+$, $K^+$-ATPase in human cancer cells

Takuto Fujii[1], Takahiro Shimizu[1], Hiroshi Takeshima[2], Hideki Sakai[1] ([1]*Dept. Pharm. Physiol., Grad. Sch. Med. Pharm. Sci., Univ. Toyama, Japan,* [2]*Dept. Biol. Chem., Grad. Sch. Pharm. Sci., Kyoto Univ., Japan*)

Cardiac glycosides, potent inhibitors of $Na^+$, $K^+$-ATPase, have been used in treatment of congestive heart failure as positive inotropic agents. In addition, epidemiological studies and *in vitro* studies using cancer cell lines suggested the anti-cancer effects of cardiac glycosides. Cardiac glycosides at submicromolar concentrations can block cancer cell growth without affecting enzyme acivity of $Na^+$, $K^+$-ATPase, however, these mechanisms have not been fully understood. We reported recently that $Na^+$, $K^+$-ATPase $\alpha$1-isoform is associated with volume-regulated anion channel (VRAC), an anion channel responsible for cell volume regulation and cell death, in membrane microdomains of human cancer cells. Nanomolar concentrations of cardiac glycosides, such as ouabain, digoxin, and digitoxin, are considered to interact with $Na^+$, $K^+$-ATPase $\alpha$1-isoform in membrane microdomains to activate VRAC via production of reactive oxygen species, thus producing anti-proliferative effects. These cardiac glycosides-induced effects were not observed in non-cancer cells. On the other hand, we have found that nanomolar concentrations of cardiac glycosides drastically inhibit glucose uptake in human liver cancer cells by decreasing the expression level of glucose transporter GLUT1 which is overexpressed at the plasma membrane in many types of cancer cells. Cardiac glycosides induce the clathrin-dependent endocytosis of GLUT1 via activation of $Ca^{2+}$/calmodulin-dependent protein kinase II, and the endocytosed GLUT1 is degraded in lysosome. In this mechanism, cardiac glycosides may affect on $Na^+$, $K^+$-ATPase $\alpha$3-isoform which is abnormally expressed in intracellular compartments of various human cancer cells including liver cancer cells. Our findings uncovered novel anti-cancer mechanisms in which cardiac glycosides and $Na^+$, $K^+$-ATPase are involved. (COI:No)

### PS27-04

#### Roles of leucine transporters in cancer and neural development

Hiroyuki Sakurai (*Dept Pharmacol, Sch Med, Kyorin Univ, Japan*)

Because branched chain amino acids, especially leucine, stimulate cell proliferation via mTORC1 pathway activation, BCAA transport proteins are considered to be important regulatory molecules for rapidly dividing cells such as embryonic or cancer cells. One of BCAA transport proteins, LAT1 encoded by SLC7A5 gene, is expressed relatively specific for cancer cells and its expression increases as the cancer evolves to more malignant phenotype. Inhibition of LAT1 reduces leucine uptake into cancer cells, which leads to growth arrest and/or apoptosis via mTOR inhibition. In fact, a specific inhibitor for LAT1, JPH203, is reported to suppress cancer cell proliferation in vitro and in vivo.

In this talk, I will present relatively unexplored roles of LAT1 and other leucine transporters in cancer and development.

1.It has been shown that LAT1 is expressed in the placenta and early embryo. Its importance in early development is evident as lat1 knockout mice is embryonically lethal. We investigated its expression and function in Xenopus embryo. Xenopus lat1 expresses in the notochord, the inducer of neural tissues, and in the eye. Its inhibition by morpholino injection resulted in defective neural tube closure, primary neurogenesis, and eye formation.

2.Some prostate cancer cells express LAT3 when their growth is dependent on androgen. We established androgen independent prostate cancer cell line from androgen dependent LNCaP cells. The newly established cell line expresses y+LAT2, a sodium dependent large neutral amino acid transporter. Its knockdown by siRNA inhibited cell growth.

3. Although the expression levels of LAT1 was comparable between 2 breast cancer cell lines MDA-MB-231 cells were more resistant to JPH203 treatment than T-47D cells. We are trying to elucidate JPH203 resistant mechanism by transcriptome analysis using T-47D cells in the presence or absence of JPH203. (COI:No)

# Planned Symposium 28

## Basic and applied researches to progress the knowledge and therapy for heat stroke

(March 19, 14:10〜16:00, Hall 6)

### PS28-01

#### Exercise capacity in hot and humid environmental conditions -Possible brain mechanisms and cooling strategies-

Hiroshi Hasegawa (*Grad Integ Arts & Sci, Hiroshima Univ, Japan*)

Hyperthermia has been demonstrated as an important factor limiting endurance performance in hot and humid environmental conditions in both human and animal studies. While temperature can affect individual peripheral physiological systems such as muscle contraction characteristics directly, a dominant role for central mechanisms for exercise impairment has been proposed over the past two decades. Exercise-induced hyperthermia may have a direct effect on the central nervous system such as brain temperature, cerebral blood flow, brain activity, cognitive function, brain neurotransmission and neuromuscular activity. In turn, these changes may affect not only the physiological capacity for exercise, but also the athlete's perception of heat stress, motivation for exercise or pacing strategy. Many major sport events are held in extremely hot environments. The recent summer Olympic games are no exception, and these took place under high ambient temperatures (such as in Atlanta 1996; Athens 2004; Beijing 2008). This trend is likely to continue as athletes begin to prepare for what will likely be Olympics and Paralympics in Tokyo 2020. Several specific approaches including fluid intake, heat acclimation, pre, per and post cooling and other practical applications have been investigated (Hasegawa and Cheung, 2013). This presentation focuses on the possible brain mechanisms at high body temperature that influence exercise performance, and the variable strategies such as body cooling for preventing hyperthermia during exercise in hot and humid environmental conditions. (COI:No)

### PS28-02

#### Blood flow distribution in hyperthermia

Manabu Shibasaki (*Nara Women's Univ.*)

Climate change has had a widespread impact on humans and natural systems. In recent decades, a number of severe heat waves have occurred throughout the Northern Hemisphere. The frequent occurrence of heat waves and the urban heat island phenomenon poses a significant threat to human health. When exposed to a hot environment for a long time, we feel dizziness or light-headedness. These symptoms indicate orthostatic intolerance. Orthostatic intolerance is considered to be markedly influenced by the regulation of blood pressure and cerebral blood flow during heat stress. While heat stressed, skin blood flow can increase from ~300 mL/min upwards to ~8 L/min and is associated with marked decreases in systemic vascular resistance. In order to maintain the blood pressure, cardiac output must increase along with increases in vascular resistance, but 50% or more of cardiac output can be directed to the skin during severe heat stress. Thus, control of skin blood flow becomes an important factor for orthostatic tolerance in heat-stressed humans. A factor contributing directly to orthostatic tolerance may be cerebral blood flow or its regulation. As well as other non-cutaneous vascular beds, heat stress also reduces cerebral blood flow. However, not only the distribution of blood but also hypocapnia due to hyperthermia-induced hyperventilation causes a reduction in cerebral perfusion. In this symposium, I will summarize the regulation of skin and cerebral blood flow in hyperthermic individuals. (COI:No)

### PS28-03

#### Central neural pathways for thermosensory information to promote heat loss responses and heat avoidance behavior

Kazuhiro Nakamura (*Dept Integrative Physiol, Nagoya Univ Grad Sch Med, Japan*)

Thermal homeostasis is maintained in hot environments by autonomic facilitation of heat loss and by behavioral avoidance of heat. These autonomic and behavioral responses for heat defense are driven by central thermoregulatory circuits stimulated by sensory information that is derived from skin thermoreceptors sensing ambient temperatures. We have revealed the ascending central neural pathways conveying cutaneous thermosensory information for autonomic and behavioral thermoregulation. Cutaneous warm-sensory and cool-sensory excitatory signals are separately transmitted from the spinal cord to the dorsal and external parts of the lateral parabrachial nucleus (LPB), respectively. Then, the warming-activated and cooling-activated neurons in the LPB separately transmit excitatory signals to the thermoregulatory center in the preoptic area. The LPB-mediated thermosensory pathways are required to elicit autonomic thermoregulatory responses including cutaneous vasomotor responses as well as to drive thermoregulatory behaviors including heat avoidance. Interestingly, lesions of the thalamus that ablate the spinothalamocortical pathway mediating thermal perception do not affect autonomic or behavioral thermoregulatory responses to environmental thermal challenges. Our results show that thermal homeostasis in hot environments requires thermosensory transmission mediated by the spinal–LPB–preoptic thermosensory pathways, distinct from the central thermosensory process for thermal perception by the spinothalamocortical pathway. (COI:No)

### PS28-04

#### Heat tolerance, not related to heat loss responses

Kei Nagashima, Yuta Masuda (*BTFL, Fac Human Sci, Waseda Univ*)

Heat stroke is one of big health problems in Japan. Besides climate changes within these 10-20 years, one of the reasons may be that residents in Japan have less ability of heat tolerance. However, it seems that many studies have considered heat tolerance as physiological response to minimize the increase in core body temperature during heat exposure and/or exercise, especially heat loss responses such as elevated skin blood flow and sweat rate. In this symposium, we introduce two studies evaluating heat tolerance in other point of view. Exp 1, male C57BL6 mice were expose to heat of 33℃ for 14 days with free access to horizontal running wheel. On the first and last days of the exposure period, abdominal temperature was monitored with rotation counts of the running wheel. The rotation counts increased on the last day compared with those on the first day. Abdominal temperature was greater on the last day than that on the first day. These results may indicate that thermal input from the periphery determines the counts of the running wheel and prevents increase of core body temperature in heat. However, continuous heat exposure may blunt the effect of the peripheral thermal input. Exp 2, young male subjects were immersed in 40℃ water up to the subclavian level for 40 min. Rectal temperature, skin blood flow and sweat rate of the forehead, and metabolic rate were continuously monitored. Rectal temperature increased by ~2.5℃, which were various among subjects. There were no relationships between the increase of rectal temperature and the skin blood flow and sweat rate; however, negative relationship was found between rectal temperature and metabolic rate. The results may suggest that metabolic response to heat is also a factor preventing increase in core body temperature. (COI:No)

### PS28-05

#### Possible central mechanism of acquired heat tolerance in heat-acclimated rats

Kentaro Matsuzaki, Md Emon Hossain, Osamu Shido (*Dept Environmental Physiol, Facult Med, Shimane Univ, Japan*)

In humans and rodents, chronic heat exposure has been well known to induce heat acclimation that improves heat tolerance. We have reported that heat exposure promotes progenitor cell proliferation and neural differentiation in the hypothalamus and the inhibition of hypothalamic neurogenesis impairs the ability of heat tolerance in rats. To elucidate the mechanisms for heat acclimation, we investigated the effects of direct heat exposure on the proliferation and differentiation of cultured neural stem/progenitor cells (NSCs/NPCs). The NSCs/NPCs isolated from the brain of 14.5-day-old rat fetuses were propagated as neurospheres at either 37.0℃ (control) or 38.5℃ (heat exposure) for four days. The effects on proliferation were investigated by cell viability assay, measurement of neurosphere diameter, and counting the total number of cells. The mRNA expressions of heat shock proteins (HSPs) and brain-derived neurotrophic factor (BDNF), cAMP response element-binding (CREB) protein and Akt phosphorylation levels, and intracellular reactive oxygen species (ROS) levels were analyzed using real time PCR, Western blotting and CM-H2DCFDA assay, respectively. Heat exposure under proliferation condition increased NSC/NPC viability, neurosphere diameter, and cell count. BDNF mRNA expression, CREB phosphorylation, and ROS level were also increased by heat exposure. Heat exposure increased HSP27 and HSP70 mRNA expressions concomitant with enhanced phospho-Akt level. Moreover, LY294002, a PI3K inhibitor, diminished the effects of heat exposure on NSC/NPC proliferation. Furthermore, heat exposure under differentiation conditions increased the proportion of cells positive for Tuj1 (a neuronal marker). These findings suggest that mild heat exposure increases NSC/NPC proliferation, possibly through activation of the Akt pathway, and also enhances neuronal differentiation. Direct effects of temperature on NSCs/NPCs may be one of the mechanisms involved in hypothalamic neurogenesis in heat-acclimated rats. (COI:No)

# Planned Symposium 29

## Recent advances in muscle physiology

(March 19, 14:10〜16:00, Hall 8)

### PS29-01

#### Microscopic heat pulses induce activation of thin filaments in striated muscle

Shuya Ishii[1, 2], Kotaro Oyama[1, 2, 3, 4], Fuyu Kobirumaki-Shimozawa[1], Shinichi Ishiwata[5], Norio Fukuda[1] (*[1]Dept Cell Physiol, Sch Med, Jikei Univ, Japan, [2]Sch Adv Sci Engn, Waseda Univ, Japan, [3]QST, [4]PRESTO, JST, [5]Fac Sci Engn, Waseda Univ, Japan*)

During excitation-contraction coupling of striated muscle, sarcomeres are activated via thin filament structural changes, i.e., from the "off" state to the "on" state, in response to a rise in the intracellular $Ca^{+2}$ concentration. We systematically investigated the effects of rapid heating by infra-red (IR) laser irradiation on the sliding of thin filaments reconstituted with human cardiac $\alpha$-tropomyosin (Tm) and bovine ventricular troponin (Tn), or rabbit fast skeletal Tm-Tn complex in the *in vitro* motility assay. Temperature was varied from 25℃ up to ∽45℃ within 2-10 s. With increasing temperature, the sliding velocity of F-actin and thin filaments reconstituted with cardiac Tm-Tn was accelerated in the presence of ATP and $Ca^{+2}$, with a temperature coefficient ($Q_{10}$) of ∽2 (between 25℃ and ∽41℃). In the absence of $Ca^{+2}$ and in the presence of ATP at 25℃, thin filaments reconstituted with cardiac Tm-Tn did not move; however, IR laser irradiation elicited movements of the reconstituted thin filaments with a $Q_{10}$ of 5.5. Likewise, IR laser irradiation elicited movements of thin filaments reconstituted with fast skeletal Tm-Tn with a $Q_{10}$ of 11.0, showing higher sensitivity to temperature. The heating-induced acceleration was observed in the presence of $Ca^{+2}$ for cardiac and fast skeletal thin filaments, with the temperature dependency >2-fold less in both cases. These findings suggest that 1) the "on-off" equilibrium of the cardiac thin filament state is partially shifted toward the "on" state in diastole at the body temperature, enabling rapid and efficient myocardial dynamics in systole, and 2) the higher temperature dependency for fast skeletal thin filaments is optimized for the muscle's physiological properties *in vivo*. (COI:No)

### PS29-02

#### Designing of sarcomere structure and direct visualization of myosin force generation using DNA nano device

Mitsuhiro Iwaki[1, 2] (*[1]BDR, RIKEN, Japan, [2]Grad Sch Front Biosci, Osaka Univ, Japan*)

To elucidate how muscle works, extensive experimental and theoretical works have proposed the swinging lever-arm model. However, the dynamic features of how the myosin head swings the lever-arm, how myosin initially interacts with actin, and how the swings coordinate with each other are not well understood even though they are essential for the force generation, contraction speed, heat production, and response to mechanical perturbations of muscle. This is because myosin heads during force generation have not been directly visualized. Here, we engineered thick filaments comprising DNA origami and human muscle myosin and are optimized for nanometer-precision single-molecule imaging to directly visualize the heads during force generation. We found that when a head diffuses, it weakly interacts with actin and then strongly binds preferentially to the forward region as a Brownian ratchet. Upon strong binding, the head cooperatively swings its lever-arm in a two-step manner and occasionally reverses direction. These results can explain the mechanical characteristics of muscle contraction and suggest that our DNA origami-based assay system can be used to dissect the mechanistic details of molecular motor assembly. (COI:No)

### PS29-03

#### Analysis of cardiac sarcomere dynamics by in vivo nano-imaging

Fuyu Kobirumaki-Shimozawa[1], Togo Shimozawa[2], Jia Li[3], William E. Louch[3], Shinichi Ishiwata[4], Norio Fukuda[1] (*[1]Dept Cell Physiol, Sch Med, Jikei Univ, Japan, [2]Tech Dev, Sch Sci, The Univ Tokyo, Japan, [3]Inst Clin Med, Fac Med, Univ Oslo, Norway, [4]Dept Phys, Fac Sci and Eng, Waseda Univ, Japan*)

Cardiac contractility is highly dependent on sarcomere length (SL), and a change of merely ~100 nm causes a dramatic change in contractile performance (see Kobirumaki-Shimozawa et al., *J Physiol Sci*, 2014). Therefore, for full understanding of the mechanism(s) of the heart's pump function, it is imperative to analyze the dynamics of cardiac sarcomeres at high precision *in vivo*. In the present study, we expressed $\alpha$-actinin-AcGFP in the Z-disks in cardiomyocytes of the left ventricle in adult mice, and applied SL nanometry (as in Shintani et al., *J Gen Physiol*, 2014; Kobirumaki-Shimozawa et al., *J Gen Physiol*, 2016) for the measurement of SL displacement at high spatial (20 nm) and temporal (10 ms) resolution under a fluorescence microscope, simultaneously with the measurements of left ventricular pressure (LVP) and electrocardiogram. First, we found that the magnitude of correlation between the movement of each sarcomere and entire myofibrillar (ventricular) contraction (as quantified by "correlation index", CI) varied between cardiac cycles. In other words, sarcomeres alternately contributed to ventricular function (average CI, ~65%). Our analysis revealed that the longer the sarcomeres during diastole, the higher the likelihood they contributed to ventricular contraction in the subsequent beat. Second, in contrast to a traditional view, a sarcomere in a myofibril repeated shortening and lengthening independently from parallel neighbors. Third, CI was coupled with LVP, indicating that ventricular contractility depends on the population of contributing sarcomeres. The present cardiac nano-imaging system has a broad range of application possibilities for unveiling sarcomere dynamics at high precision in relation to a change in LVP in health and disease. At the meeting, we will discuss (1) sarcomere synchronization as a key determinant of the ventricular function and (2) technical aspects of the application of nano-imaging to the heart *in vivo* under true physiologic conditions. (COI:No)

### PS29-04

#### Cardiac mechanoenergetics in excised, cross-circulated whole heart preparation under the alteration of thermal condition – The role of TRPV1 –

Koji Obata[1], Hironobu Morita[1], Miyako Takaki[2] (*[1]Dept Physiol, Gifu Univ, Grad Sch Med, Japan, [2]Nara Medical Univ, Japan*)

Myocardial temperature sensitively affects cardiac contractility and energy metabolism. Here, we introduced our recent studies concerning the effects of altering cardiac temperature and the role of TRPV1 on left ventricular (LV) myocardial mechanical works and energetics, *mechanoenergetics* using our unique experimental model, *the excised, rat whole heart preparation* with cross-circulation system. We analyzed the LV end-systolic pressure–volume relationship (ESPVR) and linear relationship between myocardial oxygen consumption per beat ($VO_2$) and systolic pressure–volume area (PVA; total mechanical energy per beat) in isovolumically contracting rat hearts during hypo- (32℃), normo- (37℃), and hyperthermia (42℃) in the presence of capsazepine (CPZ, a TRPV1 antagonist) or capsaicin (Cap, a TRPV1 agonist). The slope of $VO_2$–PVA linear relation represents the $O_2$ cost of PVA, reciprocal contractile efficiency, and its $VO_2$ intercept is composed of $O_2$ consumption for $Ca^{2+}$ handling in excitation–contraction (E-C) coupling and basal metabolism.

LV ESPVR shifted downward with increasing cardiac temperature, which was suppressed by CPZ. In Cap-treated hearts, LV ESPVR shifted downward, similar to hyperthermia. The $VO_2$ intercepts of $VO_2$–PVA linear relation did not change during increasing cardiac temperature because of decreased E-C coupling $VO_2$, and inversely increased basal metabolic $VO_2$, which was suppressed by CPZ, though the $VO_2$ intercepts in Cap-treated hearts significantly decreased due to the decreased E-C coupling $VO_2$. Logistic time constants evaluating LV relaxation time were significantly shortened with increasing cardiac temperature related to the acceleration of the detachment in cross-bridge (CB) cycling, indicating increased myosin ATPase activity. Western blotting analysis revealed that the levels of phosphorylated phospholamban decreased significantly in hyperthermia-hearts, as well as Cap-treated hearts.

We concluded that thermal intervention could modulate cardiac inotropism, which is, at least in part, mediated through TRPV1 signaling pathway by changing CB cycling, $Ca^{2+}$ handling, and basal metabolism in rat hearts. (COI:No)

### PS29-05

#### Cross-scale study of beating heart by using a multi-scale heart simulator

Takumi Washio[1, 2], Jun-ichi Okada[1, 2], Xiaoke Cui[1], Seiryo Sugiura[1], Toshiaki Hisada[1] (*[1]UT-Heart Inc., [2]University of Tokyo*)

In a healthy beating heart, the left ventricular pressure rises more slowly than the sharp rise of intracellular free Ca2+ concentration, and falls much more rapidly than the slow decay of free Ca2+ ions. These are important properties to avoid the risk of mechanical damage of blood vessels due to sudden rise of the blood pressure in the systolic phase, and to obtain sufficient blood-filling in the diastolic phase. In this talk, we focus on the roles of mechanical cooperativities of the myosin molecules affecting on these properties. By using a multi-scale heart simulator, we analyze how the myosin molecules communicate mechanically along the thin filament, along the myofibril, and along the fiber in the cardiac muscle to achieve these properties. (COI:No)

# Symposia

# Symposium 1

## Deeper insights into smooth muscle physiology using natural products

(March 17, 9:00～10:50, Hall 5)

### S01-01
#### Effects of inhibition of protein phosphatases 1 and 2A on smooth muscle contraction

Akira Takai (*Dept Physiol, Asahikawa Medical Univ, Japan*)

During the last three decades since okadaic acid was shown to be an inhibitor of type 1 and type 2A protein phosphatases (PP1 and PP2A), inhibitory effects on these enzymes have been reported for other various naturally occurring substances with different relative affinities to PP1 and PP2A. They are now widely used as valuable tools in physiological as well as biochemical research fields. It was the force-enhancing effect of okadaic acid on Triton-X100-skinned smooth muscle fibers that provided an initial clue to the discovery of its inhibitory action to PP1 and PP2A. However it was soon shown that, when applied at 30 – 37℃ to preparations with intact cell membrane, relatively low concentrations (<3 $\mu$M) of okadaic acid strongly inhibited rather than enhanced contractions induced in various smooth muscles by agonist stimulation or high $K^+$ depolarization. At higher concentrations (>10 $\mu$M), in contrast, okadaic acid has been constantly shown to produce or enhance contractions in intact smooth muscle preparations as well as in permeabilized preparations. The contractile effects of okadaic acid can most simply be explained by inhibition of myosin light chain phosphatase which contains PP1 as the catalytic subunit. On the other hand, recent findings obtained by experiments using highly specific PP2A inhibitors have led to the conclusion that the relaxant effect is attributable to inhibition of PP2A. An important implication is that PP2A, as well as PP1, is somehow involved in the regulation and/or maintenance of the contractility of smooth muscles. Several pieces of evidence suggest that the site of action of PP2A is some rather downstream step of smooth muscle contraction such as cross-bridge cycling. Further studies are expected to elucidate the detail of the function of PP2A in smooth muscles. (COI:No)

### S01-02
#### Relaxing effects of rubratoxin A on smooth muscles by inhibiting PP2A

Kosuke Takeya (*Dept Biochem, Vet Med, Okayama Univ Sci, Japan*)

The discovery of okadaic acid (OA) opened a new era in the smooth muscle research filed. OA was first introduced as a potent smooth muscle constrictor. Later on, Dr. Takai and colleagues discovered that it constricted smooth muscles by inhibiting Ser/Thr phosphatases. Few years later, okadaic acid was reported to have relaxing effect on intact smooth muscles at lower concentration. This unique dual effects have been attributed to different inhibition potency between PP1 and PP2A. Based on $K_i$ values for each phosphatase, OA inhibits PP2A about 5,000-times more potently than PP1. In intact smooth muscle tissues, however, the effective range is much narrower, that is, it relaxes smooth muscles at < 1 $\mu$M while it constricts them at >10 $\mu$M. This narrow gap made it difficult to study the molecular basis of relaxing effect of okadaic acid by PP2A inhibition. Recently we introduced a novel PP2A specific inhibitor, rubratoxin A (RubA), to clarify the role of PP2A in smooth muscle contractions. RubA relaxed carbachol-induced contractions as well as ionomycin-induced ones in intact bovine ciliary muscle and guinea pig taenia cecum. It should be noted that RubA did not show any contractile effects at the concentrations we examined. These results suggest that PP2A alters $Ca^{2+}$-sensitivity of smooth muscle contraction. We then examined its effect on $\beta$ –escin skinned guinea pig carotid artery. RubA at 1 $\mu$M and 10 $\mu$M significantly inhibited contraction at any $Ca^{2+}$ concentrations. The data fitting to the Hill equation in $[Ca^{2+}]$-contraction relationship indicated that RubA decreased $F_{max}$-$Ca^{2+}$ and increased $[Ca^{2+}]_{50}$, indices of $Ca^{2+}$ sensitivity for the force and myosin-actin interaction, respectively. These results suggest that PP2A inhibition causes downregulation of the myosin light chain phosphorylation and direct interference with myosin-actin interaction. (COI:No)

### S01-03
#### Natural products induced disruption of actin polymerization modulate mechanical responses of skinned smooth muscle through various pathways

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

Cytochalasin D, a fugal metabolites, and latrunculin B, a sponge toxin, are known to inhibit actin polymerization, and also to suppress smooth muscle contraction. To clarify the force inhibitory mechanisms of cytochalasin D and latrunculin B in detail, we examined the compounds effects on the myosin light chain phosphorylation-dependent and -independent contraction of beta escin skinned taenia cecum and carotid artery from guinea pig. Both cytochalasin D and latrunculin B inhibited the maximal Ca ion induced force at around 1 micro M, but enhanced sub-maximal force development induced by lower Ca ion concentrations. On the other hand, these compounds only suppressed high Mg ion induced force development. These results suggest that actin filaments disruption by cytochalasin D and latrunculin B enhances Ca ion sensitivity force through modulation of thin filaments linked Ca ion dependent pathways. (COI:No)

### S01-04 (AP-7)
#### Daikenchuto, a traditional herbal medicine, ameliorates fibrosis by activating TRPA1 channel in intestinal myofibroblasts

Rin Kurahara[1], Keizo Hiraishi[1,2], Yaopeng Hu[2], Ryuji Inoue[2], Katsuya Hirano[1] ([1]*Dept Cardiovasc Physiol, Sch Med, Kagawa Univ, Japan,* [2]*Dept Physiol, Sch Med, Fukuoka Univ, Japan*)

**Background:** Daikenchuto (DKT) is a traditional oriental herbal medicine, widely used to mitigate post-operative ileus and constipation. In this study, we investigated the anti-fibrotic effect of DKT in a murine chronic colitis model and elucidated the role of transient receptor potential ankyrin 1 (TRPA1) channels of intestinal myofibroblasts in colonic fibrosis.
**Methods:** A murine chronic colitis model was established by weekly intrarectal administration of trinitrobenzene sulfonic acid (TNBS). Inflammatory and fibrotic changes were evaluated by histopathological examination. An intestinal myofibroblast cell line (InMyoFibs) was stimulated with TGF-$\beta$1, and subsequent intracellular signaling and pro-fibrotic factors were investigated. Samples from non-stenotic and stenotic regions of Crohn's Disease (CD) patient's intestines were used for pathological analyses.
**Results:** In TNBS chronic colitis model mice, the extents of inflammation and fibrotic changes were more prominent in $TRPA1^{-/-}$ knockout than in wild-type mice. One-week enema administration of DKT suppressed fibrotic lesions in wild-type mice, but not in TRPA1 knockout mice. Active ingredients of DKT, i.e. hydroxy $\alpha$-sanshool and 6-shogaol induced $Ca^{2+}$ influxes in InMyoFib, which were antagonized by co-treatment with a selective TRPA1 channel blocker HC-030031. DKT counteracted TGF-$\beta$1-induced expression of Type 1 collagen, $\alpha$-SMA, N-cadherin, the phosphorylation level of Smad-2 and p38-MAPK and the expression level of myocardin, a well-known master transcription factor regulating fibrosis signaling at the downstream of TGF-$\beta$1 receptor. Importantly, a 24-hour incubation with another DKT active ingredient Japanese Pepper increased the mRNA and protein expressions of TRPA1, which in turn negatively regulated collagen synthesis in InMyoFibs. TRPA1 expression in the stenotic regions of CD patient's intestine was significantly greater than that in the non-stenotic regions.
**Conclusions:** DKT suppresses intestinal fibrosis by upregulating the expression and activating the channel function of TRPA1. This putative mechanism underlies the reported beneficial actions of DKT on inflammatory bowel disease. (COI:No)

# Symposium 2

## Warm-blooded cool animals: hibernation and torpor physiology

(March 17, 9:00～10:50, Hall 7)

### S02-01

#### Role of sulfide metabolism in hypoxia tolerance of deep hibernators

Fumito Ichinose (*Dept Anesthesia, Mass General Hosp, Harvard Med School, Boston, USA*)

Small-bodied hibernators such as ground squirrels successfully and repeatedly execute cycles of metabolic depression, tissue ischemia-reperfusion, and severe global hypoxemia without harm. Despite decades of intensive research, detailed mechanisms that permit such extreme mammalian physiology remain unknown. One of the many physiological mysteries of small-bodied hibernators is their marked tolerance to severe hypoxia, which occurs during rewarming arousal from deep torpor bouts. Hydrogen sulfide (H2S) is an evolutionarily conserved O2 sensor that importantly modulates metabolism and signaling in modern cells. In mice, severe hypoxia is associated with an acute increase in H2S in the brain. Pre-exposure to moderate hypoxia or low dose inhaled H2S upregulates the capability to metabolize H2S in the mouse brain, enabling them to survive lethal hypoxia. While H2S has recently been studied as a potential method to confer a "suspended animation state" to non-hibernators, its link with hypoxia tolerance has not previously been explored in a natural hibernator. Interestingly, ground squirrels express markedly higher levels of enzymes that synthesize or catabolize H2S in their brain compared to mice. Enhanced capacity to metabolize H2S may enable the hibernators to use it as an organic substrate for energy production when O2 supply is limited and/or to mitigate the inhibition of mitochondrial complex IV by hypoxia-induced H2S production; this would be a novel mechanism of hypoxia tolerance that has not previously been reported in a natural mammalian system. (COI:No)

### S02-02

#### Induction of synthetic hibernation-like state by manipulating hypothalamic neuronal circuts

Takeshi Sakurai (*Faculty of Med, Univ of Tsukuba*)

Some mammals actively lower their body temperature to reduce energy expenditure when facing food scarcity, a state known as hibernation. Hibernating animals fully recover to a normal condition with no organ or tissue damage. Because a hypometabolic state could be beneficial for many medical applications, this ability has evoked great interest. Here, we identified a novel chemically-defined neuronal population, which resides in the periventricular hypothalamic nuclei in mice, excitatory manipulation of which induced a marked and very long-lasting hypothermic state, similar to hibernation. In this state, the set-point of body temperature of mice was significantly lowered, but their behaviour and metabolism were still actively regulated, showing stark contrast to states induced by anesthesia. Functions of these cells are also necessary for daily torpor and circadian control of body temperature. This finding opens the door to the development of methods to induce a hibernation-like state in non-hibernating mammalian species including humans. (COI:No)

### S02-03

#### Development of a new pharmacological method to induce the long-term synthetic torpor in mice

Miho Sato-Hashimoto (*Dept Lab Sci, Gunma Univ Grad Sch Health Sci, Japan*)

Synthetic torpor is defined as a reversible metabolic depression that is induced artificially. Some attempts to induce synthetic torpor pharmacologically have been made so far, one of which is the potent method targeting adenosine receptor A1 (A1AR). Administration of the A1AR agonist, N6-cyclohexyladenosine (CHA), induces hypothermia and bradycardia dramatically both in non-hibernators (mice and rats) and hibernators (Syrian hamsters and ground squirrels). Moreover, one study in arctic ground squirrels showed that the sensitivity of A1AR was increased during hibernation. We report here the pharmacological method to induce the long-term torpor using CHA combined with treatments of PPAR agonists in mice. We found that pretreatments of PPAR $\alpha$ or - $\gamma$ agonists prolonged hypothermia induced by CHA. After treatments of these PPAR agonists, a single CHA injection induced hypothermia at 20 ± 1 ℃ for over 24 hours, and the heart beat also decreased by around 120 from 700 bpm. According to results from the dose-response experiments, the pretreatment with a PPAR $\alpha$ agonist increased the sensitivity of A1AR five times higher than that in control mice, while a PPAR $\gamma$ agonist also enhanced the sensitivity but not as much as a PPAR $\alpha$ agonist. This model seemed to reproduce some traits in natural torpor including hibernation, because up-regulation of PPAR gene expression was reported in hibernating animals. Furthermore, PPAR agonist treatments have been known to have the protective effects against cardiovascular and brain diseases. In this synthetic torpor model, thus, the PPAR activation may contribute to at least two changes similar to those during hibernation, that are prolonged hypothermia via enhancing the sensitivity to CHA and protection from damages caused by low temperature and hypoxia. (COI:No)

### S02-04

#### Low Temperature Tolerance of a mammalian hibernator, Syrian hamsters

Yoshifumi Yamaguchi (*Institute of Low Temperature Science, Hokkaido University*)

Mammal hibernation is a strategy for surviving during the harsh season with cold and food shortage by reducing metabolisms and body temperature for energy-sparing. Many homeothermic mammals are unable to hibernate as they suffer from organ damage under prolonged hypothermia that is experienced during hibernation, whereas some mammals including ground squirrels, chipmunk, and hamsters can survive for long periods at low body temperature. This hypothermia tolerance in hibernating animals should be based on low-temperature tolerance at the cellular level, but its molecular mechanism is unclear yet. To elucidate the molecular mechanism of hibernation in mammals, we have studied a small mammalian hibernator, Syrian hamsters, which can be induced to hibernate irrespective of season under an appropriate condition. We previously suggested that Syrian hamsters remodeled the set-point of body temperature and white adipose tissues by prolonged short photoperiodic and cold condition. Independently from these seasonal systemic remodeling, we found that primary hepatocytes of Syrian hamsters have tolerance against low temperature at the cellular level. The low temperature tolerance was observed not only in animals that hibernate but also animals that were kept at warm condition and did not hibernate, suggesting that the low temperature tolerance is an intrinsic property of the Syrian hamster. Surprisingly, however, the low temperature tolerance in primary hepatocytes was diminished by changing environmental conditions. We will discuss potential mechanisms of such environment-dependent low temperature tolerance in Syrian hamsters. (COI:No)

### S02-05

#### Daily torpor in mice as a model of active hypometabolism: transcriptome analysis of skeletal muscle during torpor

Genshiro Sunagawa A. (*BDR, RIKEN, Japan*)

Some mammals enter a hypometabolic state either daily torpor (minutes to hours in length) or hibernation (days to weeks), when reducing metabolism would benefit survival. The metabolic rate is reduced to 1~30% of normal rates, and the animal results in severe hypothermia, surprisingly without any tissue damage. The mechanisms for such hypothermia-resistance and hypometabolism-resistance is not understood. In 2016, we developed a method to induce torpor stably in mice (Sunagawa GA and Takahashi M, Sci Rep, 2016) and this introduced modern techniques in genetics such as genetical engineering to the field of mammalian hypometabolism research. Recently, we found that two genetically close inbred mouse strains C57BL/6J (B6J) and C57BL/6N (B6N) have distinct torpor phenotypes. This led us to hypothesize that the torpor phenotype in mice is regulated by relatively few genes or gene loci. We analyzed the transcriptome of soleus muscles from 38 B6J mice in torpid and non-torpid conditions and identified 287 torpor-specific genes. Among the torpor specific genes, a transcription factor ATF3 was found highly expressed during torpor deprivation and that its binding motif was enriched in torpor-specific promoters (Sunagawa GA, et al., bioRxiv 374975, 2018). In addition, the results of torpor phenotyping of atf3-KOs will be presented. Our results demonstrate that mouse daily torpor combined with powerful genetic tools have the potential to study active hypometabolism. (COI:No)

# Symposium 3

## Frontiers of biophotonics in physiology: Ion environments and molecule behaviors in the nano-space detected by Raman spectrum

(March 17, 9:00～10:50, Hall 8)

### S03-01

#### Non-invasive sample measurement derived from live tissue by Raman spectroscopic microscopy

Sakiko Akaji[1], Yoshinori Marunaka[2,3,4] (*[1]HORIBA Ltd., [2]Res Inst Clin Physiol, Kyoto Indust Health Adoc, Kyoto, [3]Res Organ Sci Technol, Ritsymeikan Univ, Kusatsu, [4]Ins Res Center Food Nutrit Safety, Jiangsu Univ. Ahenjiang*)

Raman spectroscopic technology is enable to measure samples derived from live tissue non-invasively. The research for this technology is focused as the obtained Raman spectrum is having the advantages of acquiring the molecular structural information and also for building the spectral imaging.
One example is the detection of disease-derived cells like cancer. The pathological method, one of the conventional methods, is available, however it takes time and many procedures to detect those cells. Recently, some studies have shown that Raman spectroscopic method is able to detect cancerous cells without any pretreatments.
Based on the strength point of Raman spectroscopy described above, we have tried to measure the samples derived from live tissue in order to prove the possibility of using the non-invasive measurement method for physiological phenomenon. The visual diagnosis is difficult for the molecular structural change in the samples derived from tissue. Therefore, we are going to detect the cell differentiation stage, and the difference between benign and malignant tumor by using Raman spectroscopic.
In this presentation, we would like to show the relation between the visual diagnosis and structural change detection of Raman spectroscopic regarding the cell condition. (COI:No)

### S03-02

#### Surface enhanced Raman spectroscopy probe for nanometer-scale measurement of pH and hydrogen peroxides on the outer membrane of cells

Leonardo Puppulin[1,2], Shigekuni Hosogi[3], Hideo Tanaka[4], Yasuaki Kumamoto[5,4], Eishi Ashihara[3], Yoshinori Marunaka[2,6,7] (*[1]Department of Nanometrology, WPI Nano Life Science Institute, Kanazawa University, [2]Research Center for Drug Discovery and Pharmaceutical Development Science, Research Organization of Science and Technology, Ritsumeikan University, [3]Department of Clinical and Translational Physiology, Kyoto Pharmaceutical University, [4]Department of Pathology and Cell Regulation, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, [5]Department of Applied Physics, Osaka University, [6]Research Institute for Clinical Physiology, Kyoto Industrial Health Association, [7]International Research Center for Food Nutrition and Safety, College of Food and Biological Engineering, Jiangsu University*)

Regulation of intracellular pH and reactive oxygen species (ROS) is critically important for many cellular functions. In the peculiar case of cancer cells, it has been demonstrated that pH homeostasis is crucial for biological functions such as cell proliferation, metastasis, drug resistance and apoptosis. As compared to normal cells, the extracellular surface pH of cancer cells is expected to be more acidic, mainly due to elevated cellular glycolytic activity (Warburg effect). In addition to proton concentration, also ROS play a key role in cell metabolism. In particular, hydrogen peroxide ($H_2O_2$) is recognized as one of the main second messenger molecules. In fact, it modulates signaling pathways activating downstream proteins that control cell metabolism. Endogenous $H_2O_2$ molecules involved in cell signaling are primarily generated on the extracellular space by superoxide dismutases that rapidly convert superoxide anions ($O_2$) produced by membrane-associated NADPH oxidases (NOXs enzymes). Several studies demonstrated that during tumorigenesis $H_2O_2$ stimulates cell proliferation and preservation of the transformed state. For this reason, a characteristic feature of malignant cells is upregulation of NOXs (i.e., mainly NOX1, NOX2 and NOX4), which promotes the production of $H_2O_2$ in the extracellular milieu. Since the most significant gradients of protons and $H_2O_2$ can be observed only in a very shallow region of the extracellular fluid in contact with the cell, we show here the development of a new sensor of nanometer size that can be anchored to the outer membrane of cells. The newly designed sensor is based on gold nanoparticles functionalized with pH- or $H_2O_2$-sensitive compounds that can be detected and quantified using surface enhanced Raman spectroscopy. We report here the results of quantitative analyses conducted on MKN28 gastric cancer cells, HepG2 human liver cancer cells, A549 adenocarcinomic human alveolar basal epithelial cells and human epidermal keratinocyte (HEK). (COI:No)

### S03-03

#### Raman microscopic analysis of biological tissues: toward construction of Raman histopathology

Yoshinori Harada, Hideo Tanaka (*Dept Pathol Cell Reg, Grad Sch Med, Kyoto Pref Univ Med, Japan*)

Pathological histology mainly depends on morphological analysis of tissues, and requires experts for recognition of tissue status. Raman scattering light measurement can directly visualize biomolecules by acquiring their vibrations in an unlabeled and nondestructive way. Raman microscopic imaging has potential to provide objective information on the biochemical environment as well as morphological appearance of tissues. Here, we introduce our recent studies of Raman scattering light measurements in heart and liver tissues. Evaluation of myocardial viability is important for determining the strategy for revascularization therapy. We have conducted basic studies on myocardial viability evaluation by Raman scattering light measurement. We found that it is useful for in-situ assessment of both very early myocardial ischemia and reversibility of myocardial viability in rat hearts without labeling. This was shown to be realized by mainly sensing redox states and damages of myocardial mitochondrial respiratory chain. As for studies of liver diseases, using rat nonalcoholic fatty liver disease (NAFLD) models, we incorporated machine learning with Raman spectroscopic imaging to objectively study liver tissue specimens. We extracted biochemically different regions in liver tissue, enabling quantification of chemical components. Using Raman microscopic information on the chemical composition enabled us to group tissues, providing helpful information for histopathological examination. In addition, Raman imaging combined with machine learning enhanced the diagnostic power of nascent stages of NAFLD in which morphological features were not yet apparent. We expect that Raman microscopic analysis of biological tissues can provide additional valuable microchemical information in relation to pathogenesis, taking advantage of its molecular discrimination ability. (COI:No)

### S03-04

#### High-throughput cell analysis by high-speed Raman microscopy

Yasuaki Kumamoto[1,2] (*[1]Dept Appl Phys, Grad Sch Eng, Osaka Univ, Japan, [2]Dept Pathol, Grad Sch Med, Kyoto Prefect Univ Med, Japan*)

Raman spectroscopy is becoming a viable tool for probing a physiological condition of a biological sample as it has a high sensitivity to microenvironment of a molecule. However, its combination with optical microscopy, namely Raman microscopy, is not widely used. This is because Raman scattering is extremely inefficient as well as Raman spectral measurement uses a detector with a large number of pixels in general, and consequently the spectral measurement time consisting of signal accumulation time and detector readout time often becomes as long as several tenth minutes or even one hour in Raman microscopic imaging of a single cell. Here I talk about high-speed Raman microscopy for high-throughput cell analysis and imaging. One of the strategies to accelerate Raman imaging measurement is to conduct simultaneous detection of a number of spectra for reducing the effective signal accumulation time required for obtaining one spectrum. The simultaneous spectral detection is enabled by a line illumination slit-scan confocal Raman microscope. It reduced the effective signal accumulation time by 2 to 3 orders of magnitude. To further increase the imaging speed, I conducted a narrowband and low-spectral resolution Raman measurement. With this measurement, readout time of the detector was reduced in comparison to a correspondent number of single-spectrum measurements by 2 orders of magnitude. Furthermore, the low-spectral resolution measurement increased the number of photons incident to each pixel of the detector, allowing shortage of the signal accumulation time by 4 times not in trade-off with signal to noise ratio of spectra. Overall, with the narrowband and low-spectral resolution measurement, Raman imaging of more than 200 cultured cells with a sub-500-nm spatial resolution took only 20 minutes. I will discuss the potential of this high-speed Raman microscope for physiological study. (COI:No)

# Symposium 4

## Neural mechanisms unveiled by combined *in vitro* and *in silico* approaches

(March 17, 9:00～10:50, Hall 9)

### S04-01

#### Perimeter release model: a nanoscale topographical arrangement of $Ca^{2+}$ channels and synaptic vesicles in the active zone

Yukihiro Nakamura (*Dept Pharmacol, Jikei Univ Sch Med, Tokyo, Japan*)

Synaptic efficacy and precision are influenced by the coupling of voltage-gated $Ca^{2+}$ channels (VGCCs) to synaptic vesicles. Accumulating evidence indicates that presynaptic voltage-gated $Ca^{2+}$ channels are clustered within the active zone, but the spatial distribution of readily-releasable pool vesicles has not been elucidated. To investigate this, we performed local $Ca^{2+}$ imaging and patch pipette perfusion of EGTA at the calyx of Held giant presynaptic terminal in the auditory brainstem. Reaction-diffusion simulations of presynaptic $Ca^{2+}$ constrained by the result of these experiments and following release simulations suggest that $Ca^{2+}$ sensors for vesicular release are located in the range of several tens of nanometers from the perimeter of VGCC clusters and predict that VGCC number per cluster determines vesicular release probability without altering release time course. This "perimeter release model" provides a unifying framework accounting for developmental changes as well as fast and slow release components observed at this fast central synapse. (COI:No)

### S04-02

#### In vitro modeling of structure-function relationships in neuronal networks

Hideaki Yamamoto[1], Ayumi Hirano-Iwata[1,2] ([1]*WPI-AIMR, Tohoku Univ, Japan,* [2]*RIEC, Tohoku Univ, Japan*)

In vitro experiments using dissociated neurons take irreplaceable roles in molecular and cellular neuroscience. However, its use in the systems-level study has been limited due to the substantial difference in the network organization from the actual brain. Such structural difference in the network results in the generation of an atypical dynamics in cultured neurons, such as the globally synchronized bursting. Here, we employed surface engineering technology to prepare guidance cues for the development of cultured cortical neurons and investigated how the modulation of network structure at the mesoscopic scale influences the network dynamics [1, 2]. We focus on the modular organization of brain networks, characterized by the presence of densely-connected subsystems, or modules, that are weakly interacting with each other. Induction of modular organization was found to suppress coherent bursting and promoted coexistence of coherent and incoherent states [2]. The result demonstrates that surface micropatterning provides a unique tool to constructively study the structure-function relationships in living neuronal networks.

References:
[1] H. Yamamoto et al., Phys. Rev. E 94, 012407 (2016).
[2] H. Yamamoto et al., Sci. Adv. 4, eaau4914 (2018).

(COI:No)

### S04-03

#### Estimating neural dynamics based on data-driven approach

Toshiaki Omori (*Grad Sch Eng, Kobe Univ, Japan*)

Elucidating neural dynamics is one of the important subjects in neuroscience. Recent developments in recording technologies enable us to access spatiotemporal neural responses. We have proposed data-driven approaches for estimating latent dynamics of neural systems such as neuronal intrinsic dynamics and network dynamics. In particular, combined methods with computational models and data-driven methods are employed to estimate not only latent variables but also underlying biophysical parameters. We also discuss methods to extract only substantially important nonlinear membrane currents from a number of candidate membrane currents, and estimate neuronal dynamics under nonlinear observation in imaging recording by means of combined framework with model-driven and data-driven approaches. (COI:No)

### S04-04

#### Computational neurobiology of axonal spike signaling

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

Axonal spikes are generated at the initial segment and propagate to the terminal without attenuation. Spike propagation along the axon is classically thought as stable binary code for neuronal computation. However, recent studies by subcellular recordings from the axons or the terminals have suggested that axonal spikes are subject to fine-tuning in an activity-dependent manner. Towards a comprehensive understanding of dynamic control of axonal spike signaling, a complementary approach by computer simulation has been widely used, since it covers the limitations of the experiment due to technical difficulty in recording from a small structure like the axon. We have developed a simple model of the hippocampal mossy fiber axon implemented with the microstructure of typical *en passant* morphology as well as the ionic conductance recorded experimentally from the axon terminals. The model enables realistic simulation of membrane potentials and currents from any compartments of axon arbor, and therefore can be adopted for evaluation of detailed membrane properties difficult to obtain by experiment. We have used the model to address the mechanisms underlying axonal afterdepolarization following an action potential. The hypotheses derived from subcellular recoding experiment were tested accordingly with simulation in the model. Another application of combined experimental and computational analysis will be also discussed on ectopic spiking of distal axons during epileptic bursts. Thus, mutual feedback of *in vitro* and *in silico* approaches helps to convince our understanding of axonal spike signaling and its use-dependent fine-tuning. (COI:No)

# Symposium 5

## Mitochondrial function and its roles in cellular physiology/pathophysiology

(March 17, 15:20〜17:10, Hall 6)

### S05-01

#### Characteristics of the substrate-free mitochondria

Chae Hun Leem[1], Jeong Hoon Lee[1], Quynh Mai Ho[1], Jae Boum Youm[2] (*[1]Department of Physiology, University of Ulsan College of Medicine 88 OlumpicRo43Gil SongpaKu, Seoul, KOREA, [2]Department of Physiology, College of Medicine Inje University, BokjiRo75, BusanjinKu, Busan, Korea*)

Mitochondria are critical organelle to convert the metabolites to the life-essential chemicals, ATP. For serving the role, the mitochondria have very peculiar steps from metabolites to ATP formation. Usually the Ψm was regarded as zero without the mitochondrial substrate or in the presence of uncoupler. However, we found there still considerable Ψm was existed even in those conditions. So basic scheme of the generation of Ψm is hampered and the question how to explain this phenomena remains. For attacking these problems, we used a multiparametric microfluorometric system to monitor NADH, FAD, and TMRE fluorescence, simultaneously and developed a measurement method of Ψm quantitatively. The Ψm in substrate free conditions was around -60 mV. The application of ATP generated considerable hyperpolarization. The application of $K_{ATP}$ channel opener, diazoxide (DZX), could depolarize Ψm. When $K^+$ was replaced with meglumin, the Ψm was slightly hyperpolarized and the effect of DZX was abolished. The $K_{ATP}$ channel blockers did not have any effect. The application of ATP hyperpolarize Ψm, however, ADP or AMP didn't. Oligomycin blocked the effect of ATP. Interestingly, oligomycin depolarized the resting Ψm considerably. The effect of ATP was not affected by DZX. The replacement $K^+$ with meglumin slowed the ATP-induced change of Ψm and made it transient. The return to $K^+$ medium recovered the effect of ATP. From these results, the Ψm in substrate-free conditions was not zero and $K^+$ may particpate in the formation of the resting Ψm. The F1, F0-ATPase may participate on the formation of the resting Ψm. $K^+$ may be an important modulator for F1, F0-ATPase acitivity. The F1, F0-ATPase and $K^+$ flux might contribute the formation of the resting Ψm but it still need further study on them. (NRF-2016M3C1A6936605) (COI:No)

### S05-02

#### Characteristics of $Ca^{2+}$ efflux from mitochondria

Ayako Takeuchi, Mohammed Moinul Islam, Satoshi Matsuoka (*Integr. Physiol. Fac. Med. Sci. Univ. Fukui*)

Mitochondrial $Ca^{2+}$ is determined by the influx mainly via mitochondrial $Ca^{2+}$ uniporter (MCU) and the efflux via $Na^+$-$Ca^{2+}$ exchange ($NCX_{mit}$) and $H^+$-$Ca^{2+}$ exchange ($HCX_{mit}$), with the $NCX_{mit}$ representing the major component in excitable tissues, whereas the $HCX_{mit}$ being dominant in non-excitable tissues. Previously we reported that NCLX, which belongs to a $Na^+/Ca^{2+}$-$K^+$ exchanger family and was identified as the $NCX_{mit}$, participates in regulating various cellular functions including excitable cells such as HL-1 cardiomyocytes and non-excitable cells such as B lymphocytes, by supplying $Ca^{2+}$ to sarco/endoplasmic reticulum. However, contribution of NCLX to the overall $Na^+$-dependent $Ca^{2+}$ efflux remains unclear, especially in tissues where plasma membrane $Na^+$-$Ca^{2+}$ exchanger (NCX) family exists also in mitochondria. In addition, electrophysiological characteristics of $NCX_{mit}$ are entirely unknown. We studied these issues in mouse isolated mitochondria.

Immunoblot showed that both NCLX and NCX1 are expressed in mitochondria of heart, brain and kidney. Application of $Ca^{2+}$ to isolated mitochondria rapidly decreased extra-mitochondrial $Ca^{2+}$, which was detected by Calcium Green-5N, via $Ca^{2+}$ uptake through MCU into mitochondria. Subsequent MCU inhibition by Ru360 increased extra-mitochondrial $Ca^{2+}$, by uncovering the $Ca^{2+}$ efflux, which was accelerated in the presence of $Na^+$. This $NCX_{mit}$ activity was prominent in brain and heart and less in kidney. It was inhibited by an NCLX blocker CGP-37157, but was insensitive to an NCX1 blocker, SEA0400, suggesting that $NCX_{mit}$ is mediated mainly via NCLX. Finally, we performed voltage clamp experiments using mitoplast prepared from isolated heart mitochondria. We succeeded in recording the extra-mitochondrial $Na^+$ dependent inward currents with $Ca^{2+}$ in the pipette, as well as the extra-mitochondrial $Ca^{2+}$ dependent outward currents with $Na^+$ in the pipette. These currents were inhibited by CGP-37157, demonstrating a direct evidence that $NCX_{mit}$ via NCLX is electrogenic. (COI:No)

### S05-03

#### Mitochondrial structures and function under stress

Yoichiro Kusakari (*Dept Cell Physiol, Jikei Univ, Japan*)

Mitochondria are highly dynamic organelles with high plasticity that can transiently and rapidly coordinate their shape, distribution and size. However, the diverse functions of mitochondria in cellular metabolism and stress responses continue to emerge. We will discuss mitochondrial structures and functions under mechanical stress. (COI:No)

### S05-04

#### Pathological role of non-MCU calcium-influx mechanism in the cardiac mitochondria

Jin O-Uchi (*Department of Medicine, Cardiovascular Division, University of Minnesota*)

Malignant hyperthermia (MH), frequently associated with the ryanodine receptor type 1 (RyR1), is a pharmacogenetic disorder of skeletal muscles that exhibits hypermetabolic responses to anesthetic gases. However, sudden cardiac death (SCD) in the MH families are also reported even though under the conscious condition without anesthesia. We previously reported that small amount of RyR1 is expressed in the mitochondria, but not in the sarcoplasmic reticulum in the hearts, which serves as an additional mitochondrial $Ca^{2+}$ ($mtCa^{2+}$) influx mechanism distinct from the main $mtCa^{2+}$-influx pathway, $mtCa^{2+}$ uniporter (MCU). Therefore, we hypothesize that MH-associated mutant RyR1s form "leaky channel" at cardiac mitochondria and induce $mtCa^{2+}$ overload, followed by an increase in the mitochondrial reactive oxygen species (mROS) generation, which alters the cellular $Ca^{2+}$ handing in cardiomyocytes. Using knock-in mice carrying a MH-related RyR1 mutation Y522S (YS), we found that YS hearts exhibit disrupted mitochondrial morphology and develop significantly higher number of multiple ventricular extrasystoles by $\beta$-adrenergic stimulation compared to wild-type (WT) hearts. We also found that YS mitochondria possess higher basal $mtCa^{2+}$ concentration ($[Ca^{2+}]_m$) and depolarized mitochondrial membrane potential. Moreover, pretreatment with RyR1 blocker dantrolene decreased basal $[Ca^{2+}]_m$ and hyperpolarized mitochondrial membrane potential in YS mitochondria. We next showed that YS myocytes had significantly higher basal cytosolic $Ca^{2+}$ concentration as well as slower cytosolic $Ca^{2+}$ clearance compared to WT. Pretreatment of dantrolene normalized the $Ca^{2+}$ handing profiles in YS cardiomyocytes to the levels in WT. Finally, we confirmed that YS cardiomyocytes exhibited higher mROS level and excessive cellular oxidation. These data suggest that chronic $mtCa^{2+}$ overload via leaky mutant mRyR1 increases mitochondrial and cellular oxidation, which may alter cytosolic $Ca^{2+}$ handling and increase the arrhythmogenic events in MH. The outcome of this study will lead us to design novel strategies for the pharmacological management and risk stratification of SCD in MH patients. (COI:No)

### S05-05

#### Exploring the regulation of ER-mitochondria contact and its physiological roles in mammals

Yusuke Hirabayashi (*Dept Chem. and Biotech., Grad Sch Eng, Univ Tokyo, Japan*)

A network of contact sites between the membranes of different organelles are emerging as critical platforms for various forms of intracellular signaling. The interface between ER and mitochondria is of particular interest as a signaling hub because it is thought to play critical physiological functions such as regulation of $Ca^{2+}$ homeostasis, lipid biogenesis and mitochondrial fission. In addition, changes in the number of these contacts have been reported in various mouse models of neurodegenerative disease, as well as in patients with neurodegenerative diseases. However, despite the fact that multiple proteins are enriched at ER-mitochondria contacts sites, the molecular mechanisms underlying ER-mitochondria tethering are still largely unknown in metazoans.Here, we will report the identification of PDZD8 as a novel protein required for ER-mitochondria tethering protein (Hirabayashi et al. Science 2017). Pdzd8 is an integral ER proteins present at ER-mitochondria contact sites. Using 3D FIB-serial EM reconstructions, we demonstrate that PDZD8 is required for the formation of ER-mitochondria contacts in mammalian cells. Using a series of functional loss-of-function and rescue experiments, we found that PDZD8-dependent ER-mitochondria contacts are required for proper $Ca^{2+}$ exchange between ER and mitochondria in mammalian cells. In dendrites of cortical pyramidal neurons, PDZD8 is required for $Ca^{2+}$ uptake by mitochondria following synaptically-induced $Ca^{2+}$-release from ER and thereby regulated cytoplasmic $Ca^{2+}$ dynamics. Thus, PDZD8 represents a critical ER-mitochondria tethering protein, which is involved in the regulation of dendritic $Ca^{2+}$ dynamics in mammalian neurons. (COI:No)

# Symposium 6

## Crosstalk in modifiable and non-modifiable risk factors for cardiovascular disease

(March 17, 15:20〜17:10, Hall 8)

### S06-01

#### Effects of Endurance Exercise on Arterial Stiffening with Aging

Shigeki Shibata (*Faculty of Health Science, Kyorin University*)

A notable consequence of sedentary ageing is large-vessel arterial stiffening. This pathophysiological process is characterized by the development of fibrosis and collagen cross linked products in the arterial wall. Central arterial stiffening increases the risk of cardiovascularrelated morbidity and mortality in older adults and thus, the development of strategies to forestall age-associated arterial stiffness has important clinical implications. Sustained, regular endurance exercise training is thought to be one such favorable strategy. In this field, the presenter's previous findings are summarized as follows.
i) Masters athletes who have performed near-daily (6-7 sessions per week) vigorous endurance exercise training plus competition for the majority of their adult lives maintains youthful compliant large arteries. ii) More than 4-5 weekly sessions of committed lifelong exercise is associated with a more compliant large arteries in the elderly as compared with their peers. iii) A lower frequency of lifelong exercise (2-3 sessions per week) is associated with improved carotid artery compliance and decreased left ventricular afterload. iv) Large arterial stiffening with aging was not substantially improved in previously sedentary healthy seniors even after one year of progressive and vigorous endurance exercise training when compared with life-long vigorous exercise training by Masters athletes. v) Breaking of advanced glycation end product cross links with Alagebrium was safe, and slowed but did not reverse age related large arterial stiffening in the elderly without an additive effect of exercise training.
This presentation as a part of symposium "Crosstalk in modifiable and non-modifiable risk factors for cardiovascular disease" plans to focus on effects of endurance exercise training on large arterial stiffening with aging based on recent literature and presenter's previous findings.
(COI:No)

### S06-02

#### Cognitive function and cerebral blood flow; Effects of age and exercise

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

Ageing is the primary risk factor for cognitive deterioration that is considerable to be associated with onset of dementia. However, the mechanism of ageing-related cognitive deterioration remains unclear. On the other hand, cerebral blood flow (CBF) or regulation of cerebral circulation is attenuated in the elderly. Also, CBF regulation associated with cerebral metabolism thus likely plays an important role in the preservation of cognitive function. Indeed, our recent study demonstrated that cognitive function was more strongly affected by changes in cerebral metabolism than by changes in CBF during exercise. Thus, it could be expected that ageing-induced cognitive deterioration may be affected by a decrease in CBF as a result of brain ischemia and energy depletion. Exercise is a potential therapeutic tool to postpone or prevent the onset of cognitive decline. It has been reported that onset execise and chronic exercise training improved cognitive function. This may be associated with exercise-induced modification in CBF regulation. However, there is a lack of sufficient discussion regarding the effect of exercise via CBF regulation on age-induced alteration in cognitive function. In this presentation, I would like to summarize current knowledge on previous investigations providing the possibility of effect of exercise on cognitive function in elderly compared with that in young people.
(COI:No)

### S06-03

#### Understanding the influence of the menopause on shear stress mediated cerebral artery dilation at rest and during static handgrip exercise

Stefanie Ruediger[1], Jeff S. Coombes[1], Shigehiko Ogoh[2], Daniel J. Green[3], Tom G. Bailey[1]
(*[1]School of Human Movement and Nutrition Sciences, The University of Queensland, St Lucia, Brisbane, QLD, Australia, [2]Department of Biomedical Engineering, Toyo University, Saitama, Japan, [3]School of Sport Science, Exercise, and Health, The University of Western Australia, Crawley, Western Australia, Australia*)

**Background:** Declines in circulating-oestrogen during the menopause has a wide-range of detrimental effects on vascular structure and function. These changes, coupled with elevated blood pressure, may be associated with reductions in cerebral blood flow (CBF), and cognitive decline in post-menopausal women (PMW). Exercise-induced elevation in blood flow and shear stress induces peripheral vascular adaptation. If CBF and shear stress could be enhanced with exercise, this would likely improve cerebrovascular function in PMW. However, PMW have reduced sensitivity to shear stress, and exhibit exaggerated blood pressure responses to exercise, which may limit increases in CBF. Whether exercise-induced increases in blood pressure alter flow to the brain in PMW is unknown. The aim of this study is to compare the CBF response to acute exercise in PMW, to pre-menopausal women. Secondly, we aim to understand the influence of the menopause on CBF and shear stress flow-mediated-dilation (FMD), as a marker of cerebrovascular function.
**Methods:** 15 post- and 15 pre-menopausal women (40-65 years), will be recruited. Participants will undergo a static handgrip-exercise for 3 minutes at 30% of their maximum voluntary contraction. CBF velocity of the middle and posterior cerebral artery will be assessed continuously, using Transcranial Doppler. We will use Duplex ultrasound to measure internal carotid (ICA) and vertebral artery (VA) blood flow, and shear stress at each artery will be estimated. At baseline, cognitive function, brachial and cerebral FMD will be assessed.
**Discussion:** We hypothesize that cerebral FMD will be reduced in post- compared with pre-menopausal women. Further, the CBF and shear stress response to static handgrip-exercise will be attenuated in post- compared with pre-menopausal women, and this will be associated to larger blood pressure responses in PMW. These results will help to understand differences in brain vascular function at rest, and in response to acute static exercise in PMW.
Data collection is currently underway and will be analysed in February 2020.
(COI:No)

### S06-04

#### Exercise pressor reflex in type 2 diabetes-the role of insulin in circulatory control during exercise

Norio Hotta[1], Masaki Mizuno[2] (*[1]Chubu Univ, Japan, [2]UT Southwestern Medical Center, USA*)

Type 2 diabetes mellitus (T2DM) is one of the diseases in the world requiring prevention and a medical cure. Insulin resistance is a principal feature of T2DM. Exercise therapy has been widely known to be effective in improving insulin resistance in T2DM. However, patients with T2DM display an exaggerated blood pressure response to physical exercise. The potentiated pressor response to exercise increases cardiovascular event risks such as heart attack and stroke, resulting in a reduction of safety of exercise prescription. To date, the mechanism underlying T2DM-induced augmented cardiovascular response to exercise has not been fully investigated. Central command originating from the higher centers and the exercise pressor reflex originating from active muscles play a crucial role in cardiovascular regulation during exercise. We have recently reported that both renal sympathetic nerve activity and the blood pressure response to electrical stimulation of the mesencephalic locomotor region and the isolated L4 and L5 ventral roots were significantly augmented in the combination of high-fat diet-fed and low-dose streptozotocin-treated T2DM rats as compared with control (Kim et al., Am J Physiol Regul Integr Comp Physiol, 317:R270-R279, 2019). This suggests that central command and the exercise pressor reflex generate the exaggerated rise in sympathetic activity and blood pressure during exercise in this disease. Furthermore, evidence suggests that hyperinsulinemia precedes the development of T2DM and insulin receptors are expressed in the peripheral nerve and dorsal root ganglia neurons. To this end, we hypothesized that insulin sensitizes thin-fiber muscle afferents mechanically and metabolically, contributing to heightened exercise pressor reflex in T2DM (Hotta et al., J Physiol, doi: 10.1113/JP278527). Here we discuss alterations in the exercise pressor reflex function in T2DM at tissue and cell levels. Further, we demonstrate evidence suggesting an association between insulin resistance and exercise blood pressure in non-diabetic elderly subjects.
(COI:No)

### S06-05

#### Cerebrovascular endothelial function: Aging, sex hormone, and exercise

Erika Iwamoto (*Dept Health Sci, Sapporo Med Univ, Japan*)

Endothelial dysfunction is associated with cerebrovascular events such as chronic stroke and vascular dementia. In peripheral conduit arteries, endothelial function is commonly assessed by a noninvasive method, ischemia-induced flow-mediated dilation (FMD). Interestingly, recent studies measured carotid shear-mediated dilation using hypercapnia to assess the cerebrovascular function. Analogous to the FMD in the peripheral conduit arteries, the decreased carotid shear-mediated dilation could serve as a potentially useful measure of cerebrovascular endothelial function.
There is a considerable amount of evidence reporting that aging represents the major risk factor for the development of cardio and cerebrovascular diseases. We previously reported that carotid shear-mediated dilation is attenuated with healthy aging (Iwamoto E et al., Am J Physiol Heart Circ Physiol. 2018). The enhanced sympathetic tone may be one of the potential causes of blunted sheer-mediated dilation with aging (Iwamoto E et al, J Appl Physiol. 2018). Moreover, the change in sex hormones is a natural part of aging. In women, menopause results in a rapid loss of estradiol. In our recent data, we found that there is a positive relationship between serum estradiol concentrations and carotid shear-mediated dilation in pre-, peri-, and postmenopausal women. In contrast, exercise-induced increases in shear stress release the vasoactive substance from the endothelium and have beneficial effects on vascular function. In fact, exercise training has been shown to improve endothelial function in cardiovascular patients. In addition, exercise training increases resting cerebral blood flow in ischemic lesions and improve cognitive function via endothelium-dependent mechanisms. However, it requires further investigation to elucidate the optimal mode of exercise (aerobic or resistance exercise, continuous or interval exercise) and intensity to improve cerebrovascular function. In this symposium, I would like to show our ongoing data investigating the effects of aging, sex hormone, and mode and intensity of exercise on carotid shear-mediated dilation.
(COI:No)

# Symposium 7

## Molecular and Neural basis for appetite and food preference

(March 17, 15:20〜17:10, Hall 10)

### S07-01

#### Molecular mechanisms to generate various types of taste cells

Makoto Ohmoto (*Bio-Center, Tokyo Tech, Japan*)

Taste substances are detected by taste cells in taste buds in the oral epithelium. Individual taste cells contribute to evoke one of five taste qualities: sweet, umami, bitter, sour, and salty tastes. They are continuously replaced every few weeks by new ones derived from local epithelial stem cells. We have been interested in the molecular mechanisms underlying the generation of various types of taste cells. We found that a POU homeodomain protein Skn-1a is expressed in sweet, umami, and bitter taste cells and that Skn-1a knockout mice lack these cells in taste buds. These results suggest that Skn-1a is a determinant to sweet, umami, and bitter taste cells and that some transcription factors other than Skn-1a would also be involved in the generation and differentiation of taste cells. In this symposium, I will show our recent works focused on the transcriptional factors expressed in taste tissues to study how various types of taste cells are generated. (COI:No)

### S07-02

#### Hypothalamic neuronal circuits regulating hunger-induced taste modification

Ou Fu (*Nips, Okazaki, Japan*)

The gustatory system plays a critical role in sensing appetitive and aversive taste for the evaluation of food quality. Although taste sensitivity and preference are known to change depending on internal states, such as hunger, the mechanistic insight remains unclear. Here we examine the neuronal mechanisms regulating hunger-induced taste modification in the mouse brain.

Starved mice exhibit an increased preference for sweet taste and tolerance for aversive taste. This phenomenon is recapitulated by activating the orexigenic AgRP neurons in the hypothalamus, especially that projecting to the lateral hypothalamus. Glutamatergic neurons in the lateral hypothalamus innervate AgRP neurons and they are sufficient and necessary to modulate taste preferences. Furthermore, two distinct pathways from Lateral hypothalamus to the lateral septum or the lateral habenula regulate the preference for appetitive and aversive tastes, respectively. Our results suggest that these hypothalamic circuits for taste modification would be important for optimizing feeding behavior under energy deficiency. (COI:No)

### S07-03

#### Hypothalamic regulation of cortical activity for appetitive behavior

Ikue Kusumoto-Yoshida, Jihao Ma, Ran Yamaguchi, Tomoyuki Kuwaki (*Dept Physiol, Grad Sch Med Dent, Kagoshima Univ, Japan*)

Appropriately regulating food intake is essential for health maintenance. Rising rates of obesity, diabetes, and cachexia pose significant threats to health, and thus understanding brain regions that underlie aberrant food intake is critically important. It is well known that the hypothalamus regulates both food intake and energy homeostasis. However, interactions between the hypothalamus and other brain areas such as cortical regions require further elucidation.

We have been exploring neural circuits downstream of hypothalamic orexin neurons using histological and genetic approach. In study of restricted feeding induced c-fos mapping, increased c-fos signal positive neurons were observed not only hypothalamic orexin neurons but also in the insular cortex. During the restricted feeding paradigm, mice showed increased locomotion just before feeding time that resemble food anticipation similar to the previous study. The insular cortex is known as the higher order sensory cortex that integrate multiple modalities and play an important role in establishing homeostasis within the body. These results suggest an important role of correlated activity for insular and orexin neurons in food anticipatory behavior. Optogenetic stimulation of orexin neuron axon terminals in the insular cortex on food intake showed the increase of both food intake and c-fos expression in the insular cortex, suggesting an important role for the insular cortex in orexin-regulated food intake. (COI:No)

### S07-04

#### Neuronal SIRT1 regulates simple sugar selection through FGF21 and oxytocin signalling in mice

Sho Matsui[1,2] ([1]*Div Food Sci. Biotechnol, Grad Sch Agr, Univ Kyoto, Japan,* [2]*Lab. of Metabolic Signal, IMCR, Univ Gunma, Japan*)

Diet affects health through ingested calories and macronutrients, and macronutrient balance affects health span. The mechanisms regulating macronutrient-based diet choices are poorly understood. Previous studies had shown that NAD-dependent deacetylase sirtuin-1 (SIRT1) in part influences the health-promoting effects of caloric restriction by boosting fat use in peripheral tissues.

First, we analyzed diet selection behavior in neuron-specific Sirt1 overexpression (NS-OE) and knockout (NS-KO) mice. The results indicated that neuronal SIRT1 promotes fat preference, whereas it suppresses sucrose preference. Therefore, neuronal SIRT1 shifts diet choice from sucrose to fat in mice, matching the peripheral metabolic shift.

Next, we identified that SIRT1 positively regulates the expression of oxytocin (Oxt), which is known to specifically suppress the preference to simple sugar, but not fat. We proved the necessity of oxytocin signaling for the regulation of sucrose preference by SIRT1 with pharmacological blockade of Oxt receptor and pharmacogenetic inhibition of Oxt neuronal activation. We also analyzed Oxt neuron-specific Sirt1 overexpression (OS-OE) and knockout (OS-KO) mice to prove that SIRT1 in Oxt neurons is sufficient for regulating sucrose preference in mice. Therefore, SIRT1-mediated suppression of simple sugar preference requires oxytocin signalling, and SIRT1 in oxytocin neurons drives this effect.

Finally, we searched for the metabolic signal that represents simple sugar ingestion. We found that the hepatokine FGF21 acts as an endocrine signal to oxytocin neurons, promoting neuronal activation and Oxt transcription and suppressing the simple sugar preference. SIRT1 promotes FGF21 signalling in oxytocin neurons by up-regulating the expression of beta-klotho, a co-receptor for FGF21, and stimulates Oxt transcription.

Taken together, these data suggest that SIRT1 suppresses simple sugar selection by potentiating the negative feedback by FGF21 via Oxt. (COI:No)

# Symposium 8

## Physiological importance of environmental temperatures in ectotherms

(March 18, 9:00～10:50, Hall 6)

### S08-01

#### Ambient temperature sensing with TRPA1 channel in mosquito

Makoto Tominaga (*Div Cell Signaling, Natl Ist Physiol Sci, Japan*)

Temperature and odors profoundly affect the behavior of animals. Transient receptor potential channel, subfamily A, member 1 (TRPA1) functions as a polymodal nociceptor for sensing both vital environmental cues in insects. Mosquitoes are recognized as disease vectors, and many efforts have been devoted to investigations of their host-seeking behaviors and repellents. However, the physiological characteristics of mosquito TRPA1 have not been systematically studied. We identified multiple alternative splice variants of the TrpA1 gene from Anopheles gambiae (Ag), Anopheles stephensi (As), Aedes aegypti (Aa) and Culex pipiens pallens (Cp) mosquitoes. And we performed comparative analyses of the responses of mosquito TRPA1s to heat or chemical stimuli with calcium-imaging and whole-cell patch-clamp methods. Comparison of TRPA1 among four mosquito species from different thermal niches revealed that TRPA1 of mosquitoes inhabiting the temperate zone had a lower temperature threshold for heat-evoked activation, which was supported by the in vivo heat-avoidance test. Notably, the chemosensitivity of mosquito TRPA1 channels revealed differences not only between variants but also among orthologues. Moreover, we discovered 3 novel mosquito TRPA1 agonists. We described that thermal niches and evolutionary trajectories significantly affect the functional properties of mosquito TRPA1, which represents a hallmark of the behaviors that may permit the design of improved mosquito control methods. (COI:No)

### S08-02

#### Thermo-sensing mechanisms underlying temperature preference in fruit flies

Takaaki Sokabe[1,2] ([1]*Cell Signaling, NIPS, Japan,* [2]*Thermal Biology, ExCELLS, Japan*)

Every animal actively seeks for favorable temperatures in dynamic thermal landscapes depending on their innate preferences. It has been a long-lasting question as to how we sense the environmental temperatures and a subset of TRP channels have been identified as a physiological thermosensor in the last two decades, whose activity is directly regulated by temperature changes. This machinery appears to be conserved among a wide range of species including endotherms and ectotherms. Due to the small body size, insects equilibrate their body temperature with the environments quickly, which differs from mammals with their autonomous body temperature regulation. Therefore, insects are highly sensitive to temperature fluctuation and show a variety of temperature-dependent physiological behaviors.

We recently found that fruit fly (*Drosophila melanogaster*) larvae could discriminate subtle temperature differences and displayed a development-dependent shift in their thermal preference. This thermal preference switch includes rhodopsins, lipid signaling and TRPA1. TRPA1 has been known to respond to temperature changes directly, however, thermo-sensing occurs upstream of TRPA1, possibly through rhodopsins, in this context. These signaling components are expressed together in the central and the peripheral nervous system to regulate behaviors. The roles of rhodopsins outside of phototransduction have been reported in other sensory modalities or other animals, implying that the unconventional role of the light sensor may be evolutionarily conserved. I will also discuss our recent concepts including functional roles of membrane lipids in sensory processes. (COI:No)

### S08-03

#### Effect of temperature on seasonal adaptation mechanism in medaka fish

Takashi Yoshimura[1,2] ([1]*WPI-ITbM, Nagoya Univ, Japan,* [2]*Grad Sch Bioagricult Sci, Nagoya Univ, Japan*)

The appropriate timing of various seasonal processes, such as reproduction, migration and hibernation, is crucial to the survival of animals living in temperate regions. However, underlying mechanisms of seasonal adaptation are not well understood.

Medaka fish (*Oryzias latipes*), an excellent model for studying seasonal adaptation, are active and exhibit clear phototaxis in conditions simulating summer, but remain at the bottom of the tank and failed to exhibit phototaxis in conditions simulating winter. Mate preference tests using virtual fish created with computer graphics demonstrated that medaka are more attracted to orange-red-colored model fish in summer than in winter. Transcriptome analysis of the eye reveals dynamic temperature-dependent seasonal changes in the expression of genes encoding photopigments and their downstream pathways, suggesting that plasticity in phototransduction pathway is crucial for the seasonal changes in color perception.

Depression is considered an adaptation to a harsh environment. Seasonal changes in environment also lead to depression-like behaviors in animals and humans. We observed decreased sociability and increased anxiety-like behavior in medaka exposed to short day and cool temperature, winter-like conditions. Whole brain metabolomic analysis revealed seasonal changes in 68 metabolites, including serotonin and glutamate. Transcriptome analysis identified 3,306 differentially expressed transcripts, including circadian clock genes. A broad-spectrum chemical screen identified a drug that reverse the winter behavior. Our chemical genomics study provides insights into winter induced depression-like behaviors. (COI:No)

### S08-04

#### Molecular mechanisms underlying temperature-dependent sex determination in reptiles

Shinichi Miyagawa (*Dept Bio Sci Tech, Faculty Indust Sci Tech, Tokyo Univ Sci, Japan*)

Sex determination is a critical element in development that greatly influences the individual on multiple levels, including physiological, reproductive and behavioral phenotype. In contrast to sex determination based on intrinsic genotypic factors, as commonly seen in many vertebrates, certain reptiles, including the crocodilians and turtles, display temperature-dependent sex determination (TSD), in which the temperature of the surrounding environment during embryonic development determines the sexual fate of the individual. However, much of the details concerning its underlying molecular mechanism remain to be elucidated, such as how the developing embryo initially detects the external temperature signals and directs the gonadal fate accordingly. We have investigated several thermosensory factors, and particularly focused upon transient receptor potential (TRP) channels as main initiation candidate. Functional characterization of alligator TRPV4 channel reveals that it is activated in temperatures proximate to alligator TSD. We also found that selective inhibition and activation of TRPV4 channel induces both down and upregulation, respectively, of male gene expression cascade, and higher prominence of Müllerian duct in males by TRPV4 inhibition. In addition, we have investigated the effects of several TRP agonists and antagonists on the turtle gonadal differentiation. Our findings provide several insights to genetic framework underlining TSD, and our potential novel findings serve as a basis for further understanding gonadal fate pathway during vertebrate sex determination. (COI:No)

# Symposium 9

## Reverse engineering the brain functions for the control of adaptive behaviors

(March 18, 9:00～10:50, Hall 7)

### S09-01
#### Cryogenic approaches to reveal neural mechanisms for sophisticated feedback motor control

Tomohiko Takei[1,2], Stephen Lomber G.[3], Douglas Cook J.[2], Stephen Scott H.[2] (*[1]Hakubi Cent, Kyoto Univ, Japan, [2]Cent Neurosci, Queen's Univ, Canada, [3]Dept Psychol, Western Univ, Canada*)

Feedback corrections of goal-directed motor action are surprisingly fast and complex, but little is known how such flexible feedback motor actions are generated in the central nervous system. Neurophysiological studies implicate a broad network in fronto-parietal cortices in these feedback corrections, but the specific role of each region is unknown. Here we investigated the function of dorsal premotor cortex (PMd) and parietal area 5 (A5) in feedback control by combining a neural deactivation (cooling deactivation) in non-human primates with a model simulation. To give functional implications to the behavioral results, we generated an optimal feedback control model to observe how deactivations (i.e. reductions) of model parameters impacted feedback responses. Results showed that deactivation of the "feedback controller" impaired both response speed and accuracy, whereas deactivation of "state estimator" impaired only accuracy but not speed of the response. Next, we trained a rhesus monkey to perform a unilateral arm postural task, in which the monkey was required to maintain arm posture while responding to mechanical perturbations. Under normal conditions, the monkey made a quick and accurate perturbation response to return to the original position. When we deactivate PMd, the monkey showed impairments in both response speed and accuracy. On the other hand, when we cooled A5, monkey showed impairment of response accuracy, but not response speed. These results suggest that PMd and A5 have different functions in feedback control: feedback controller and state estimation, respectively. This study demonstrates for the first time that feedback processing for voluntary control involves cortical circuits beyond primary motor cortex. (COI:No)

### S09-02
#### Ventral striatum as a potential therapeutic target for functional recovery after spinal cord injury

Michiaki Suzuki (*Neural Prosthesis Project, Dementia and Higher Brain Function, Tokyo Metropolitan Inst of Med Sci*)

Neuronal mechanisms underpinning functional recovery after spinal cord injury (SCI) have been investigated widely in humans and animal models. Following SCI at the mid-cervical segment in non-human primates, plastic changes in motor cortices and spinal circuits are associated with recovery of dexterous finger movements. However, the contribution of structures up-stream of the motor cortices to recovery after SCI remains unclear. Recently, we demonstrated that the ventral striatum (VSt), which is largely known as a key subcortical node for processing motivation and reward, causally contributes to functional recovery of dexterous finger movements after SCI in monkeys. In addition, with brain imaging study using positron emission tomography we clarified that the neuroplastic functional reorganization of the VSt-motor networks occurs after SCI. These results suggest that the VSt is the pivotal node of the cortical reorganization required for functional recovery of finger dexterity, and that the VSt could be a critical target for therapeutic interventions that aim to promote functional recovery. To test whether the VSt is a useful target for facilitation of motor performance, high frequency electrical stimulation was delivered to the VSt while an intact monkey performed the force-tracking task. VSt stimulation enhanced performance such as number of trials. This result suggests that the VSt might be a key target for the therapeutic intervention to promote functional recovery after SCI. (COI:No)

### S09-03 (AP-1)
#### The local network in the striatum tail contributes to the behavioral switching

Jun Kunimatsu[1,2], Okihide Hikosaka[2] (*[1]Faculty of Med, Univ Tsukuba, Tsukuba, Japan, [2]National Eye Institute, NIH, MD, U.S.A.*)

Although, in our daily life, the object values may change in different environments and we can switch our behavior accordingly, underlying neuronal mechanism is unclear. To address it, we devised a new value procedure: scene-based value task. The monkey viewed 8 fractal objects in 2 scenes (A and B); 4 of them were good (with large-reward) in scene A and bad (with small-reward) in scene B, while the other 4 were good in scene B and bad in scene A. After experiencing this procedure repeatedly, the monkey became able to choose whichever objects were good. Since scenes A and B were presented in a random sequence, the monkey's choice was switched abruptly depending on the scene-context.

We then recorded neuronal activity in striatum tail while the monkey passively viewed these objects in different scenes. We found differences between medium spiny neurons (MSNs) and fast spiking interneurons (FSIs). Many of MSNs responded to the fractal objects differently depending on their values. Importantly, this object-value coding was stronger in either scene A or B. In contrast, FSIs showed no object-value coding. Instead, many of them responded to the scenes selectively (stronger to scene A or B). These results suggested that the object-value coding of MSNs, which is basically stable, is modulated by the inhibitory inputs from the scene-selective FSIs.

To test the causal role of FSI, we locally injected IEM-1460, an inhibitor of GluA2-lacking AMPARs, in the recording sites to selectively block the excitation of FSIs but not MSNs. After injection, monkeys were unable to learn new scene-object value association. On the other hand, object-value learning (no scene) was not affected. This result indicated that the local network of striatum tail regulates the scene-object association learning. These mechanisms may support the monkey's flexible switching based on stable long-term experiences of various environments. (COI:No)

### S09-04
#### Functional and anatomical dissociations between corticostriatal and corticosubthalamic neurons

Yoshihisa Tachibana (*Div Syst Neurosci, Grad Sch Med, Kobe Univ, Japan*)

The cortico-basal ganglia (BG) circuits are important for motor, cognitive, and motivational control of our actions. To achieve these functions, cortical information deriving from the motor, associative, and limbic cortices is processed in BG local networks and finally transmitted to thalamocortical networks and brain stem networks. In these circuits, the activation of cortical neurons is considered to have opposing effects on neuronal activity of BG output nuclei (i.e., the substantia nigra pars reticulata and the internal globus pallidus): the excitatory modulation through the corticostriatal projection and the inhibitory modulation through the corticosubthalamic projection. However, anatomical and functional dissociations between corticostriatal and corticosubthalamic neurons have not been fully elucidated. In this talk, first, I would like to talk about the viral tracing study to test whether the same or different populations of cortical neurons in the primary motor cortex (M1) of mice project to the striatum and subthalamic nucleus. Second, I would like to talk whether the optogenetic manipulation of M1 corticostriatal and corticosubthalamic neurons has opposing effects on motor behavior using self-initiated lever-pull task. Finally, I would like to discuss whether such cortical neurons fire differentially based on the data obtained from different layers of M1 using *in vivo* two-photon calcium imaging. (COI:No)

### S09-05
#### Neural mechanism of time perception

Koji Toda[1], Saya Yatagai[1], Kota Yamada[2], Kohei Yamamoto[1], Katsuyasu Sakurai[3], Warren Meck[4], Henry Yin[4] (*[1]Dept. Psychol., Keio Univ., Japan, [2]Dept. Psychol., Keio Univ., [3]IIIS, Univ. Tsukuba., [4]Dept. Psychol. & Neurosci., Keio Univ.*)

Time perception is a subjective experience observed across many species. Animals need to create the subjective sense of time based on the integration of multiple sensory-motor information of self and perceivable objective events in the external world. Although many researchers have tried to understand the psychological and neurobiological mechanisms of the subjective experience of physical time, theories of time perception have been controversial, contradictory, and confusing. Here we designed a novel experimental setup that combined behavioral, neurobiological, and computational approaches in investigating interval timing. Head-fixed mice were trained on a fixed-time schedule Pavlovian conditioning task. We administered sucrose solution every 10s. No external conditioned stimulus was presented throughout the experiment. Mice could learn to anticipate the timing of the scheduled reward delivery. We found that the pattern of anticipatory licking is significantly modulated by motivational state. In addition, we also used a peak procedure task, in which regular trials are mixed with probe trials with non-rewarded long interval, to assess the internal representation of the expected time of reward delivery. Mice showed a peak response around the trained 10s after the previous reward delivery. To investigate the neurobiological substrates for the timing behavior observed, we used integrative approach that combined immunohistochemistry, chemogenetics, and optogenetics. This novel multi-disciplinary apparoach paves a way forward to study the neural mechanism of the timing behavior. (COI:No)

# Symposium 10

## A new world of physiology developed by peptide hormones

(March 18, 9:00～10:50, Hall 8)

### S10-01
#### Searching of the novel bioactive peptides using various methods

Takanori Ida (*Frontier, Miyazaki Univ, Japan*)

G-protein-coupled receptors (GPCRs) constitute a large protein superfamily that shares a 7-transmembrane motif as a common structure. Human genome sequencing has identified several hundred orphan GPCRs for which ligands have not yet been identified. GPCRs play crucial roles in cell-to-cell communication involved in a variety of physiological phenomena and are the most common target of pharmaceutical drugs. Therefore, the identification of endogenous ligands for orphan GPCRs will lead to clarification of novel physiological regulatory mechanisms and potentially facilitate the development of new GPCR-targeted therapeutics. But in the last 10 years there has been little discovery of novel bioactive peptides for orphan receptors. To break this situation, we searched for model organisms and discovered several novel bioactive peptides. Various applied researches are in progress for these peptides. In parallel, a new peptide extraction method is currently under development. We want to further develop peptide search research. (COI:No)

### S10-02
#### Regulation of insulin-like activities in response to each amino acid

Daisuke Yamanaka[1], Haruka Nagata[2], Yuka Toyoshima[3], Hiroki Nishi[2], Lila Otani[4], Yuki Goda[2], Fumihiko Hakuno[2], Asako Takenaka[5], Hisanori Kato[4], Shin-Ichiro Takahashi[2], Koichi Ito[1] (*[1]Dept Vet Med Sci, Grad Sch Agri Life Sci, Univ Tokyo, Japan, [2]Dept Appl Ani Sci, Grad Sch Agri Life Sci, Univ Tokyo, Japan, [3]Dept Bioreg, Inst Adv Med, Nippon Med Sch, Japan, [4]Dept Appl Bio Chem, Grad Sch Agri Life Sci, Univ Tokyo, Japan, [5]Dept Agri Chem, Sch Agri, Meiji Univ, Japan*)

Insulin-like growth factors (IGF) and insulin are peptide hormones highly homologous in structure and function. IGF and insulin synthesis/secretion is controlled by quality and/or quantity of protein in diets, leading to regulation of growth and metabolism. We have investigated roles of amino acids in IGF/insulin systems using growing rats fed a low protein (LP) diet.

It is known that these animals show reduction in gene expression of IGF-I in liver, a major IGF-producing organ. Our recent analysis using a cultured hepatocyte cell line showed that deprivation of essential amino acids (EAAs) decreased IGF-I mRNA levels, indicating that EAAs are required for upregulation of IGF-I gene expression. Diet-induced insulin secretion is also decreased in LP-fed rats; however, insulin secretion was restored to normal levels by supplementation of the LP diet with three branched chain amino acids, suggesting an important role of BCAAs in insulin secretion. On the other hands, neutral lipids (triglyceride) were accumulated in the liver of LP-fed rats. Analysis using single amino acid-deficient diets indicated that arginine- or threonine-deficient diets increased hepatic triacylglyceride content. Our further research showed that LP and arginine-deficient (dArg) diets regulated lipid accumulation through different mechanisms; insulin signaling and *de novo* lipid synthesis were enhanced in LP-fed rats, while triglyceride release from liver was attenuated in dArg-fed rats. To investigate the underlying mechanisms, we cultured hepatocytes in amino acid-sufficient or deficient medium. Surprisingly, intracellular triacylglyceride level was increased by amino acid deficiency without addition of any lipids or hormones, indicating that hepatocytes themselves monitored the extracellular amino acid concentrations to induce lipid accumulation in a cell-autonomous manner.

Taken together, we concluded that each amino acid play distinct roles in regulation of insulin/IGF systems and induction of insulin-like activities, coordinately controlling growth and metabolism. (COI:No)

### S10-03
#### Intervention effect on glucose intolerance by supplementation with methyl modulator

Takahiro Nemoto (*Department of Physiology, Nippon Medical School*)

According to the Developmental Origins of Health and Disease (DOHaD) theory, low birth weight infants due to malnutrition in the prenatal period acquire a thrifty phenotype. When these babies grow up in a eutrophic environment, there is a mismatch between constitution and environment, and there is a risk of developing various metabolic diseases. We aimed to develop an interventional method by creating a rat model that is low birth weight due to low carbohydrate-caloric restriction during pregnancy and presents hyperinsulinemia with a high fat diet after growth. Metabolomic analysis of rat blood revealed various metabolic changes, and we investigated whether to improve insulin resistance caused by environmental mismatch caused by methyl modulator intervention. Offspring were obtained from dams fed a low carbohydrate-calorie restricted diet (LC) throughout the gestation period. Methyl modulator diet was prepared according to previous reports and fed to dams during late pregnancy or lactating. Each of the offspring was divided into a high fat diet (HFD) group and a standard chow group at the age of 4 weeks, and was reared for 18 weeks. Even if the methyl modulator diet was given to pregnant and postpartum lactating dams, there was no significant increase in body weight or body fat with a HFD-exposure. The blood insulin concentration after oGTT, which was significantly higher in HFD-exposed LC rats, was reduced by methyl modulator supplementation. Insulin receptor expression levels in liver and adipose tissues, which were significantly lower in HFD-fed LC offspring, were normalized by supplementation with methyl donor during pregnancy or after birth. Methyl donor supplementation during pregnancy or after birth in rats with low-calorie intake during fetal life resulted in metabolic changes and normalized gene expression regulation abnormalities. Currently, we are analyzing the effects on the next generation offspring. (COI:No)

### S10-04
#### Regulatory mechanism of neuropeptide "PACAP" on exocrine system

Tomoya Nakamachi (*Lab Regul Bio, Grad Sch Sci Eng, Univ Toyama, Japan*)

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic neuropeptide belonging to the vasoactive intestinal polypeptide (VIP)/secretin/glucagon superfamily. PACAP and VIP bind to PAC1 receptor (PAC1R), VPAC1 receptor (VPAC1R), and VPAC2 receptor (VPAC2R). Mammalian PACAP has the highest binding affinity for PAC1R, almost 1000 times greater than that for VPAC1R or VPAC2R.

Our study was started based on the finding of a new phenotype in PACAP null mice, which show dry eye-like symptoms, corneal keratinization and tear reduction. PACAP and its receptor mRNA were expressed in mouse lacrimal glands. PACAP immunoreactivity was merged with parasympathetic neuronal marker, and PAC1R immunoreactivity was observed in basal side of acinar cells. PACAP eye drop significantly elevated tear secretion and it was canceled by co-treatment with PAC1R antagonist in mouse. By signaling analysis, PACAP eye drops stimulated tear secretion via an adenylate cyclase/cAMP/PKA cascade. PACAP stimulated phosphorylation of aquaporin 5 (AQP5), and its translocation from the cytosol to the membrane in lacrimal acinar cells. Moreover, AQP5 siRNA treatment to lacrimal gland attenuated PACAP-induced tear secretion. These results suggest that PACAP act as an endogenous regulator of tear secretion through AQP5 translocation.

AQP5 is also expressed in salivary gland and sweat gland. Therefore, we investigated the effect of PACAP on saliva and tear secretion. In three major salivary gland, PACAP mRNA was dominantly expressed in sublingual gland and submandibular gland. VPAC1R is dominantly expressed in the three major salivary gland. Intravenous administration of PACAP significantly increased saliva secretion and co-treatment with VPAC1R antagonist suppressed the effect. Meanwhile, sweat gland expressed PAC1R and subcutaneous administration of PACAP significantly increased sweat secretion. The PACAP-induced sweat secretion was suppressed by co-treatment with PAC1R and VPAC2R antagonist. These data suggest that PACAP has a stimulating effect on exocrine glands that express AQP5 via various PACAP receptors. (COI:No)

### S10-05
#### Development of adrenomedullin derivative

Sayaka Nagata (*Faculty of Medicine, Univ of Miyazaki, Japan*)

Human adrenomedullin (hAM) is a hypotensive peptide hormone that exerts powerful anti-inflammatory effects. We recently showed that hAM significantly reduces the clinical severity of the 2, 4, 6-trinitrobenzene sulfonic acid-induced colitis in animal models. Furthermore, in a clinical study, hAM treatment reduced the Disease Activity Index in ulcerative colitis. However, these therapies required continuous administration of hAM as the half-life of native hAM is quite short in blood.

To resolve this problem, we developed two kinds of AM derivatives.

Frist, we conjugated the hAM N-terminal with 60 kDa polyethylene glycol (PEG-AM). A single subcutaneous administration of PEG-AM reduced the total inflammation score in mice with the dextran sulphate sodium (DSS)-induced colitis. In addition, the plasma half-life of PEG-AM was much longer than native hAM. Moreover, we examined the effect of PEG-AM on four-vessel occlusion model rats, which exhibit vascular dementia. PEG-AM treatment prevented memory loss and learning disorders in dose-dependent manner.

Furthermore, we designed of human IgG1 Fc fusion protein containing hAM (Fc-AM). Sufficient concentrations of Fc-AM was observed in blood two days after a single subcutaneous administration. Fc-AM stimulated cAMP production in HEK-293 cells stably expressing the AM1 receptor. Treatment with Fc-AM inhibited blood pressure increase in spontaneously hypertensive rats. In addition, Fc-AM reduced total inflammation scores in the DSS colitis model. These data indicate that PEG-AM and Fc-AM are a possible therapeutic agent for the treatment of inflammatory bowel disease or vascular dementia. (COI:No)

# Symposium 11

## AMED support for medical R&D: From basics to practice

(March 18, 9:00～10:50, Hall 10)

### S11-01

#### Relations between coronary microvascular dysfunction and the development of diastolic dysfunction in prediabetic and diabetic rats

James Pearson[1,2], Hirotsugu Tsuchimochi[1], Takashi Sonobe[1], Jennifer Ngo[1], Mark Waddingham[3], Masaki Wakabayashi[4], Manabu Shirai[4] (*[1]Dept Cardiac Physiol, Res Inst, NCVC, Japan, [2]Dept Physiol, Monash Univ, [3]Dept Adv Medical Res Pulm Hypertension, NCVC, [4]Omics Center, NCVC*)

Diastolic dysfunction of the left ventricle is an early factor in the onset of heart failure. Here we investigated the relations between coronary and myocardial function in the development of diastolic dysfunction associated with diabetes and the roles played by chronic inflammation and oxidative stress. Currently we are utilizing synchroton based microangiography, in vivo cross-bridge dynamics and immunoblotting assays to investigate the role of these factors in non-obese, insulin resistant Goto-Kakizaki rats and obese, hypertensive diabetic stroke-prone SHR rats. Further, the gene expression of sedentary control and GK rats is being compared to aged-matched rats following regular, medium-intensity exercise training using RNAseq analysis. Results to date show that both severe hypertension and diabetes impair cardiomyocyte contractile function and relaxation through myofilament posttranslational modifications independent of coronary endothelial dysfunction but is exacerbated by such progressive changes in coronary endothelial and smooth muscle function. (COI:No)

### S11-02

#### A new biomarker for abdominal aortic aneurysm

Yoshihiro Ishikawa[1], Utako Yokoyama[2] (*[1]CVRI, Grad Sch Med, Yokohama City University, [2]Dept Physiology, Tokyo Medical Univ*)

Abdominal aortic aneurysm is a common disease among elderly people, and is most commonly found in men with smoking history. Epidemic survey has shown that the disease may be found in four to six percent of men above age sixty five. The majority of such patients were found during physical examinations using abdominal ultrasound. Alternatively, in some cases, the presence of aneurysm is not found until it ruptures and the patient is transferred to hospital under critical conditions. Thus, it is important to establish an easy method of detecting abdominal aneurysm. We have identified myosin heavy chain 11 as a biomarker of abdominal aortic aneurysm. We will explain how it was identified and what is the potential use of this biomarker in the future. (COI:No)

### S11-03

#### Development of human arterial graft with mechanically functional extracellular matrices

Utako Yokoyama[1,2], Tomoyuki Kojima[2,3], Junichi Saito[2], Takanori Yoda[2], Takashi Nakamura[2], Yoshinobu Sugo[3], Kentaro Kurasawa[3], Etsuko Miyagi[3], Yoshihiro Ishikawa[2] (*[1]Dept Physiol, Tokyo Med Univ, Japan, [2]Cardiovasc Res Inst, Yokohama City Univ, Japan, [3]Dept Ob/Gyn, Yokohama City Univ, Japan*)

Elasticity and stiffness are necessary for vascular integrity. Therefore, biological tissue-engineered blood vessels with mechanically functional extracellular matrices are desired. Fibronectin fibrillogenesis plays a critical role for assembling elastic fibers and collagen fibers which provide elasticity and stiffness. Recently, we found that periodic hydrostatic pressure (PHP) with extremely low frequency promoted fibronectin fibrillogenesis on the surface of vascular cells. We successfully fabricated implantable human arterial graft by PHP, and examined PHP-induced mechanosensing mechanisms in vascular smooth muscle cells. We seeded human umbilical arterial smooth muscle cells (hUASMCs) on cell culture disk to make the first cell layer. Twenty-four hours after seeding, cells were exposed to PHP for 24 h, and then cells for the next layer were seeded on the top of the first layer, followed by repeating the same procedure ten times to construct multi-layer cell sheet. The burst pressure of the construct was over 1200 mmHg. The multi-layered construct was trimmed as a patch graft. The graft was sutured at the abdominal aorta of nude rat in which the same size of aortic tissue was resected. Five months after implantation, echocardiography confirmed complete patency and histological analysis revealed that all patch grafts were completely endothelialized. Host-derived cells markedly infiltrated into the graft. To examine the PHP-responsive genes, we performed RNAseq analyses and found that angiopoietin-like 4 (ANGPTL4) and insulin-like growth factor binding protein 5 (IGFBP5) that binds to fibronectin were highly increased by PHP. In conclusion, implantable human arterial graft consisting of vascular smooth muscle cells was constructed by exposure to PHP. (COI:No)

### S11-04

#### Development of Japan originated neuro-modulation system treating cardiovascular disease

Keita Saku (*Dept Cardiovasc, Grad Sch Med, Kyushu Univ, Japan*)

The imbalance of autonomic nervous system, disproportional sympathetic upregulation and parasympathetic downregulation, is involved in the pathogenesis of various cardiovascular diseases, such as hypertension, myocardial infarction (MI) and heart failure. Based on the solid preclinical evidence of therapeutic effects, several devices to restore the autonomic balance by stimulating or denervating the nerves have been developed in worldwide. We recently develop the several neuro-modulation devices for cardiovascular disease.

The infarct size in MI predicates survival outcomes, therefore therapies that reduce infarct size beyond early coronary reperfusion are critical to prevent the subsequent development of heart failure. Vagal verve stimulation, which directly activates parasympathetic nervous system, has been reported to exert multiple cardio-protective effects. Despite attractive preclinical studies of vagal nerve stimulation for acute MI, its clinical translation remained unestablished. We have been developing an intravenous vagal nerve stimulation (iVNS) catheter system which can stimulate the right vagal nerve with minimally invasive technique. We confirmed that iVNS during acute MI markedly reduces the infarct size and prevents subsequent heart failure in a dog model of ischemia-reperfusion. We are now proceeding the strategies to obtain the regulatory approval of iVNS catheter system as a medical device.

In this session, we will introduce our challenges in device development and address the barriers against the development of invasive medical devices in Japan by referring to our experiences. (COI:No)

### S11-05

#### The research and development of a robot that manages anesthesia

Kenji Shigemi[1], Osamu Nagata[2], Yuka Matsuki[1], Yoshihiro Ogino[3] (*[1]Dept Anesthesiol Reanimatol, Sch Med, Fukui Univ, Japan, [2]Dept Anesth, Ctr Hosp Natl Ctr Global Health Med, [3]Nihon Kohden Corp*)

University of Fukui, Center Hospital of the National Center for Global Health and Medicine (NCGM), and Nihon Kohden Corporation are jointly researching and developing a robot device that manages general anesthesia which is necessary for surgical operation. This study was accepted by Japan Agency for Medical Research and Development (AMED) as a three-year project from 2018, and the results are scheduled to be applied for regulatory affairs at Pharmaceutical and Medical Devices Agency (PMDA) in 2021. From the induction of general anesthesia to the recovery from general anesthesia, this device under development controls the intravenous infusion rate of sedative (propofol), analgesic (remifentanil), and muscle relaxant (rocuronium) based on information from the electroencephalogram (BIS processor QE-910P, Nihon Kohden Co., Tokyo), the muscle relaxation monitor (TOF Watch SX, Nihon Kohden Co., Tokyo) with three syringe pumps (TE-SS835T and TE-SS800N, Terumo Co., Tokyo) through a personal computer. The required dose of propofol is estimated on the dose-response curve which is determined with the relationship between BIS value and the effect site concentration (Ce) of propofol. The dose of remifentanil is determined with the balance of the doses of propofol and remifentanil. Rocuronium is administered to maintain the level of surgical muscle relaxation (%T1 from 3% to 10%). This device is fundamentally based on the closed-loop drug delivery system to maintain sedation, analgesia and muscle relaxation appropriately, and is expected to prevent human error and drug overdose/underdose, and to improve anesthesia safety as well as to contribute to doctors' work style. (COI:No)

# Symposium 12

## Control of endogenous regulatory responses in glial cells in neuroinflammation – From embryonic development to pathogenesis of brain diseases

(March 18, 15:20～17:10, Hall 7)

### S12-01

#### Microglia affect animal behaviors; neuroinflammatory processes in the normal mature brain

Kazuya Miyanishi (*Dept Mol Cell Physiol, Grad Sch Med, Ehime Univ, Japan*)

Microglia are not resting cells even in normal mature brains, but they constantly move their ramified processes to monitor the activity states of neural circuits. The physiological functions of microglia are yet obscure. We have found that microglia in substantia nigra pars reticulata (SNr) display more activated morphology rather than those in the SN pars compacta in a Parkinson's disease rat model. Microglia may compensate the disordered neurotransmissions by eliminating glutamatergic syanapses in the SNr from hyperactive subthalamic nuclei. Furthermore, microglia were found to be weakly activated around the onset of sleep. The weakly activated microglia may contribute to sleep/wake cycle by phagocytosing synapses. The activation signals may be mediated by JAK/STAT/IRF -dependent signaling pathway. These findings suggest that neuroinflammatory processes may be responsible for activation of microglia even in the normal mature brains. We have further noticed that microglia may affect social interaction between males and females. Behavioral tests have revealed that male Wistar rats prefer females that are rather inactive and anxious, but friendly. We divided the ovariectomized female rats into two groups "Winner" and "Loser" by examining the preference by male rats; Winners were more popular among male rats than Losers. In the Winners' prefrontal cerebral cortex, higher expression of mRNA for phagocytosis-related proteins were found relative to that in the Losers'. Flow cytometrical analyses showed that microglia from Winners displayed higher forward scatter values than those from Losers. Inactive microglia were also found in a rat strain that shows characteristic behaviors similar to cases with attention deficit/hyperactive disorder. In conclusion, microglia may play a role in the behavior by modulating neural circuits while neuroinflammatory processes may affect microglial function even in the normal mature brain. (COI:No)

### S12-02

#### Activating a non-neuronal cardiac cholinergic system consolidates the blood brain barrier associated with upregulation of anti-inflammatory action

Yoshihiko Kakinuma[1], Shino Oikawa[1], Yuko Kai[1], Asuka Mano[1], Shuei Sugama[1], Naoko Mizoguchi[2], Masayuki Tsuda[3], Kazuyo Muramoto[2] ([1]*Dept Bioregulatory Sci (Physiol), Grad Sch Med, Nippon Medical School, Japan,* [2]*Dept Physiol Sch Dentistry, Meikai University,* [3]*Inst Lab Anim Res, Kochi Medical School*)

We previously reported that the heart-specific choline acetyltransferase (ChAT) gene overexpressing mice (ChAT) demonstrate beneficial phenotypes, i.e., tolerance against ischemia and CNS stress . In this study, we focused on mechanisms responsible for systemic and localized anti-inflammatory phenotypes of ChAT. ChAT were resistant to systemic inflammation induced by lipopolysaccharides due to an attenuated cytokine response. In addition, ChAT, equipped with less reactive Kupffer cells, were refractory to brain cold injury, with decreased blood brain barrier (BBB) permeability and reduced inflammation. This is because ChAT brain endothelial cells expressed more claudin-5, and their astrocytes were less reactive with decreased hypertrophy. Moreover, reconstruction of the BBB integrity in vitro confirmed the consolidation of ChAT BBB. ChAT were also resistant to 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) neuronal toxicity due to lower mortality rate, fewer neuronal loss of substantia nigra and attenuated BBB disruption, as evident from reduced sodium fluorescein levels in the brain parenchyma. The activated central cholinergic pathway of ChAT lead to anti-convulsive effects like vagus nerve stimulation. However, DSP-4, a noradrenergic neuron-selective neurotoxin against the locus ceruleus, abrogated the beneficial phenotype and vagotomy attenuated expression of claudin-5, suggesting the link between the cholinergic pathway and BBB function. These findings indicate that ChAT possess an anti-inflammatory response potential, associated with upregulated claudin-5, leading to the consolidation of BBB integrity. These characteristics protect ChAT against systemic and localized inflammatory pathological disorders, which targets the CNS. (COI:No)

### S12-03

#### Periodontal disease bacteria-induced neuroinflammation and Alzheimer's disease

Zhou Wu[1,2] ([1]*Dept .Phamacol, Grad Sch Dent, Kyushu Univ, Japan,* [2]*OBT Research Center, Grad Sch Dent, Kyushu Univ, Japan*)

Dementia is increasing rapidly in Japan with the super-aged society. Alzheimer's disease (AD), which accounts for 70% of dementia, could not been treated yet since its onset, resulting in enormous medical costs and a serious social burden. The cognitive decline in AD due to neuronal death which is caused by amyloid $\beta$ (A$\beta$) accumulation, excessive tau phosphorylation in neurons, and microglia- dependent neuroinflammation accumulates A$\beta$ and tau phosphorylation. On the other hand, the positive correlations between AD and periodontitis (the most common periodontal disease) have been reported by the western clinical studies, and P. gingivalis (Pg) LPS, the major periodontal disease bacteria, has been detected from the autopsy brain of AD patients. The involvement of periodontitis in AD has been attracting attention, because periodontitis is treatable oral disease.

In exploring the impacts of systemic inflammation on brain functions constantly, we have found that chronic systemic inflammation induces an age-dependent neuroinflammation, which causes age-dependent cognitive decline. In investigating the involvement of periodontitis in AD, we have found that chronic exposure to PgLPS induces AD-like pathological phenotypes, including microglia-dependent neuroinflammation, A$\beta$ accumulation in neurons and learning memory impairment in the middle-aged mice. Cathepsin (Cat) B, a lysosomal proteolytic enzyme, has been determined as a related enzyme in the PgLPS-induced AD-like pathogenesis. Furthermore, we have revealed that CatS is an enzyme related to the Pg LPS- induced systemic inflammation. In the present symposium, I will explain the involvement mechanism of periodontitis in AD onset and pathological processes, and introduce the clinical research for improvement of cognitive functions by regulating systemic inflammation and neuroinflammation. The early intervention in oral/systemic inflammation is a feasible medical approach for prevention or delaying the onset and progression of AD. It is also important to develop the specific inhibitors of CatB and CatS for regulating systemic inflammation and neuroinflammation. (COI:Properly Declared)

### S12-04

#### Stress-induced microglial activation occurs through beta-adrenergic receptors: Noradrenaline as key neurotransmitter in microglial activation

Shuei Sugama (*Dept Physiol, Nippon Med School, Japan*)

The involvement of microglia in neuroinflammatory responses has been extensively demonstrated. Recent animal studies have shown that exposure to either acute or chronic stress induces robust microglial activation in the brain. In this presentation, we tried to investigate the underlying mechanism of brain microglial activation by acute stress. At first, we looked at the spetial distribution of noradrenaline-synthesizing enzyme, DBH, in comparison with noradrenaline receptors, b1, b2, and b3 adrenergic receptors after which we examined the effects of the beta-blocker propranolol and alpha-blockers prazosin and yohimbine on stress-induced microglial activation. Finally, we compared stress-induced microglial activation between wild-type (WT) mice and double-knockout (DKO) mice. The results demonstrated that microglial activation occurred in most studied brain regions, including hypothalamus, thalamus, and hypothalamus; that b1- and b2-, but not b3-, adrenergic receptors are colocalized with microglial cells, as observed by laser scanning microscopy; that beta-blocker treatment inhibited microglial activation, with alpha-blocker showing no such effect; and that unlike WT mice, DKO mice exhibited substantial inhibition of stress-induced microglial activation in the brain. We demonstrate here that neurons/microglia may interact with noradrenaline via b1- and b2-adrenergic receptors. In this symposium, we discuss a possible mechanism for stress-induced microglial activation. (COI:No)

# Symposium 13

## New strategies for understanding functions and mechanisms of ion channels/transporters/pumps in the upcoming post-structure era

(March 18, 15:20～17:10, Hall 9)

### S13-01

#### Roles of the pore helix in the regulation mechanisms of gating of GIRK channels

I-Shan Chen[1], Yoshihiro Kubo[1,2] (*[1]Div. Biophys and Neurobiol, NIPS, Okazaki, Japan, [2]Physiol Sci. SOKENDAI, Hayama, Japan*)

G-protein-gated inwardly rectifying $K^+$ (GIRK) channels control various physiological functions. For example, GIRK1/2 heterotetramers in the brain regulate neuronal excitability; GIRK1/4 heterotetramers in the heart regulate heart rate. We previously identified a novel GIRK activator, ivermectin, and more recently a novel GIRK inhibitor, terfenadine. We reported that a GIRK1-specific amino acid residue located in the pore helix behind the selective filter, Phe137, is critical for the terfenadine-mediated inhibition. Here, by electrophysiological analyses and docking simulations, we present our observations of mutants at the pore helixes, including Phe137 in GIRK1 subunit and the corresponding residue Ser148 in GIRK2 subunit, and some amino acid residues which are close to this region. We found that the pore helix residue plays multiple roles in the function of GIRK channels as follows: (1) it couples the terfenadine induced conformational change with the channel closure and its side chain volume is crucial; (2) it contributes to the gating behaviour upon membrane potential hyperpolarization; and (3) it regulates the ion selectivity and the polarity of the residue is important. The results provide us with a novel insight into the gating mechanisms of GIRK channels by the pore helix. (COI:No)

### S13-02

#### Analysis of the Inactivation mechanism in a Mutant of the Voltage-Gated Potassium Channel Kv1.2

Hiroko X. Kondo[1], Norio Yoshida[2], Gen Masumoto[3], Matsuyuki Shirota[4,5,6], Yu Takano[7], Kengo Kinoshita[5,6,8] (*[1]Fac Eng, Kitami Inst Tech, Japan, [2]Grad Sch Sci, Kyushu Univ, Japan, [3]RIKEN ISC, [4]Grad Sch Med, Tohoku Univ, Japan, [5]Grad Sch Info Sci, Tohoku Univ, Japan, [6]ToMMo, Tohoku Univ, Japan, [7]Grad Sch Info Sci, Hiroshima City Univ, Japan, [8]IDAC, Tohoku Univ, Japan*)

Voltage-gated potassium (Kv) channels conduct potassium ions selectively in response to membrane depolarization, and ion permeation through the pore domain is dynamically regulated by several types of gating mechanisms. "C-type" inactivation is one of well-defined inactivation processes in the Kv channels. This inactivation is primarily caused by conformational changes around the selectivity filter (SF) and/or outer mouth of the channels. The W366F mutant of Kv1.2 channel (corresponding to W434F in Shaker) is known as a fast-inactivating mutant and is in a C-type inactivated state at a depolarizing membrane potential. In order to clarify the structural properties of C-type inactivated protein, we performed molecular dynamics simulations of the wild-type and W366F mutant of the Kv1.2-2.1 chimera channel and carried out the structural analysis by using the statistical methods. In our simulation the W366F mutant was in a nearly nonconducting state with a depolarizing voltage and recovered from inactivation with a reverse voltage. The result of the principal component analysis for SF structures shows a conformational difference at V375 and G376 between channels in the 'active' and 'inactive' states. To evaluate a correlation between SF conformation and ion occupancy state in SF, we analyzed the probability distributions of potassium ions in SF of the WT and W366F mutant by using the three-dimensional reference interaction site model (3D-RISM) theory. Our simulations and 3D-RISM analysis suggested that structural changes in the SF upon membrane depolarization trap potassium ions around the inner mouth of the SF and prevent ion permeation. This pore restriction is involved in the molecular mechanism of C-type inactivation. (COI:No)

### S13-03

#### Structural and molecular basis of $Na^+$/D-glucose co-transporters hexose specificity

Kazuyo Kamitori[1,2], Yuichiro Fujiwara[1,2] (*[1]Dept Mol Physiol and Biophys, Fac Med, Kagawa Univ, Japan, [2]Int Inst Rare Sugar Res and Ed*)

$Na^+$-dependent sugar transporters utilize the transmembrane sodium gradients for cellular sugar uptake. Human $Na^+$/D-glucose cotransporters (SGLTs) are abundant in small intestine and kidney where they contribute to the D-glucose absorption and reabsorption, respectively. Tumor cells highly express SGLTs to facilitate D-glucose uptake and glycolysis, resulting in the unlimited tumor growth. These physiological and pathological functions made SGLTs the targets for diabetes or cancer treatment, and several SGLT2 inhibitors are already used as anti-diabetic drugs. Each SGLT transports specific hexoses in specific tissues, therefore the hexose specificity should be precisely regulated depending on their roles. This specificity is determined by the structure around the hexose binding pocket. Understanding structural basis of SGLTs hexose specificity would provide important information for the drug design targeting these molecules. Although crystal structures of SGLTs has not been reported, here we performed homology modeling of human SGLT1 using the template structure of *Vibrio parahaemolyticus* $Na^+$/D-galactose cotransporter.

We have reported human SGLT1 transport capacity for various hexoses using the two-electrode voltage-clamp method in *Xenopus laevis* oocytes. We observed that both D-glucose and D-galactose were cotransported with $Na^+$ as previously reported, and that none of other hexoses were transported. This suggests that SGLT1 has tolerance for the orientation of OH group in the 4-position but intolerance for the 2-, 3-positions. We performed mutation studies based on the structure model, toward the understanding of hexose specificity. Interestingly, some of the point mutants at amino acids in the sugar binding pocket changed the substrate specificity; that is, D-mannose and D-allose were transported, or transportation of D-glucose or D-galactose was diminished. These results shed light on the relationship between the sugar orientation and the hexose specificity in SGLT1. Present analyses would contribute to the clinical strategies targeting SGLTs, on the points of controlling efficacy and side effects. (COI:No)

### S13-04

#### Structural basis of the proton extrusion mechanism of the gastric proton pump

Kazuhiro Abe[1,2] (*[1]CeSPI, Nagoya Univ, Japan, [2]Grad Sch Pham Sci, Nagoya Univ*)

The gastric proton pump, H+, K+-ATPase is a P-type ATPase that is responsible for acidifying the gastric juice down to pH 1, and is thus an important drug target for treating gastric acid-related diseases. It mediates electro-neutral exchange of H+ and K+ coupled with ATP hydrolysis, but with an as yet undetermined transport stoichiometry. Here we present the crystal structures of the H+, K+-ATPase in complex with two acid blockers, vonoprazan and SCH28080, in the luminal-open E2P state. These drugs have partially overlapped, but clearly distinguishable, binding modes, which are defined in the middle of a conduit running from the gastric lumen to the cation-binding site. The crystal structures also revealed a conserved lysine residue that points to the juxtaposed carboxyl residues in the cation-binding site. The unusual configuration of the cation-binding site enables the extrusion of a single proton even into the pH1 solution of the stomach (Abe et al., 2018, Nature).

We also show crystal structures of E2-P transition state, in which the counter-transporting K+ is occluded. We found a single K+ bound to the cation-binding site of H+, K+-ATPase, indicating an exchange of 1H+/1K+ per hydrolysis of one ATP molecule. This fulfils the energy requirement for the generation of a six pH unit gradient across the membrane. The structural basis of K+ recognition is resolved, supported by molecular dynamics simulations. These results establish how the gastric pump overcomes the energetic challenge to generate an H+ gradient of more than a million-fold - the highest cation gradient known in any mammalian tissue – across the membrane (Yamamoto et al., 2019, eLife). (COI:No)

# Symposium 14

## The new sunrise of zinc physiology and zinc pathophysiology

(March 19, 9:00～10:50, Hall 7)

### S14-01

#### Integrated omics analysis revealed mechanisms underlying improvement of mouse hippocampal function with zinc-enriched breeding over generations

Kumiko Sakai[1], Seiichi Chiba[2], Kenji Sakai[3] (*[1]Res Promo Inst, Fac Med, Oita Univ, Japan, [2]Dept Anatomy, Fac Med, Oita Univ, Japan, [3]Dept Biosci Biotech, Grad Sch Biore Bioenvtl Sci, Kyushu Univ, Japan*)

Zinc is a necessary element for neuromodulation in brain and its homeostasis is strictly controlled under healthy conditions. Relationships between zinc-deficiency and neurological disorders have been well-investigated to date, however, little is known about the implication of an excess but physiologically normal level of zinc for neuronal metabolism in the brain. In this study, integrated analysis of metabolomics and transcriptomics were performed improve comprehensive understanding of the molecular mechanism of brain zinc homeostasis and its neurological and psychological function.

We continued to administer zinc 3 times the normal level to mice over generations. Behavioral experiments of their offspring showed increases in some brain functions such as social behavior, learning and memory levels compared to control mice. Metabolomic profiling of hippocampus tissue revealed distinguishable profiles of metabolites and the pathway analysis indicated specific relationship to catechol amine biosynthesis by the chronic zinc treatment. Microarray analyses that were conducted to identify differential expressed genes and their Gene Ontology analyses using Metascape highlighted genes with functional terms of **Behavior, GPCR, Transmembrane, Neuronal System,** etc. We also applied an integrated analysis of the metabolomics and transcriptomics data together with the term **Zinc** using the system biology tool IPA to analyze the significantly altered molecular and bio function networks. The integrated omics profiles indicated that the function categories of **Nervous System Development and Function** and **Cell-To-Cell Signaling and Interaction** were strongly facilitated, whereas the diseases categories of **Neurological Disease** and **Psychological Disorder** were significantly inhibited by zinc-related molecules and genes. This comprehensive study of neurological functions and networks would provide new insights of zinc into advanced and healthy functions of brain. (COI:No)

### S14-02

#### Zinc complex to treat type 2 diabetes with improving insulin resistance

Hiroyuki Yasui[1], Yuki Naito[1], Yutaka Yoshikawa[2] (*[1]Dept Anal Bioinorg Chem, Kyoto Pharm Univ, Japan, [2]Kobe Women's Univ, Japan*)

We have synthesized and investigated transition metal complexes including manganese (Mn), copper (Cu), zinc (Zn), and platinum (Pt) for the purpose of new drug development treating intractable diseases, based on bioinorganic medicinal chemistry. Especially, Zn(II) compounds have been clinically used for the treatment of skin injury, gastric ulcer, Wilson disease, and very recently hypozincemia resulting from low zinc status. Additionally, Zn(II) ion attracts higher attention because of both the various physiological functions and discovery of many zinc transporter proteins.

In this paper, we would like to introduce our research of potent Zn(II) complexes to treat type 2 diabetes mellitus (DM) with improving insulin resistance. It is well known that the number of DM patients increases gradually in the world. Therefore, the prevention and treatment for DM with new kinds and novel actions of medicines are much needed such as GLP-1 like analogs, DPP4 and SGLT2 inhibitors. Before now, we have synthesized over 250 kinds of Zn(II) complexes with different chemical-structures of ligans/coordination modes of atoms, and evaluated the *in vitro* insulin-mimetic/enhancing actions, pharmacokinetics (PK), and anti-DM therapeutic effects with respect to rational drug design concept.

From the results, we first found that zinc complexes coordinated with both sulfur and oxygen atoms exhibit high insulin-mimetic activities, preferable PK properties after oral administration, desirable bio-distribution to targeted organs, and effective therapeutic action for DM including the protection of pancreatic islets. Additionally, we address the action mechanism for anti-diabetic activities of Zn(II) complexes, which directly activate intracellular PI3-K within the insulin signaling pathway in an insulin-independent manner. Then, it is concluded that our developed Zn(II) complexes have high potential to treat type 2 DM with improving insulin resistance in peripheral organs, and that Zn(II) complexes can be insulin-enhancer suppressing insulin requirements. (COI:No)

### S14-03

#### Molecular basis on lung pathogenesis in cystic fibrosis - dysregulated splicing switches of zinc transporter ZIP2 and anti-inflammatory molecule SIGIRR

Tsuyoshi Shuto (*Global Cent Nat Resources Sci, Grad Sch Pharm Sci, Kumamoto Univ, Japan*)

Airway mucus hyperproduction and hyperinflammation are important hallmarks of cystic fibrosis (CF). Dysregulated expression of airway ion transporters CFTR and ENaC have been implicated as causes of CF-associated mucus hypersecretory and hyperinflammatory phenotypes. However, the roles of $Zn^{2+}$ and $Zn^{2+}$ transporters as well as anti-inflammatory molecules in the regulation of CF airway pathogenesis remain unelucidated. First, we identified a novel connection between CFTR/ENaC expression and the intracellular $Zn^{2+}$ concentration in the regulation of mucin MUC5AC. CFTR-defective and ENaC-hyperactive airway epithelial cells specifically and highly expressed a unique, alternative splice isoform of the zinc importer ZIP2/SLC39A2 (ΔC-ZIP2), which lacks the C-terminal domain. Importantly, ΔC-ZIP2 levels correlated inversely with wild-type ZIP2 and intracellular $Zn^{2+}$ levels. Moreover, the splice switch to ΔC-ZIP2 as well as decreased expression of other ZIPs caused zinc deficiency, which is sufficient for induction of MUC5AC. We next explored the possible involvement of single immunoglobulin interleukin-1 receptor (IL-1R)-related molecule (SIGIRR), a membrane protein essential for suppressing TLRs- and IL-1R-dependent signals, in the regulation of CF airway hyperinflammation. We showed that cell surface expression and anti-inflammatory function of SIGIRR were specifically and remarkably down-regulated in CFTR-defective CF airway epithelial cells compared to non-CF cells. Notably, CF airway epithelial cells specifically and highly expressed a unique, alternative splice isoform of SIGIRR that lacks exon 8 (Δ8-SIGIRR), which results in the production of truncated form of SIGIRR protein. Δ8-SIGIRR protein was expressed intracellularly and its over-expression abolished plasma membrane expression and function of SIGIRR in non-CF cells, indicating that the splice switch to Δ8-SIGIRR is sufficient for reduced surface SIGIRR expression. Taken together, we demonstrate the unique splicing switches of ZIP2 and SIGIRR genes control mucus hypersecretory and hyperinflammatory phenotypes in cystic fibrosis airway epithelial cells, respectively. (COI:No)

### S14-04

#### Innovative insight into defense against progressive neurodegeneration caused by synaptic $Zn^{2+}$ dysregulation

Atsushi Takeda (*Dept Neurophysiol, Sch Pharmaceutical Sciences, Univ Shizuoka, Japan*)

The causes of progressive neurodegenerative disorders, i.e., Alzheimer's disease (AD) and Parkinson's disease (PD) are unknown. The basal (static) concentration of intracellular $Zn^{2+}$ is estimated to be approximately 100 pM and is extremely lower than that of intracellular $Ca^{2+}$ (approximately 100 nM), suggesting that intracellular $Zn^{2+}$ homeostasis is crucial for neural function. Moreover, the basal concentration of extracellular $Zn^{2+}$ is estimated to be approximately 10 nM and is age-relatedly increased based on age-related increase in brain extracellular Zn. We postulated that progressive neurodegeneration is due to age-related intracellular $Zn^{2+}$ dysregulation, which is induced by rapid influx of extracellular $Zn^{2+}$. Neuronal amyloid $\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) accumulation is considered an upstream event in the AD pathogenesis. Here we report that Zn-$A\beta_{1\text{-}42}$ oligomers formed in the extracellular compartment are synaptic activity-independently taken up into neurons, followed by rapid intracellular $Zn^{2+}$ dysregulation. PD is characterized by selective loss of dopaminergic neurons in the substantia nigra pars compacta of the brain. Here, we report a unique mechanism of nigral dopaminergic degeneration, in which rapid intracellular $Zn^{2+}$ dysregulation via the production of reactive oxygen species, especially hydrogen peroxide causes PD in rats, which is induced by paraquart and 6-hydroxydopamine. I will talk about novel defense strategy against progressive neurodegeneration by controlling intracellular $Zn^{2+}$ dysregulation. (COI:No)

# Symposium 15

## Aging-related changes in physiological functions induced by the space environment

(March 19, 9:00～10:50, Hall 8)

### S15-01

#### Vestibular plasticity and orthostatic hypotension

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

Daily activity-induced changes in the gravitational vector one of the major disturbances that affect the cardiovascular system. For example, a postural change from a recumbent to an upright position induces an increase in the hydrostatic pressure gradient, a footward fluid shift, reduced venous return and cardiac output, and reduced arterial pressure (AP). This reduction in AP is sensed by baroreceptors in the blood vessels, and AP is thought to be stabilized by the arterial baroreflex, an important negative feedback process. On the other hand, postural changes stimulate the peripheral vestibular organs. Stimulation of the peripheral vestibular organs by head movement or changes in gravitational forces is known to induce sympathoexcitation (vestibulo-sympathetic reflex). We have reported that the combination of both the baroreflex and the vestibulo-sympathetic reflex is important for maintaining AP during postural change. Daily gravitational change is important for maintaining the vestibular function. Elderly people and astronauts are known to show a plastic alteration of the vestibular system because of reduction in vestibular inputs (less activity in elderly people and no gravity in astronauts). This plasticity might participate in the increase in risk of orthostatic hypotension in elderly people and astronauts. This possibility will be discussed in this symposium. (COI:No)

### S15-02

#### Ameliorating effect of noisy galvanic vestibular stimulation on body balance

Chisato Fujimoto, Tatsuya Yamasoba, Shinichi Iwasaki (*Dept Otolaryngol Head Neck Surg, Grad Sch Med, Univ Tokyo, Japan*)

Noisy galvanic vestibular stimulation (nGVS) is a procedure that applies electrical current as zero-mean current noise to the vestibular system through electrodes placed over the bilateral mastoid process. An optimal level of nGVS facilitates the processing of subthreshold stimuli in neural systems, such as the autonomic, motor or postural control systems. With regard to the postural control system, stability in standing posture and gait performance were improved during the application of imperceptible optimal level of nGVS in healthy subjects and in patients with bilateral vestibulopathy (BV). On the other hand, a further increase of nGVS intensity degrades stability of standing posture. The proposed mechanism behind these effects is stochastic resonance, in which the existence of an optimal amount of noise can enhance subthreshold signals in a non-linear system. We recently found that a 30-min application of nGVS at optimal intensity led to a post-stimulation ameliorating effect of the postural stability that lasts for several hours in healthy elderly adults and BV patients, even after the cessation of the stimulus. nGVS is a promising candidate for a novel treatment of refractory postural instability due to vestibulopathy. Further clinical studies are needed to increase the evidence level of the therapeutic effects of nGVS. (COI:No)

### S15-03

#### Roles of Dkk2 in the effects of gravity change on the interactions between muscle and bone in mice

Naoyuki Kawao[1], Hironobu Morita[2], Hiroshi Kaji[1] ([1]*Dept Physiol & Regene Med, Kindai Univ Fac Med, Japan,* [2]*Dept Physiol, Gifu Univ Grad Sch Med, Japan*)

Long term space flight simultaneously induces muscle and bone loss, and the interactions between muscle and bone have been recently noted. Previous studies indicate that inhibitors of canonical Wnt signal, Dkks, play crucial physiological and pathophysiological roles in bone metabolism. Although canonical Wnt signal is involved in aging-related muscle atrophy, its roles in the interactions between muscle and bone have remained unclear. We therefore examined roles of Wnt signal-related factors in the effects of gravity change on the muscle/bone relationships in mice. Mice were exposed to 3 g hypergravity environment using centrifuge. Hindlimb unloading was achieved by the tail suspension. Mice were kept in hypergravity and HU for 4 and 3 weeks, respectively. In a comprehensive DNA microarray analyses, Dkk2 was identified as a soluble factor whose expression was most increased and reduced by hypergravity and HU in the soleus muscle of mice, respectively. Hypergravity significantly reduced levels of Dkk2 mRNA in the soleus muscle and serum Dkk2 in mice, although HU increased the levels of Dkk2 in serum and the soleus muscle of mice. Moreover, shear stress reduced Dkk2 expression in mouse C2C12 myotubes. Simple regression analysis showed that serum Dkk2 levels and Dkk2 expression in the soleus muscle were negatively related to trabecular bone mineral density in mice. As for the effects of Dkk2 on bone cells, Dkk2 decreased the expressions of osteogenic genes, alkaline phosphatase activity, and mineralization in mouse primary osteoblasts, although it enhanced RANKL expression. In conclusion, we showed that Dkk2 contributes to the interaction between muscle and bone in response to gravity change and mechanical unloading in mice. An increase in Dkk2 expression in the skeletal muscle may be involved in microgravity- and disuse-induced bone and muscle loss. (COI:No)

### S15-04

#### Mechanical load-regulated expression of periosteal Osteocrin promotes CNP-dependent bone formation

Haruko Takano [1], Hiroki Ochi[2], Shigetomo Fukuhara[3], Yasuhiro Sawada[4], Shingo Sato[2], Akihiro Yasoda[5], Naoki Mochizuki[1] ([1]*Dept Cell Biol., Natl. Cerebral and Cardiovasc. Ctr. Res. Inst.,* [2]*Dept. of Physiol. and Cell Biol., Grad. Sch of Med. and Dent. Sci. Tokyo Med. and Dent. Univ.,* [3]*Dept. of Mol. Pathophysiol., Inst. of Adv. Med. Sci., Nippon Med. Sch.,* [4]*Dept. of Rehab. for Motor Func., Natl. Reha. Ctr. for Persons Disabil.,* [5]*Clin. Res. Ctr., Natl. Hosp. Org. Kyoto Med. Ctr.*)

Genetic regulation followed by mechanical stimuli including loading after birth affects long bone growth. However, little is known about how mechanical factors modulate bone growth. Here, we show that a periosteal osteoblast-derived secretory peptide, Osteocrin (OSTN), is induced by mechanical loading and promotes long bone growth. OSTN expression in tibial periosteum was suppressed by mechanical unloading and restored by subsequent re-loading. The mice lacking OSTN showed less bone mass in trabecular and cortical regions of long bones than the control mice, suggesting the contribution of OSTN to bone development. We found that OSTN regulated growth plate and trabecular bone formation by inducing proliferation and maturation of chondrocytes through enhancing CNP-dependent signaling. Besides chondral growth, OSTN together with CNP induced osteoblast differentiation of periosteum-derived multipotent progenitor cells leading to bone hypertrophy. OSTN-dependent bone growth was ascribed to enhancement of CNP signaling by inhibiting of CNP clearance of NP receptor 3 (NPR3) by OSTN, because OSTN bound to NPR3. Collectively, we demonstrate that OSTN functions as a mechano-transducer that links mechanical loading to CNP-dependent bone growth. (COI:No)

# Symposium 16

## State-of-the-art physiology in urinary continence

(March 19, 9:00～10:50, Hall 9)

### S16-01
#### Cyclic nucleotide-dependent pathways as targets for managing bladder dysfunction

Christopher Fry[1], Basu Chakrabarty[1], Anthony Kanai[2] ([1]*University of Bristol, UK,* [2]*University of Pittsburgh, USA*)

Cyclic nucleotides such as cGMP and cAMP are recognised as smooth muscle relaxants and regulators of intracellular [$Ca^{2+}$] regulation. However, it is increasingly understood that they also determine, directly or indirectly, several other functional bladder properties that, when these are deranged, are associated with conditions such as detrusor overactivity. For example, cGMP and cAMP regulate several purinergic pathways, such as ATP release from postganglionic parasympathetic nerves to detrusor smooth muscle or from the urothelium; as well as afferent nerve firing. Thus, they can influence the ability of the bladder to void and also to respond to bladder filling. Cellular cyclic nucleotide levels can be manipulated either by altering the extent of production, or the rate of degradation and both offer attractive routes for drug development. This talk will consider how cyclic nucleotides regulate functional properties of bladder wall tissues and how their metabolism can be manipulated as potential drug targets. Cyclic nucleotides play a fundamental role in other tissue processes such as generation of fibrosis, a fundamental problem in the bladder that decreases its contractile ability and increases its stiffness. Strategies to reverse this pathological condition will also be considered in the context of cyclic nucleotide regulation. (COI:No)

### S16-02
#### Circadian micturition rhythm coordinated by the bladder clock

Hiromitsu Negoro[1,2], Jin Kono[2] ([1]*Dept Urology, Univ Tsukuba, Japan,* [2]*Dept Urology, Grad Sch Med, Kyoto Univ, Japan*)

Nocturia (micturition at night) is one of the most bothersome lower urinary tract symptoms. The cause of nocturia is multifactorial, but it can be divided into three factors, including sleep disorder, increased urine production rate in the kidney and decreased functional bladder capacity (FBC) at night. In healthy individuals, the urinary bladder increases its functional volume at night compared with that at daytime. The mechanism of day-night changes of FBC was unknown, but it has been elucidated gradually after the discovery of the circadian clock system. The center of the circadian clock exists in the suprachiasmatic nucleus of the brain, which orchestrates the circadian clocks in most organs and cells, termed peripheral clock. We reported that the peripheral clock also exists in the bladder, and that connexin43 (Cx43), not only a major gap junction protein in the bladder detrusor muscle but also a hemichannel for ATP release in the urothelium, is one of the clock-controlled genes to act as a regulator of FBC. Urothelial cells sense changes in stretch and transmit mechanotransduction signals to the afferent nerve by releasing various neurotransmitters such as ATP. ATP activates purinergic receptors expressed by afferent nerves in the suburothelium, whereas ATP activates various purinergic receptors on the urothelium in an autocrine/paracrine manner to form a positive feedback loop of ATP secretion. The Cx43 function varied according to the circadian rhythm in the urothelium as well as in the bladder detrusor muscles, which can be involved in the coordination of circadian micturition rhythm. In the urothelium, circadian expressions of mechano-sensor molecules have been also reported, and ATP concentration in patients with overactive bladder is known to be increased. These findings can shed a light on the physiology of day-night change in micturition. Modulating the circadian clock can be a novel approach to treat nocturia. (COI:No)

### S16-03
#### Corticotrophin-Releasing Hormone neurons of Barrington's nucleus: Probabilistic, spinally-gated control of bladder pressure and micturition

Hiroki Ito (*Dept. Urology, Yokosuka Kyosai Hospital, Kanagawa, Japan*)

Micturition requires the co-ordinated control of bladder and urethral sphincter via lumbosacral parasympathetic, sympathetic and somatic motoneurons. In adult mammals, this involves a spino-bulbar-spinal loop and a critical site in this chain of command is Barrington's nucleus in the pons (Barr). The afferent signal arising from the bladder is conveyed to the spinal cord via A delta and C fibre of the pelvic nerve. The spinal cord relays the signal to the periaqueductal gray (PAG) in the midbrain where it is integrated. Upon the initiation of voiding, this signal is summoned to Barr which transmits motor commands to the lumbosacral parasympathetic neurons controlling the bladder and urethral sphincter motoneurons. Lesion of Barr or acute transection of the pons abolishes micturition, while supra-collicular decerebration or transection of PAG does not stop reflex voiding in cats and rats. Thus, Barr is believed to be the pre-parasympathetic control center of voiding reflex. The largest cell group within this nucleus are pontospinal glutamatergic neurons that express corticotrophin-releasing hormone (BarrCRH). While there is agreement that they can generate bladder contractions, it is unclear whether they act as high-fidelity pre-parasympathetic motor drive enabling to determine the degree of bladder contractions. Combined opto- and chemo-genetic manipulations along with in vivo high-density multiunit recordings in mice shows that BarrCRH neurons provide a timing signal that probabilistically generates non-voiding contractions during the storage phase or a voiding (including urethral sphincter bursting) depending on the phase of the voiding cycle. Additionally, they release CRH at a spinal level that acts as a negative feedback brake on the excitatory effects of BarrCRH neurons. These findings define the roles of the BarrCRH neurons in voiding and emphasise the importance of the state of priming of a downstream spinal gating circuit in determining the response of the lower urinary tract. (COI:No)

### S16-04
#### Mechanical stimulation of afferent nerves as a cause of bladder storage dysfunction

Hikaru Hashitani, Retsu Mitsui (*Dept Cell Physiol, Grad Sch Med Sci, Nagoya City Univ, Japan*)

During the storage phase, the urinary bladder does not remain relaxed, rather it maintains a basal tone by developing micromotions resulting from 'asynchronous' spontaneous phasic contractions (SPCs). Importantly, spontaneous 'transient' rises in the intravesical pressure arising from SPCs appear to have a much larger influence than the baseline pressure rises on afferent nerve firing. Thus, increased SPCs would cause urinary urgency reported in patients with bladder storage dysfunction known as an overactive bladder (OAB). Besides detrusor smooth muscle (DSM), the major contractile element in the bladder wall, the contractility of bladder mucosa predominantly arising from muscularis mucosa (MM) has become evident. The MM expressing $\alpha$-smooth muscle actin immunoreactivity forms a discontinuous layer in the lamina propria that is clearly distinct from DSM. The MM generates bursting spontaneous action potentials, and is capable of developing approximately 8 times greater SPCs per section area compared to DSM. However, nerve-mediated contractions of the MM are rather modest. Considering such contractile properties of the MM as well as its much smaller volume compared with DSM, it is unlikely that the contractility of MM contributes to bladder voiding contractions. The MM may function to prevent excessive stretching of the blood vessels and urothelium during bladder filling. Nevertheless, it is envisaged that the MM have a larger mechanical impact on afferent nerves compared to the DSM because of its anatomical proximity. Thus, aberrant SPCs in the MM could be a primary cause of the hyperactivity of mechanosensitive afferent nerves. Unlike the DSM, the MM receives inhibitory innervation, namely nitrergic as well as CGRP-containing afferent nerves that may stabilise the MM excitability. On the contrary, the sensitivity of the MM to angiotensin II is more than 100 times higher compared with the DSM. Neurohumoral modulation of the MM contractility could be a novel therapeutic target of OAB. (COI:No)

# Symposium 17

## Current advance in endocrine disruptor research

(March 19, 9:00～10:50, Hall 10)

### S17-01

#### The effect of perinatal exposure to Gadolinium-based contrast agents on cognitive function of the offspring

Noriyuki Koibuchi[1], Miski Khairinisa A.[1], Winda Ariyani[1], Wataru Miyazaki[3], Asahi Haijima[4], Izuki Amano[1], Yoshito Toshima[2] (*[1]Dept Integrative Physiol, Gunma Univ. Grad Sch Med, Japan, [2]Dept Diagnostic Radiol & Nucl Med, Gunma Univ. Grad Sch Med, Japan, [3]Dept Biosci Lab Med, Hirosaki Univ Grad Sch Health Sci, Japan, [4]Dept Health Sci & Social Welfare, Sch Human Sci, Waseda Univ, Japan*)

Gadolinium-based contrast agents (GBCA) are commonly used to enhance the image during magnetic resonance imaging (MRI). Although its exact adverse effect in the developing central nervous system has not yet been reported even after repetitive injection during pregnancy, the accumulation of GBCA in various brain regions such as the dentate gyrus has recently been reported. To examine the effect of GBCA exposure during perinatal period, several different types of GBCA was intravenously administered to pregnant or lactating mice. Measurement of gadolinium of the whole brain showed an accumulation of gadolinium in the offspring whose mother received gadolinium either prenatally or postnatally, indicating gadolinium can cross the placenta and excreted in the milk. Behavioral analysis revealed that these animals showed an increased anxiety, cognitive impairment and motor coordination defect. In the primary culture of rodent cerebellum, GBCA disrupted dendrite arbolization of Purkinje cell. In transient transfection-based reporter gene assay, GBCA augmented thyroid hormone receptor-mediated transcription with lower dose and suppressed it with higher dose. Throughout the experiment, GBCAs containing a linear chelate structure showed relatively greater effect than those containing a macrocyclic chelate structure. These results indicate that GBCA, particularly linear GBCA, may cause an adverse effect in the developing brain. It may be administered during pregnancy or lactating period only when the benefit significantly outweighs the risk of exposure.
(COI:No)

### S17-02

#### Direct Actions of Xenoestrogens on Thyroid Hormone Receptors

Wataru Miyazaki[1], Ariyani Winda[2], Noriyuki Koibuchi[2] (*[1]Dept Biosci Lab Med, Grad Sch Health Sci, Hirosaki Univ, Japan, [2]Dept Integrative Physiol, Grad Sch Med, Gunma Univ, Japan*)

Isoflavones (genistein, daidzein), bisphenol A (BPA), and 4-nonyl-phenol (4NP) are known as xenoestrogens which modulate the actions of estrogen receptors (ERs). These compounds also act on the other nuclear receptors including thyroid hormone (TH) receptors (TRs). THs have essential roles for the development and functional maintenance of many organs, and the actions of THs are directly or indirectly modulated by xenoestrogens. These compounds may affect TH system at multiple levels such as synthesis, secretion, transport and degradation of THs, expression of TRs and TR-mediated transcription. Isoflavones have been considered as healthy supplements that reduce risk of the specific diseases, for example, coronary heart disease, cancers, and hot flashes. However, in the clinical field, the effects of isoflavones are controversial. Under certain conditions, isoflavones may worsen the symptoms of subclinical hypothyroidism, whereas they may improve thyroid functions in Hashimoto disease patients. Recently, we have reported that isoflavones can bind to TRs directly, and augment TR-mediated transcription. On the other hand, synthetic xenoestrogens such as BPA and 4NP have been considered as toxic endocrine-disrupting chemicals, even though the similar augmentation was also induced by 4NP and BPA in recent studies. In our presentation, we summarize the effects of xenoestrogens in thyroid hormone action, and discuss why such clarification was established. Although we need further investigation of the relationships of ERs-mediated actions with TRs, the direct modulation of xenoestrogens is one of the clues to prevent the adverse effects of these compounds.
(COI:No)

### S17-03

#### Estrogen receptor activation or inhibition induced by next-generation bisphenols

Ayami Matsushima (*Dept Chem, Fac Sci, Kyushu Univ, Japan*)

Bisphenol A (BPA) is a raw industrial material for polycarbonate plastics and epoxy resins; however, a series of studies showed that BPA induces adverse effects on experimental animals as endocrine-disrupting chemicals (EDCs) even at low doses. The abnormal aspect of BPA is reported not only on reproductive organs but in their nervous systems, although the molecular mechanisms of these low-dose effects remain unknown. EDCs including BPA are presumed to directly bind nuclear receptors such as estrogen receptors (ERs), and elicit their harmful effects. Forty-eight nuclear receptors are reported in humans, with all of these representing potential targets of EDCs. These findings result in avoiding the usage of BPA in daily products including baby bottles. As a result, emerging number of bisphenol derivatives, (i.e., next-generation bisphenols) are utilized as substitutes for BPA, although few methodical risk assessments on next-generation bisphenols have been carried out. BPA and most of new-generation bisphenols have two phenol groups in their chemical structures. A biphenyl moiety is a common privileged structure in the field of drug discovery, therefore we considered that a bisphenol moiety is a privileged structure for nuclear receptors. Indeed, we found that BPA bound strongly to estrogen-related receptor $\gamma$. To find new ligands for ER$\alpha$, we therefore screened a library of ca. 200 bisphenol derivatives and found that 20 compounds bind to ER$\alpha$ with higher affinity than BPA. Most of these activate ER$\alpha$ as its agonists; however, four compounds, including bisphenol M and bisphenol P act as novel antagonists. These structures harbor three benzene rings in tandem with terminal hydroxy groups, that is, tricyclic bisphenol structure, representing a novel privileged structure for an ER$\alpha$ antagonist. The tricyclic bisphenol structure has a potential to be new mother structure for a new breast cancer drugs.
(COI:No)

### S17-04

#### New developments of thyroid hormones analysis for the field of environmental chemistry and environmental toxicology

Kei Nomiyama[1], Hazuki Miazukawa[2], Akifumi Eguchi[4], Shouta Nalayama[3], Yoshinori Ikenaka[3], Mayumi Ishizuka[3], Rumi Tanoue[1], Tatsuya Kunisue[1] (*[1]CMES, Ehime Univ, Japan, [2]Grad Sch Agr, Ehime Univ, Japan, [3]Sch Vet Med, Hokkaido Univ, Japan, [4]Cent Prev Med Sci, Chiba Univ, Japan*)

Thyroid hormones (THs) play critical roles in neural development, protein synthesis, and the normal growth of bones in addition to regulating energy and lipid metabolism.
Recent studies have reported that exposure to endocrine disrupting chemicals such as polychlorinated biphenyls (PCBs) alter TH levels in the blood. THs are involved in the regulation of early brain development. Since the fetal thyroid gland is not fully functional until week 18–20 of pregnancy, neuronal migration and other crucial early stages of intrauterine brain development largely depend on the supply of maternal TH. Therefore, it is important to accurately measure the maternal THs concentrations.
In addition, recent updates to OECD developmental/reproductive toxicology guidelines and other regulatory guidelines and guidance require the measurement of TH levels in the blood of mammalian laboratory species during development. However, preliminary analyses indicate that there is a wide variability across laboratories in the methods being used to measure THs in young rodents, as well as in the success of obtaining reliable data. Until now, the measurement of THs has mainly been immunoassay. However validity, accuracy, sensitivity and reproducibility of the assays are issues of concern.
This presentation will introduce a high-sensitivity and high-accuracy THs analysis method using LC-MS/MS that can be measured regardless of animal species, and discuss the development of THs analysis in the fields of environmental chemistry and environmental toxicology.
(COI:No)

# Symposium 18

## History and Up-to-date stories on the mechanism of smooth muscle contraction in health and disease

(March 19, 14:10〜16:00, Hall 4)

### S18-01

#### Novel signaling molecules which regulate both $Ca^{2+}$-sensitization of vascular contraction and cancer cell migration

Sei Kobayashi, Bochao Lyu, Min Zhang, Ying Zhang, Hiroko Kishi, Tomoka Morita
(*Dept. of Molecular and Cellular Physiol., Yamaguchi Univ. Grad. Sch. of Med.*)

Previously we found for the first time that in the complete absence of cytosolic $Ca^{2+}$, Rho-kinase (ROK) can induce contraction of membrane-permeabilized vascular smooth muscle (VSM) and myosin phosphorylation. The Rho-kinase (ROK)-mediated $Ca^{2+}$-sensitization of VSM contraction contributes to abnormal vascular contractions, such as vasospasm, which is one of the major causes of the sudden death. As the causative molecule for the ROK-mediated $Ca^{2+}$-sensitization of VSM contraction, we discovered sphingosylphosphorylcholine (SPC) and further identified the "SPC/Fyn/Rho-kinase" pathway as the pathogenic pathway. The translocation of Fyn and Rho-kinase from the cytosol to the cell membrane activates these kinases and thereby induces the $Ca^{2+}$-sensitization of VSM contraction.
We also found that eicosapentaenoic acid (EPA), a component derived from fish oil, can inhibit the translocation and activation of Fyn induced by SPC and specifically suppress abnormal vasocontraction without affecting physiological $Ca^{2+}$-dependent contraction of VSM. Indeed, EPA abolished cerebral vasospasm after subarachnoid hemorrhage of the patients. However, because EPA is lipid-soluble, it cannot be either administrated intravenously or used for emergency treatment. Therefore, we tried to identify water-soluble food components as an alternative to EPA. After extensive screening, we found that food extracts/components and Chinese traditional medicine (CTM) selectively inhibit the SPC-induced $Ca^{2+}$-sensitization of VSM contraction without affecting the physiological $Ca^{2+}$-dependent contraction of VSM.
Recently we found that the "Fyn/Rho-kinase" pathway also mediates cell migration, which is critical for cancer invasion and metastasis. We found that food components and CTM also can suppress cancer cell migration remarkably.
In summary, our results suggest that food extracts/components and CTM would be novel protective and therapeutic agents for the most lethal diseases, vasospasm and cancer.

(COI:No)

### S18-02

#### Too many links indeed linking the missing link in the Ca2+-sensitization pathway regulating vascular smooth muscle contractions

Ko Momotani, Kumiko Sakai (*Facl of Pharm Sci*)

It is well known that the Ca2+-sensitization pathway is a significant contributor to vascular smooth muscle (VSM) contractions. The pathway is initiated by multiple agonists through G protein-coupled receptors (GPCRs), which further activate RhoA and Rho-kinase. Active Rho-kinase then inhibits Myosin light chain (MLC) phosphatase, resulting in an increase in MLC phosphorylation and thereby inducing VSM contractions. The player(s) between the GPCRs and RhoA in this signaling cascade had been unidentified for a long time. Here, we present a brief history of how this missing step has been elucidated and the resulting impact on the understanding of vascular physiology. Multiple RhoA GTP exchange factors (GEFs), some are to do with pathological outcomes, in between GPCRs and RhoA have been reported in recent years. A good example is leukemia-associated RhoGEF (LARG) in salt-dependent hypertension reported by Offermanns group. The other is p63RhoGEF. Its role in VSM contractions was first reported by our group. Our unpublished data further point to direct activation of p63RhoGEF in response to an increase in the internal pressure of small vessels mimicking physiological cues of vascular tone, a major regulator of blood pressure. The involvement of another GEF, PDZ-RhoGEF in VSM contractions, has also been suggested. Thus, the implication of multiple GEFs has been reported, which indeed leads to another layer of complexity in regulatory mechanisms of VSM contractions. Why different GEFs are necessary for signal transduction with a single outcome, VSM contraction in the end. One of the current views suggests that each GEF is responsible for a specific physiological and possibly pathological cue. In other words, multiple GEFs are backing diversity in physiological responses in vasculatures. If this hypothesis stands, it opens the doors to develop specific drags each for a specific pathophysiological condition by targeting a particular GEF while circumventing unwanted side-effects. (COI:No)

### S18-03

#### Role of CPI-17, an endogenous myosin phosphatase inhibitory protein in gastrointestinal motility

Qunhui Yang (*Dept Vet.Pharmacology, Grad Sch Agr. and Life Sci, the Univ of Tokyo, Japan*)

We reported that CPI-17, an endogenous myosin phosphatase inhibitory protein, played an essential role to maintain blood pressure using wild type mice (WT), CPI-17 deficient mice (KO) and phospho-resistant mutant of CPI-17 at threonine 38 (Thr38) to alanine knocked-in mice (TA). Aim of this study is to clarify role of CPI-17 in gastrointestinal motility. High concentration of KCl (64.5 mM)-induced contractions were not different among WT, KO and TA of ileal and colonic circular muscles strips. In contrast, carbachol (5 $\mu$M)-induced contractions were decreased in KO and TA than WT. Gastric emptying rate measured by the 13C-octanoic acid breath test and intestinal transit measured by oral administrated FITC-dextran solution movement did not change between WT, KO and TA. While colonic transit measured by colonic bead expulsion test revealed that colonic transit in KO and TA was significantly slower than that in WT. The colonic transit in TA was same with in KO. CPI-17 protein expression was much higher in large intestine than small intestine. In a conclusion, The CPI-17 phosphorylation signaling at Thr38 is important for maintenance of colonic transport ability than in upper gastrointestinal tract. (COI:No)

### S18-04

#### Role of Vascular Smooth Muscle PPARγ, a Transcriptional Factor

Masashi Mukohda (*Lab Vet Pharm, Faculty Vet Med, Okayama Univ of Science*)

Synthetic agonists of peroxisome proliferator-activated receptor $\gamma$ (PPAR $\gamma$), thiazolidinediones (TZDs) are currently prescribed to patients with type 2 diabetes to improve insulin resistance. TZDs have been shown to protect against vascular diseases such as atherosclerosis and lower blood pressure. In contrast, loss of PPAR $\gamma$ function in patients carrying PPAR $\gamma$ mutations causes insulin resistance and hypertension. These evidences suggest PPAR $\gamma$ has protective effect on cardiovascular diseases. Sigmund laboratory (University of Iowa) generated transgenic mice (S-DN) expressing dominant negative (DN) mutant form of PPAR $\gamma$ (P467L) specifically in vascular smooth muscle cell (SMC) (*Cell Metab.* 2008). S-DN mice are hypertensive, exhibit severe vascular dysfunction, and display reduced expression of a novel PPAR $\gamma$ target gene, RhoBTB1. In addition, S-DN mice exhibited exacerbated atherosclerosis associated with elevated NF-$\kappa$B-mediated inflammatory markers when bred with ApoE-deficient mice (*Am J Physiol Regulatory.* 2013). In this study, we examined the mechanisms by which vascular SMC PPAR $\gamma$ protects cardiovascular diseases. First, we hypothesized that PPAR $\gamma$ has protective effect on vascular disease through inhibiting inflammation in SMC. To test this, S-DN were bred with mice expressing luciferase controlled by an NF-$\kappa$B–responsive promoter. NF-$\kappa$B activity induced by tumor necrosis factor $\alpha$ (TNF-$\alpha$) was increased in vessels from S-DN mice. Next, we hypothesized that RhoBTB1 plays a protective role in vascular function that is disrupted in S-DN mice. To test this, we generated triple transgenic mice (S-RhoBTB1/S-DN) expressing DN PPAR $\gamma$ in SMC along with tamoxifen-inducible, Cre-dependent expression of RhoBTB1 in SMC. S-DN exhibited severely impaired vasodilation to acetylcholine and sodium nitroprusside and hypertension, which was reversed by restoration of RhoBTB1 in SMC. We conclude that 1) SMC-PPAR $\gamma$ has anti-inflammatory effects mediated through inhibition of NF-$\kappa$B activity, and 2) a novel PPAR $\gamma$ target gene, RhoBTB1, functions in SMC to facilitate vasodilation and mediates a protective anti-hypertensive effect. (COI:No)

### S18-05

#### The therapeutic effects of EPA on pulmonary hypertension partly manifest via inhibition of tyrosine kinase FYN

Rin Kurahara[1], Keizo Hiraishi[1,2], Ying Zhang[3], Aya Yamamura[4], Hiroko Kishi[3], Sei Kobayashi[3], Ryuji Inoue[2], Katsuya Hirano[1] ([1]*Dept Cardiovasc Physiol, Sch Med, Kagawa Univ, Japan,* [2]*Dept Physiol, Sch Med, Fukuoka Univ, Japan,* [3]*Dept Mol Cell Physiol, Grad Sch Med, Yamaguchi Univ, Japan,* [4]*Dept Physiol, Aichi Med Univ, Japan*)

**Background and Purpose:** Pulmonary arterial hypertension (PAH) is a multifactorial disease characterized by pulmonary arterial remodeling. The Src family non-receptor tyrosine kinases including Fyn play critical roles in vascular function. Eicosapentaenoic acid (EPA) is known to inhibit Fyn kinase activity. In this study, we investigated the therapeutic potential of EPA and its metabolite resolvin E1 (RvE1) for PAH.
**Method:** Cardiodynamic parameters of the monocrotaline-induced PAH rats (MCT rats) were assessed with echocardiography. Contractile responses of isolated pulmonary arteries were evaluated by the isometric tension measurement. Proliferation of human pulmonary artery smooth muscle cells (hPASMCs) derived from PAH patients were evaluated by the MTT assay. Endothelial to mesenchymal transition (EndoMT) in HPAECs and STAT3 phosphorylation in hPASMCs were examined by immunofluorescence staining and western blot analyses, respectively.
Results: Administration of EPA to MCT rats significantly improved the pulmonary arterial thickening, right ventricle dysfunction and cardiovascular fibrosis. Pulmonary arteries isolated from MCT rats exhibited enhanced contractile responses to serotonin compared with those seen with control rats. EPA administration attenuated the enhanced contractile responses in MCT rats. EPA or RvE1 treatment decelerated the enhanced proliferation of PASMCs derived from the patients with PAH. Knockdown of Fyn with siRNA prevented TGF-$\beta$2-induced EndoMT in HPAECs and IL-6-induced STAT3 phosphorylation in HPASMCs. EPA and RvE1 suppressed Src family activity by modulating phosphorylation status.
**Summary:** EPA significantly improved PAH-associated pathophysiology and cardiac dysfunction. These therapeutic effects are likely mediated at least in part via Fyn inhibition. Fyn is suggested to be a potential target for the treatment of PAH. (COI:No)

# Educational Programs

# Lunchtime Sessions

# Luncheon Seminars

# Educational Program 1

## Educational Lecture 1

(March 18, 9:00～10:50, Hall 3)

**EP1-1** Diversity of $Ca^{2+}$ entry mechanisms associated with vascular functions

Ryuji Inoue
Department of Physiology, Fukuoka University School of Medicine

**EP1-2** Regulation of body fluid and electrolytes: Physiological significance of interstitial fluid

Yoshinori Marunaka
Research Institute for Clinical Physiology, Kyoto Industrial Health Association / Research Center for Drug Discovery and Pharmaceutical Development Science, Ritsumeikan University / International Research Center for Food Nutrition and Safety, Jiangsu University

**EP1-3** Circadian clocks based on basic to applied research

Shigenobu Shibata
Advanced Science and Engineering, Waseda University

# Educational Program 2

## Educational Lecture 2

(March 18, 15:20～17:10, Hall 3)

**EP2-1** Fun to study body temperature regulation: from the observation of the whole-body response

Kei Nagashima
Waseda University

**EP2-2** Why humans get obese?
- An introduction to nutrient metabolism

Tomohiro Tanaka
Nagoya City University

**EP2-3** Physiology of Pain

Jun Sato
Chubu University

# Educational Program 3

## Model Lecture on Physiology

(March 19, 9:00～10:50, Hall 3)

**EP3-1** The sense of taste

Noriatsu Shigemura
Section of oral neuroscience, graduate school of dental sciences, Kyushu University

**EP3-2** Team-Based Learning (TBL) for Education of Physiology

Mariko Miyata
Tokyo Women's Medical Univ., Dept. of Neurophysiology

**EP3-3** Homeostatic Control of blood glucose

Michiko Tanaka
Miyazaki Prefectural Nursing University

# Educational Program 4

## Educational Workshop

(March 19, 14:10～16:00, Hall 10)

## Lunchtime Session 1

### History and future directions of physiology in association with biology and medicine

(March 17, 12:10～13:10, Hall 6)

**LS1-1** Beautiful Harmony in Physiology: From Molecule to Body and Behavior

Yoichi Ueta
Department of Physiology, School of Medicine, University of Occupational and Environmental Health

**LS1-2** Research on the physiological functions of basal forebrain cholinergic neurons

Sae Uchida
Department of Autonomic Neuroscience, Tokyo Metropolitan Institute of Gerontology

## Luncheon Seminar 1

[ Supported by Medtronic Co., Ltd. ]

### Acquiring knowledge of the heart failure from physiological points of view

(March 17, 12:10～13:10, Hall 2)

**LCS1-1** Osamu Yamaguchi

Ehime University Graduate School of Medicine, Organ and Morphology Department of Cardiology, Pulmonology, Hypertension and Nephrology

## Lunchtime Session 2

### Now is the time to make use of your power ! Importance of diversity in research life

(March 18, 12:10～13:10, Hall 10)

**LS2-1** Let's us understand unconscious bias

Yukari Date
Vice President, Director, University of Miyazaki

**LS2-2** Steps for your own successful career

Yumiko Oishi
Department of Biochemistry and Molecular Biology, Nippon Medical School

## Luncheon Seminar 2

[ Supported by Nippon Boehringer Ingelheim Co., Ltd. ]

### Management of Atrial Fibrillation Based on the Extent of Atrial Fibrosis

(March 17, 12:10～13:10, Hall 3)

**LCS2-1** Takanori Yamaguchi

Department of Cardiovascular Medicine, Saga University

## Luncheon Seminar 3

[ Supported by Mandom corp. ]

### Thermosensitive TRP channels and their physiological functions

(March 17, 12:10～13:10, Hall 4)

**LCS3-1** Makoto Tominaga
Thermal Biology Group, Exploratory Research Center on Life and Living Systems, National Institutes of Natural Sciences
Division of Cell Signaling, National Institute for Physiological Sciences, National Institutes of Natural Sciences
Department of Physiological Sciences, SOKENDAI, (The Graduate University for Advanced Studies)

**LCS3-2** Kaori Otsuka-Saito
Fundamental Research Institute, Mandom Corp.
Osaka University, Graduate school of Pharmaceutical Sciences

## Luncheon Seminar 4

[ Supported by The Japanese Plasmalogen Society ]

### Plasmalogen: A new frontier of the research in brain and cardiac diseases

(March 17, 12:10～13:10, Hall 5)

**LCS4-1** Toru Maruyamai
Department of Hematology, Oncology and Cardiovascular Medicine, Kyushu University Hospital

## Luncheon Seminar 5

[ Supported by Bayer Yakuhin Co., Ltd. ]

### Acquiring knowledge of the heart failure from physiological points of view

(March 18, 12:10～13:10, Hall 2)

**LCS5-1** Naohiko Takahashi
Department of Cardiology and Clinical Examination, Faculty of Medicine, Oita University

## Luncheon Seminar 6

[ Supported by Century Medical, Inc. ]

### Anatomically Left atrial appendage management

(March 18, 12:10～13:10, Hall 3)

**LCS6-1** Hidenori Sako
Oita Oka Hospital Cardiovascular surgery

## Luncheon Seminar 7

[ Supported by TSUMURA & CO. ]

### The physiological role of Oxytocin on social behavior

(March 18, 12:10～13:10, Hall 4)

**LCS7-1** Takashi Maruyama
Department of Physiology, University of Occupational and Environmental Health

## Luncheon Seminar 8

[ Supported by Nepa Gene Co., Ltd. ]

### Principles and applications of electroporation(NEPA21) in genome editing gene analysis

(March 18, 12:10～13:10, Hall 5)

**LCS8-1** Kazunori Hirakawa
Regional General Manager, Kyushu, NEPA GENE CO., LTD.

## Luncheon Seminar 9

[ Supported by DAIICHI SANKYO Co., Ltd. ]

### Brugada syndrome and Early repolarization syndrome

(March 19, 12:10～13:10, Hall 2)

**LCS9-1** Naohiko Takahashi
Department of Cardiology and Clinical Examination, Faculty of Medicine, Oita University

## Luncheon Seminar 10

[ Supported by Kracie Pharmaceutical Co., Ltd. ]

### Ninjin'yoeito stimulates appetite center NPY neurons and counteracts anorexia and weight loss: anti-frailty strategy

(March 19, 12:10～13:10, Hall 3)

**LCS10-1** Toshihiko Yada
Kansai Electric Power Medical Research Instituter, Center for Integrative Physiology /
Kobe University Graduate School of Medicine, Division of Diabetes, Metabolism and Endocrinology

# Award Presentations

（March 18, 14:20～15:20）

| | |
|---|---|
| **AP-1～AP-2** | 21st Promotion Award of the Physiological Society of Japan for Young Scientists |
| **AP-3～AP-6** | 10th Hiroshi and Aya Irisawa Memorial Promotion Award for Young Physiologists |
| **AP-7** | 10th Aya Irisawa Memorial Promotion Award for Excellence by Women Physiologists |
| **AP-8** | 10th Hiroshi and Aya Irisawa Memorial Award for Excellent Papers in The Journal of Physiological Sciences |
| **AP-9** | 10th Hiroshi and Aya Irisawa Memorial Award for Excellent Papers on Research in Circulation in The Journal of Physiological Sciences |

## AP-1 (S09-03)

### The local network in the striatum tail contributes to the behavioral switching

Jun Kunimatsu[1,2], Okihide Hikosaka[2] ([1]*Faculty of Med, Univ Tsukuba, Tsukuba, Japan,* [2]*National Eye Institute, NIH, MD, U.S.A.*)

Although, in our daily life, the object values may change in different environments and we can switch our behavior accordingly, underlying neuronal mechanism is unclear. To address it, we devised a new value procedure: scene-based value task. The monkey viewed 8 fractal objects in 2 scenes (A and B); 4 of them were good (with large-reward) in scene A and bad (with small-reward) in scene B, while the other 4 were good in scene B and bad in scene A. After experiencing this procedure repeatedly, the monkey became able to choose whichever objects were good. Since scenes A and B were presented in a random sequence, the monkey's choice was switched abruptly depending on the scene-context.

We then recorded neuronal activity in striatum tail while the monkey passively viewed these objects in different scenes. We found differences between medium spiny neurons (MSNs) and fast spiking interneurons (FSIs). Many of MSNs responded to the fractal objects differently depending on their values. Importantly, this object-value coding was stronger in either scene A or B. In contrast, FSIs showed no object-value coding. Instead, many of them responded to the scenes selectively (stronger to scene A or B). These results suggested that the object-value coding of MSNs, which is basically stable, is modulated by the inhibitory inputs from the scene-selective FSIs.

To test the causal role of FSI, we locally injected IEM-1460, an inhibitor of GluA2-lacking AMPARs, in the recording sites to selectively block the excitation of FSIs but not MSNs. After injection, monkeys were unable to learn new scene-object value association. On the other hand, object-value learning (no scene) was not affected. This result indicated that the local network of striatum tail regulates the scene-object association learning. These mechanisms may support the monkey's flexible switching based on stable long-term experiences of various environments. (COI:No)

## AP-2 (1P-035)

### Synaptic plasticity at cortico-striatal pathway in functional recovery after cortical damage

Susumu Jitsuki[1], Sayaka Kogami[1], Aoi Jitsuki-Takahashi[2], Tomomi Yamanoue[1], Takuya Takahashi[1] ([1]*Dept Physiol, Grad Sch Med, Yokohama City Univ, Japan,* [2]*Dept Biochem, Sch Med, Tokyo Women's Med Univ, Japan*)

Brain damage such as stroke is a devastating neurological condition that may severely compromise patient quality of life. Restoration of motor impairment after brain damage is considered to be the result of compensative neural plasticity in intact brain regions, mediated by the reorganization of cortical motor maps. Experience-dependent synaptic AMPA ($\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid) receptor (AMPAR) delivery underlies behaviors that require neural plasticity such as learning. We have previously found that rehabilitation-dependent synaptic delivery of AMPAR in the peri-injured area facilitate functional recovery after cortical damage. For execution of appropriate motor function, it requires involvement of motor-related brain regions such as striatum, thalamus or brain stem. However, it remains unclear that the role of other brain region in recovery after brain damage. Here, using cortical injury rat model, we found that AMPAR-mediated miniature EPSC (mEPSC) of the layer 5 pyramidal neurons in the periinjured cortex were positively correlate with recovery rate of forelimb reaching motor performance after rehabilitative training. On the other hand, mEPSC of the medium spiny neurons in the periinjured striatum were negatively correlate with recovery rate of motor performance. Furthermore, mIPSC of the periinjured cortex showed no correlation with motor performance. These results suggest that changes of excitatory input in the peri-injured region could contribute functional recovery after cortical damage. (COI:No)

## AP-3 (PS13-02)

### PI(3, 4)$P_2$- and voltage-dependent gating of two-pore $Na^+$ channel 3

Takushi Shimomura[1,2], Ki-ichi Hirazawa[1,2], Yoshihiro Kubo[1,2] ([1]*Div Biophys and Neurobiol, Natl Inst Physiol Sci,* [2]*Dept Physiol Sci, SOKENDAI*)

Two-Pore $Na^+$ Channels (TPCs) contain two domains (DI and DII) of a functional unit of voltage-dependent cation channels. Each domain has its own voltage sensor domain that possesses three positively charged arginine residues in helix S4. Characteristically, TPC3 shows the shift of the voltage dependence by long depolarization stimulus, so called as "induction", in *Xenopus* oocyte expression system.

The structural basis of this "induction" mechanism was investigated using multiple approaches based on two-electrode voltage-clamp technique and the structural model of TPC3. We found the correlation between $PIP_2$ level and "induction" currents. Simultaneous recordings of TPC3 current and the fluorescence from specific $PIP_2$ sensors showed that PI(3, 4)$P_2$ concentration is increased by long depolarization stimulus, possibly through any endogenous system in *Xenopus* oocytes. The "induction" kinetics of TPC3 is well correlated with the fluorescent change of PI(3, 4)$P_2$ sensors, but not with that of PI(4, 5)$P_2$. The PI(3, 4)$P_2$ sensitivity of TPC3 was confirmed using direct phosphoinositide injection method and the excised-patch membrane. These results reveal that "induction" is PI(3, 4)$P_2$-induced modulation of voltage dependence of TPC3. We also found that a cluster of basic amino acid residues in the cytosolic side of DI is critical for PI(3, 4)$P_2$ sensitivity. Interpretation of the mutational effects based on the TPC3 structural model explained how TPC3 selectively recognizes PI(3, 4)$P_2$ in DI region. While DI recognizes PI(3, 4)$P_2$, DII is considered to be mainly responsible for voltage-sensing. The voltage-dependent movement of DII-S4 was verified using the voltage clamp fluorometry, in which the voltage-dependent fluorescent change was detected from the fluorophore incorporated into some residues in DII-S4. These electrophysiological data of various approaches, combined with the structural model, revealed the detailed voltage-dependent gating mechanism of TPC3. (COI:No)

## AP-4 (PS22-02)

### Molecular characterization of the arrhythmogenic trigger unique to pulmonary vein cardiomyocytes

Yosuke Okamoto[1], Yoshinobu Nagasawa[2], Aung Naing Ye[3], Yukiko Himeno[4], Akinori Noma[4], Akira Amano[4], Daichi Takagi[1], Kuniaki Ishii[5], Kyoichi Ono[1] ([1]*Dept Cell Physiol, Grad Sch Med, Akita Univ, Japan,* [2]*Dept Pharmacol Ther, Fac Pharm Sci, Toho Univ, Japan,* [3]*Dept Pathol Diagn, Fac Med, Yamagata Univ, Japan,* [4]*Dept Bioinfomat, Coll Life Sci, Ritsumeikan Univ, Japan,* [5]*Dept Pharmacol, Fac Med, Yamagata Univ, Japan*)

Pulmonary veins (PVs) are the major origin of atrial fibrillation. We have reported that $IP_3R_2$ in rat PV cardiomyocytes cooperates with $Na^+$-$Ca^{2+}$ exchanger (NCX) on T-tubule in triggering the norepinephrine (NE)-induced automaticity, and identified a unique hyperpolarization activated $Cl^-$ current, $I_{Cl,\ h}$, that potentially facilitates the automaticity. The mathematical model which incorporated the interaction between $IP_3R_2$ and NCX, and electrophysiological characteristics of $I_{Cl,\ h}$ successfully reproduced the NE-induced automaticity. Here, we show two further topics for the properties of rat PV cardiomyocytes in relation to its arrhythmogenicity. The first one is that a $Ca^{2+}$-stimulable adenylyl cyclase (AC) is involved in the NE-induced automaticity. Microarray, RT-PCR and immunohistochemistry uncovered that one of $Ca^{2+}$-stimulable AC was expressed regionally in the supraventricular area including PV. In particular, enriched expression of the AC was detected along T-tubule of PV myocytes, while atrial myocytes hardly displayed T-tubules. HEK293 cells exhibited sustained $Ca^{2+}$ oscillation in response to UTP under isoproterenol pre-application. Gene-knockout of our interest in the cells impaired the ability to keep the $Ca^{2+}$ oscillation. The NE-induce automaticity in PV cardiomyocytes was reversibly arrested by AC inhibitor. The second topic is related to the structural basis of $I_{Cl,\ h}$. Mass spectrometry identified HSPA8 as the CLCN2 interacting protein from rat PV. The auxiliary subunit of CLCN2 was subcloned and introduced into PC12 cell. With co-expression of the HSPA8, CLCN2 current exhibited the unique voltage-dependency similar to $I_{Cl,\ h}$. All these findings suggest that unique molecular interaction among NCX, $IP_3R_2$, and the AC along T-tubule potentiates the arrhythmogenicity of rat PV, and that rat heart possessed HSPA8 as the auxiliary subunit of the hyperpolarization activated $Cl^-$ channel. (COI:Properly Declared)

## AP-5 (O03-3)

### Drp1-mediated mitochondrial dynamics in cardiac remodeling

Akiyuki Nishimura[1], Kakeru Shimoda[2], Tomohiro Tanaka[2], Kazuhiro Nishiyama[1], Motohiro Nishida[1,2] ([1]*Grad Sch Pharm Sci, Kyushu Univ, Japan,* [2]*Dept cardiocirculatory Signal, NIPS, Japan*)

Mitochondria are dynamic organelles that interchanges their morphology between fusion (elongation) and fission (fragmentation) form. Proper mitochondrial quality control is indispensable for cardiac homeostasis and defects in mitochondrial dynamics are implicated in the development of cardiac diseases. Our group has investigated the molecular mechanism underlying the development of maladaptive cardiac remodeling, especially myocardial early senescence that is observed in cardiac disease patients and mice models and has been suggested as a major cause of cardiac dysfunction. We found that defective mitochondrial dynamics through aberrant interactions between mitochondria and actin cytoskeleton are a key determinant of cardiac remodeling and fragility. Dynamin-related protein 1 (Drp1), a mitochondrial fission-accelerating protein, was activated in myocardium after myocardial infarction, which induced mitochondrial fission-associated myocardial early senescence. We also found that the actin-binding protein filamin A acted as a guanine nucleotide exchange factor for Drp1. Hypoxic stress induced the interaction of filamin A with Drp1, leading Drp1 activation and mitochondrial fission-associated myocardial senescence in an actin binding-dependent manner in cardiomyocytes. We previously reported that polysulfidation of Drp1 at $Cys^{624}$, a redox-sensitive cysteine residue, negatively regulates its activity. Drp1-filamin A interaction was regulated by polysulfidation-depolysulfidation cycle of Drp1 $Cys^{624}$. Electrophile-mediated depolysulfidation of Drp1 promoted the interaction with filamin A and induced mitochondrial hyperfission. In addition, pharmacological perturbation of the Drp1-filamin A interaction by cilnidipine suppressed mitochondrial fission-associated myocardial senescence and improved chronic heart failure in mice. Our results suggest therapeutic potential targeting pathology-dependent Drp1-filamin A interaction for the treatment of chronic heart failure. (COI:No)

## AP-6 (PS17-02)

### The role of TRPC3 and TRPC6 in a stretch-induced slow force response in cardiomyocytes

Yohei Yamaguchi[1], Gentaro Iribe[1], Keiji Naruse[2], Akira Takai[1] ([1]*Dept Physiol, Med, Asahikawa Med Univ, Japan,* [2]*Dept Cardio Physiol, Grad Sch Med, Okayama Univ, Japan*)

An increase in preload induces a biphasic active force enhancement in the heart. The short-term increase in preload rapidly augments contractile force due to the Frank-Starling mechanism, which accelerates $Ca^{2+}$ sensitivity of contraction proteins. A further long-term increase in preload for several minutes to hours causes the increase in $[Ca^{2+}]_i$, leading to a slow force response to stretch (SFR), and a further increase in the contractile force. The stretch-induced release of angiotensin II has been implicated in the SFR, to raise intracellular $Na^+$ levels, followed by an increase in intracellular $Ca^{2+}$ levels via the $Na^+/Ca^{2+}$ exchanger. However, the extracellular cation influx pathway is poorly understood. To better understand the cation influx pathway, we focused on TRPC3 and TRPC6, receptor-operated, mechanosensitive non-selective cation channels. In our studies, cardiomyocytes were enzymatically isolated from mouse hearts, and both cell ends were held by two carbon fibers to apply stretch to the cells. Using this stretch device, we found that TRPC3 and TRPC6, regulated by the angiotensin II type 1 (AT1) receptor via diacylglycerol produced by phospholipase C, played a pivotal role in the SFR. Our recent data also showed that stretch-induced activation of TRPC3 increased intracellular $Ca^{2+}$ influx, causing the accumulation of $Ca^{2+}$ in the sarcoplasmic reticulum, which in turn increased $Ca^{2+}$ release and, consequently, the twitch force, suggesting that a functional sarcoplasmic reticulum (SR) is necessary for the SFR. Other data indicate that a myocardial stretch stimulated the AT1 receptor, followed by endothelin release to increase nicotinamide adenine dinucleotide phosphate oxidase (NOX)-derived reactive oxygen species, regulating TRPC3 and TRPC6 to increase $Na^+$ influx, leading to the SFR. These data suggest the possibility that two AT1 receptor-operated cation influx pathways via TRPC3 and TRPC6, causing the enhancement of $Ca^{2+}$ release from the SR, may cooperate in the SFR. (COI:No)

## AP-7 (S01-04)

### Daikenchuto, a traditional herbal medicine, ameliorates fibrosis by activating TRPA1 channel in intestinal myofibroblasts

Rin Kurahara[1], Keizo Hiraishi[1,2], Yaopeng Hu[2], Ryuji Inoue[2], Katsuya Hirano[1] ([1]*Dept Cardiovasc Physiol, Sch Med, Kagawa Univ, Japan,* [2]*Dept Physiol, Sch Med, Fukuoka Univ, Japan*)

**Background:** Daikenchuto (DKT) is a traditional oriental herbal medicine, widely used to mitigate post-operative ileus and constipation. In this study, we investigated the anti-fibrotic effect of DKT in a murine chronic colitis model and elucidated the role of transient receptor potential ankyrin 1 (TRPA1) channels of intestinal myofibroblasts in colonic fibrosis.
**Methods:** A murine chronic colitis model was established by weekly intrarectal administration of trinitrobenzene sulfonic acid (TNBS). Inflammatory and fibrotic changes were evaluated by histopathological examination. An intestinal myofibroblast cell line (InMyoFibs) was stimulated with TGF-$\beta$1, and subsequent intracellular signaling and pro-fibrotic factors were investigated. Samples from non-stenotic and stenotic regions of Crohn's Disease (CD) patient's intestines were used for pathological analyses.
**Results:** In TNBS chronic colitis model mice, the extents of inflammation and fibrotic changes were more prominent in TRPA1$^{-/-}$ knockout than in wild-type mice. One-week enema administration of DKT suppressed fibrotic lesions in wild-type mice, but not in TRPA1 knockout mice. Active ingredients of DKT, i.e. hydroxy $\alpha$-sanshool and 6-shogaol induced $Ca^{2+}$ influxes in InMyoFib, which were antagonized by co-treatment with a selective TRPA1 channel blocker HC-030031. DKT counteracted TGF-$\beta$1-induced expression of Type 1 collagen, $\alpha$-SMA, N-cadherin, the phosphorylation level of Smad-2 and p38-MAPK and the expression level of myocardin, a well-known master transcription factor regulating fibrosis signaling at the downstream of TGF-$\beta$1 receptor. Importantly, a 24-hour incubation with another DKT active ingredient Japanese Pepper increased the mRNA and protein expressions of TRPA1, which in turn negatively regulated collagen synthesis in InMyoFibs. TRPA1 expression in the stenotic regions of CD patient's intestine was significantly greater than that in the non-stenotic regions.
**Conclusions:** DKT suppresses intestinal fibrosis by upregulating the expression and activating the channel function of TRPA1. This putative mechanism underlies the reported beneficial actions of DKT on inflammatory bowel disease. (COI:No)

## AP-8

### Overexpression of neuronal $K^+$-$Cl^-$ co-transporter enhances dendritic spine plasticity and motor learning

Kayo Nakamura[1,2], Andrew John Moorhouse[3], Dennis Lawrence Cheung[3], Kei Eto[1], Ikuko Takeda[1], Paul Wiers Rozenbroek[3], Junichi Nabekura[1,2] ([1]*National Institutes for Physiological Sciences,* [2]*Sokendai,* [3]*University of New South Wales*)

The neuronal $K^+$-$Cl^-$ cotransporter KCC2 maintains a low intracellular $Cl^-$ concentration and facilitates hyperpolarizing $GABA_A$ receptor responses. KCC2 also plays a separate role in stabilizing and enhancing dendritic spines in the developing nervous system. Using a conditional transgenic mouse strategy, we examined whether overexpression of KCC2 enhances dendritic spines in the adult nervous system and characterized the effects on spine dynamics in the motor cortex in vivo during rotarod training. Mice overexpressing KCC2 showed significantly increased spine density in the apical dendrites of layer V pyramidal neurons, measured in vivo using two-photon imaging. During modest accelerated rotarod training, mice overexpressing KCC2 displayed enhanced spine formation rates, greater balancing skill at higher rotarod speeds and a faster rate of learning in this ability. Our results demonstrate that KCC2 enhances spine density and dynamics in the adult nervous system and suggest that KCC2 may play a role in experience-dependent synaptic plasticity. (COI:No)

## AP-9

### Epac1 deficiency inhibits basic fibroblast growth factor-mediated vascular smooth muscle cell migration

Yuko Kato[1,2], Utako Yokoyama[1], Takayuki Fujita[1], Masanari Umemura[1], Tetsuo Kubota[2], Yoshihiro Ishikawa[1] ([1]*Yokohama City University,* [2]*Tokyo Medical and Dental University*)

Vascular smooth muscle cell (VSMC) migration and the subsequent intimal thickening play roles in vascular restenosis. We previously reported that an exchange protein activated by cAMP 1 (Epac1) promotes platelet-derived growth factor (PDGF)-induced VSMC migration and intimal thickening. Because basic fibroblast growth factor (bFGF) also plays a pivotal role in restenosis, we examined whether Epac1 was involved in bFGF-mediated VSMC migration. bFGF-induced lamellipodia formation and migration were significantly decreased in VSMCs obtained from Epac1$^{-/-}$ mice compared to those in Epac1$^{+/+}$-VSMCs. The bFGF-induced phosphorylation of Akt and glycogen synthase kinase 3$\beta$ (GSK3$\beta$), which play a role in bFGF-induced cell migration, was attenuated in Epac1$^{-/-}$-VSMCs. Intimal thickening induced by the insertion of a large wire was attenuated in Epac1$^{-/-}$ mice, and was accompanied by the decreased phosphorylation of GSK3$\beta$. These data suggest that Epac1 deficiency attenuates bFGF-induced VSMC migration, possibly via Akt/GSK3$\beta$ pathways. (COI:No)

# Oral Presentations

# Oral Session 1

## Kidney・Urination・Digestion・Absorption

(March 17, 15:20〜16:20, Hall 7)

### O01-1

#### The Physiological Roles of Moesin, a Cytoskeletal Protein, in thick ascending limb of loop of Henle *via* NKCC2

Kotoku Kawaguchi[1], Ryo Hatano[2], Shinji Asano[1] (*[1]Dept Mol Physiol, Col Pharm Sci, Ritsumeikan Univ, Japan, [2]Dept Med Physiol, Grad Sch Med, Chiba Univ, Japan*)

Tubular reabsorption of electrolytes in the kidney is an essential function in regulating fluid balance in the body. $Na^+/K^+/2Cl^-$ cotransporter type 2 (NKCC2) is specifically expressed in luminal cell surface of the thick ascending limb of the loop of Henle (TAL). The reabsorption of NKCC2 plays important roles in regulating fluid balance. In general, luminal surface expression level of NKCC2 is regulated by intracellular membrane trafficking involving exocytosis and endocytosis. It is well known that exocytosis of NKCC2 is promoted by the cAMP/PKA pathway *via* some hormone stimulation. On the other hand, the molecular mechanisms of endocytosis of NKCC2 are not clear. Recently, moesin, which is a crosslinker between membrane proteins and actin cytoskeleton, was reported to interact with NKCC2. However, the physiological roles of moesin in the kidney remain unclear. Here, we examined the roles of moesin in the regulation of renal function *in vivo* by using male moesin-null ($Msn^{-/y}$) mice.

To evaluate the cell surface expressions and endocytosis of NKCC2, we analyzed these changes using protein biotinylation in tubular suspension including TAL. We investigated the distribution of NKCC2 in lipid raft. To examine the renal physiological roles, we performed biochemical analysis of plasma and urine.

In these results, we found that apical surface expression of NKCC2 was significantly increased in $Msn^{-/y}$ TAL. Internalized NKCC2 was significantly reduced in the $Msn^{-/y}$ TAL. Lipid raft expression of NKCC2 was significantly decreased in $Msn^{-/y}$ mice. Increase of plasma $Na^+$ and $Cl^-$ concentration was observed in $Msn^{-/y}$ mice. Urinary absolute excretions of $Na^+$ and $Cl^-$ in $Msn^{-/y}$ mice were lower than those of WT mice. These results suggest that moesin regulates the apical surface expression level of NKCC2 by targeting NKCC2 to lipid raft and plays important roles in the renal electrolyte handling. (COI:No)

### O01-2

#### Elucidation of pace maker cells of spontaneous activities in detrusor smooth muscle

Kentaro Kawagoe[1], Eljamal Kareman[1], Tomoko Maki[2], Shinsuke Nakayama[3], Shunichi Kajioka[1], Toshiyuki Sasaguri[1], Masatoshi Eto[4] (*[1]Dept Clin Pharmacol, Grad Sch Med, Kyushu Univ, Japan, [2]Spinal Injuries Center, [3]Department ofCell Physiology, Graduate School of Medicine, Nagoya University, [4]Department of Urology, Graduate School of Medical Sciences, Kyushu University*)

Pacemaker cells of spontaneous activities of bladder smooth muscle were believed to c-kit+ interstitial cells of Cajal (ICC) like cells as well as gastrointestinal. Recently, however, PDGFR $\alpha$ + but c-kit negative interstitial cells have been described in bladder wall as a new candidate of pace maker cells. Thus, class of pace maker cells of bladder is still under controversial. The aim of this study is to clarify the pathological modification of probable pace maker cells of overactive bladder using bladder outlet obstruction (BOO) mouse. In micturition reflex, the number of urination per day significantly increased in BOO compared to sham. The daily urination volume and one void volume were significantly decreased in BOO compared to sham. The bladder weight was significantly larger in BOO compared to sham. There was no significant difference in water intake between the two groups. In cystometry, BOO mice's non-voiding contractions significantly increased. In BOO, mRNA expression of c-kit, CD34 and PDGFR $\alpha$ was significantly elevated, however interestingly immunostaining indicated significant reduction of PDGFR $\alpha$ + cells. In MEA recording system, sham mice were hardly seen spontaneous activities. Small waves of spontaneous action potential followed by relatively slow large waves could be seen in BOO mice. Spontaneous activities were enhanced with 0.3$\mu$M carbachol and completely suppressed with 2$\mu$M nifedipine.BOO model mice show significant difference in voluntarily voiding behavior analysis among urination frequency, daily urination volume, and single void volume. The result of significant reduction of PDGFR $\alpha$ + cells despite of the obvious increase of its mRNA expression suggests that BOO might interrupt protein syntheses of PDGFR $\alpha$ or that the reduction of PDGFR $\alpha$ by BOO might provoke the increase of its mRNA. In MEA recording, we demonstrated that lower urinary tract obstruction increases the spontaneous electrical activity in bladder smooth muscle. (COI:No)

### O01-3

#### Common Ca2+-dependent inactivation (CDI)-based mechanism may exist in FSGS mutations in the N-terminal domain of TRPC6 channels

Masayuki Mori X.[1], Onur Polat K.[2], Ryo Okada[1], Ayaka Suzuki[1], Sayo Ohmura[1], Ryuji Inoue[3] (*[1]Dept Biomaterials and Chemistry, Sch Med, UOEH, Japan, [2]Dept Synthetic and Biological Chem, Grad Sch Engr, Kyoto Univ, [3]Dept Physiol, Sch of Med, Fukuoka Univ*)

Transient Receptor Potential Canonical member 6 (TRPC6) is a receptor activated nonselective cation channel, and its mutations are known to be associated with focal segmental glomerulosclerosis (FSGS), a glomerular disease leading to the end-stage renal failure. Our recent finding that impairment of Ca2+-dependent inactivation (CDI) in TRPC6 FSGS-associated mutations (Polat OK et al., JASN, 2019) raises the question as to whether disrupted CDI could be a unifying mechanism to explain the dysfunctionality of FSGS mutations in the coiled-coil C-terminus domain which dimerization is presumed to critical for Ca2+/calmodulin binding. To address this point, we examined the N-terminal mutations (in the ankyrin domains) as well as those on the C-terminal, out-side of the coiled-coil domain, by using whole-cell patch-clamp and Ca2+ imaging experiments. The newly tested FSGS mutations had also significant delay of inactivation, including L780P, reportedly attenuated Ca2+ response phenotype in C-terminal. Therefore, the CDI mechanism could have substantial potential to unify the pathogenesis of FSGS mutations, and may thus deserve further investigation in the future. (COI:No)

### O01-4

#### Study of Spatiotemporal electrical activity in the colon of mice with hypoganglionosis

Shinsuke Nakayama[1], Kouichirou Yoshimaru[2], Takayoshi Yamaza[3], Shunichi Kajioka[4] (*[1]Dept Cell Physiol, Grad Sch Med, Nagoya Univ, Japan, [2]Dept Pediatric Surg, Kyushu Univ, Grad Sch Med Sci, [3]Dept Mol Cell Biol and Oral Anat, Kyushu Univ, Grad Sch Dent Sci, [4]Dept Clin Pharmacol, Kyushu Univ, Grad Sch Med Sci*)

Hypoganglionosis, such as Hirschspring's disease (HSCR) and its allied disorders, interpreted by prenatal deficiency of tropic factors with the detailed etiology still unclear, causes refractory alimentary disorders associated with functional ileus. In this study, in order to explore possible effective therapy, we characterized functional alterations in JF1 mice with hypoganglionosis. JF1 mice are piebald mice, which possess the classic piebald s allele of Ednrb with two nucleotide substitutions, and reduce the expression of endothelin receptor type B (EDNRB) in the colon, causing a mild case of megacolon. Spatio-temporal electrical activity is the basis of smooth and elaborate motility in the gastrointestinal tract including the colon. We thus monitored the field potential in isolated muscle sheets of the proximal colon, using 8 × 8 microelectrode array (MEA) covering ~1 × 1 mm2 area. In wild-type B6 mice, samples from the proximal colon displayed basal slow electrical oscillations with the period of ~3-4 s in a majority of sensing electrodes, and occasionally large synchronized potentials occurred. On the other hand, the proximal colon of JF1 mice, basal rhythmic oscillations were negligible, and electric complexes consisting of rapid and slow potentials occurred frequently. It is noted that the rapid component of prolonged bursting was a characteristic feature, and the bursting rapid potentials propagated only in limited regions of MEA recording area. The frequency of rapid potentials was 1-2 Hz. Also, the magnitude of electric potentials was significantly smaller in JF1 mice than in wild-B6 mice. In organ bath experiments, isolated colon samples exhibited spontaneous contractions corresponding to the electrical activity measured with MEA. In the light of histological and blood examinations, we discuss possible mechaisms that account for the prominent differences in electrical and mechanical activity. (COI:No)

### O01-5

#### Fibroblast growth factor-23 and high calcium exposure inhibits intestinal calcium absorption in Caco-2 monolayer

Mayuree Rodrat, Kannikar Wongdee[2,4], Narattaphol Charoenphandhu[1,2,3] (*[1]Department of Physiology, Faculty of Science, Mahidol University, Thailand, [2]Center of Calcium and Bone Research (COCAB), Faculty of Science, Mahidol University, Thailand, [3]Institute of Molecular Biosciences, Mahidol University, Thailand, [4]Faculty of Allied Health Sciences, Burapha University, Thailand*)

Excessive calcium intake and intestinal calcium hyperabsorption often lead to a variety of adverse effects such as vascular calcification, nephrolithiasis, and dementia. Therefore, the intestinal epithelial cells probably possess a negative feedback mechanism to prevent an excessive calcium transport. Although 1, 25-dihydroxyvitamin $D_3$ [1, $25(OH)_2D_3$] and parathyroid hormone (PTH) are well recognized as calcium-regulating hormones, a few of endocrine and paracrine factors can inhibit or counterbalance calcium absorption. Previously, fibroblast growth factor (FGF)-23 has been reported to abolish the 1, $25(OH)_2D_3$-enhanced intestinal calcium absorption in mice. We herein hypothesized that FGF-23 locally produced by the intestinal epithelial cells regulated calcium absorption as part of negative feedback mechanism. The present study also aimed to determine whether FGF-23 was able to alter the changes in epithelial electrical properties and electrogenic ion transport in 1, $25(OH)_2D_3$-treated Caco-2 monolayer, as indicated by short-circuit current ($I_{sc}$) and transepithelial resistance (TER) in Ussing chamber. The results demonstrated that 1, $25(OH)_2D_3$ significantly increased $I_{sc}$ across Caco-2 monolayer, suggesting an increase in the electrogenic ion transport, while TER was decreased in 1, $25(OH)_2D_3$-treated monolayer. In addition, calcium transport across intestinal epithelium-like Caco-2 monolayer enhanced by 1, $25(OH)_2D_3$ disappeared after prolonged exposure to high apical ionized calcium. Apical and/or basolateral exposure to FGF-23 completely abolished the 1, $25(OH)_2D_3$-enhanced calcium transport as well as epithelial electrical parameters, i.e., $I_{sc}$ and TER, while pretreatment with FGF-23-neutralizing antibody could restore the 1, $25(OH)_2D_3$-enhaced calcium transport even in the presence of high apical calcium. Moreover, FGF-23 was found in both apical and basolateral culture media of 1, $25(OH)_2D_3$- and CaCl2-treated groups, but not the untreated group. In conclusion, prolonged exposure to high apical calcium and excessive calcium absorption probably induces local production of FGF-23 as a part of negative feedback loop, which, in turn, inhibits calcium transport, and modulates epithelial integrity and electrogenic transport of ions across the intestinal mucosa. (COI:No)

# Oral Session 2

## Environmental Physiology

(March 17, 16:20～17:20, Hall 7)

### O02-1

#### Replicative senescent human cells possess altered circadian clocks with a prolonged period and delayed peak-time

Yasukazu Nakahata[1], Rezwana Ahmed[1,2], Atsushige Ashimori[1,2], Kazuyuki Shinohara[1], Yasumasa Bessho[2] (*[1]Dept Neurobiol and Behavi, Nagasaki Univ, Japan, [2]Gene Regulation, Dev Biol Sci, NAIST, Japan*)

Over the last decade, a wide array of evidence has been accumulated that disruption of circadian clock is prone to cause age-related diseases and premature aging. On the other hand, aging has been identified as one of the risk factors linked to the alteration of circadian clock. These evidences suggest that the processes of aging and circadian clock feedback on each other at the animal level. However, whether these two processes influence each other at the cellular level is still largely unknown. Our lab has recently revealed that the primary fibroblast cells derived from Bmal1-/- mouse embryo, in which circadian clock is completely disrupted, do not demonstrate the acceleration of cellular aging, i.e., cellular senescence. Hence, in my study I asked the reverse question, i.e. whether cellular senescence affects the circadian clock. Interestingly, we found that senescent cells possess a longer circadian period with delayed peak-time and that the variability in peak-time is wider in the senescent cells compared to their proliferative counterparts, indicating that senescent cells show alterations of circadian clock. From these results, it is also reasonable to propose that investigation at cellular level is a powerful and useful approach to dissect molecular mechanisms of aging in the circadian clock. (COI:No)

### O02-2

#### Thyroid hormone (TH) induced cell proliferation and migration through TH receptor (TR)-dependent and -independent pathways

Ariyani Winda[1], Wataru Miyazaki[1,2], Izuki Amano[1], Noriyuki Koibuchi[1] (*[1]Department of Integrative Physiology, Graduate School of Medicine, Gunma University, Japan, [2]Department of Bioscience and Laboratory Medicine, Graduate School of Health Sciences, Hirosaki University, Japan*)

Thyroid hormone (TH) plays an important role for controlling organ development and functional maintenance. During development, TH may promote cellular proliferation and migration. The actions of TH are mainly mediated by TH receptor (TR)-dependent pathway, which requires binding of TH to TR that may localize in the nucleus, mitochondria, and plasma membrane. TH also exert the actions through TR-independent pathway through binding to membrane protein, such as integrin $\alpha V \beta 3$. However, the mechanism of TH action on cell proliferation and migration have not yet been clearly determined. In this study, we examined the effects of TH derivatives (T3, T4, rT3, and Triac) on cell proliferation and migration. A short exposure of THs induced cell proliferation in C6 and Neuro-2A cells. Flow cytometry showed that total cell number in G2 phase increased after THs exposure. The expression of p-ERK1/2 proteins and Ccnd, Ccna, Ccnb, Cdk1, Cdk4, and c-Fos mRNA increased by TH derivatives. TH derivatives also increased F-actin cytoskeleton remodelling, that leads to cell migration in C6 and 3T3 cells. Our study also showed that THs exposure induced F-actin rearrangement overlapped with the upregulation of p-Akt, p-Rac1/cdc-42, Rho A, Cdc42, or Rac1/2/3 protein expression level. Knockdown of TRs by RNAi or co-exposure integrin $\alpha V \beta 3$ with XJ735, a selective integrin $\alpha V \beta 3$ inhibitor, reduced the cells proliferation and migration. These results indicate that THs exposure exerts their action in cell proliferation and migration through both TR–dependent and –independent signaling pathway. (COI:No)

### O02-3

#### Mental stress does not impair aerobic exercise performance after whole body cold exposure

Daiki Imai[1,2], Ryosuke Takeda[1], Eriko Kawai[2], Kosuke Saho[2], Akemi Ota[2], Emiko Morita[2], Yuta Suzuki[1,2], Hisayo Yokoyama[1,2], Kazunobu Okazaki[1,2] (*[1]Res Ctr for Urban Health & Sports, Osaka City Univ, Osaka, Japan, [2]Dept of Environ Physiol for Exerc, Osaka City Univ Grad Sch of Med, Osaka, Japan*)

Whole body cold exposure or mental load applied before exercise is known to decrease exercise performance. However the effects of simultaneous application of these stresses on exercise performance remain unknown. We investigated the effects of simultaneous application of whole body cold exposure and mental load on aerobic exercise performance compared with cold exposure alone. Seven healthy young male participants underwent the stroop color word test (500 stimuli/set × 2) as mental stress trial (MS), while watched a documentary DVD as non-stress trial (NS) during 60 min of cold exposure using a water-perfused suit. Water temperature in the suit was maintained at 15℃ for 10 min then at 10℃. They undressed the suit then performed a cycling test at 80% $VO_{2max}$ to the exhaustion. Esophageal and skin temperatures ($T_{es}$ and $T_{sk}$, respectively), plasma adrenaline ($[Ad]_p$), noradrenaline ($[Nord]_p$), and cortisol ($[Cortisol]_p$) concentrations, and subjective fatigue index (SFI) were measured before and immediately after mental stress and exercise. Exercise duration was evaluated as aerobic performance. We found that $T_{sk}$ was lower after cooling than baseline, while $T_{es}$ remained unchanged. $T_{sk}$ and $T_{es}$ were not different between trials. A significant effect of interaction (time × trial) was found on $[Ad]_p$ ($p=0.023$), $[Nord]_p$ ($p=0.047$), and SFI ($p=0.040$), while not on $[Cortisol]_p$. Exercise duration was not different between trials ($p=0.762$). In conclusion, the simultaneous application of mental load accompanied with whole body cold exposure exacerbated tiredness via the enhanced sympathetic nerve activity compared with cold exposure alone, while did not impair aerobic exercise performance. (COI:No)

### O02-4

#### An Examination of The Factor of Heat Tolerance During Passive Heat Stress

Yuta Masuda, Kaito Kawashima, issei Kato, Shuri Marui, Kei Nagashima (*Body Temperature and Body Fluid Lab, Fac Human Sci, Waseda Univ, Japan*)

**Introduction:** It has been reported that many factors such as age, gender, and exercise habit, etc. affect heat tolerance of individuals; however, the mechanism remains unclear yet. Previous studies have suggested that factors involved in heat dissipation such as sweating are important in determining heat tolerance, although the clear evidences have not been presented. In the present study, we tested the hypothesis that reduction of metabolic heat production is one of mechanisms increasing heat tolerance. Therefore, we assessed thermoregulation during bathing in a hot water, where responses of heat dissipation are largely suppressed.
**Methods:** Healthy male participance (age, 23.73 ± 2.89 y ; bw, 65.75 ± 8.58 kg; and % fat, 17.18 ± 6.14 %)immersed in water of 41℃ to the clavicle level, where room temperature was at 33℃ with 50% relative humidity. We continuously measured rectal temperature ($T_{rec}$), temperature of 4 skin sites, metabolic heat production ($H_{prod}$) by calorimetry, sweat rate (SR) at back, and skin blood flow (SkBF)at face. The duration of bathing was determined till $T_{rec}$=39.0℃ or for 60min if $T_{rec}$ <39.0℃.
**Results:** $T_{rec}$ increased during bathing in all participants and reached 38.25 ± 0.51℃ ranging from 36.85 to 39.04℃ at 30 min after the onset of bathing. The value of cumulative $H_{prod}$ is correlated with the increase in $T_{rec}$ ($P<0.01$, $r = 0.83$). There was no correlation between the increase in $T_{rec}$, and mean$T_{sk}$ and cumulative values of SR and AUC-SkBF and % body fat.
**Conclusion:** Metabolic heat production during bathing is different among participants. In addition, as lower the metabolic heat production was, increase in body core temperature was suppressed. The results may suggest that suppression of heat production is one of mechanisms involved in heat tolerance. (COI:No)

### O02-5

#### Chronic treatment with the synthetic glucocorticoid methylprednisolone in Sprague-Dawley rats induces anhedonic behaviour and dysregulation of the circadian tryptophan hydroxylase mRNA expression profile in the dorsal and median raphe nuclei

Nidia M. Reyes-Prieto[1], Mitsuhiro Yoshimura[2], Yvonne M. Kershaw[1], Christopher A Lowry[3], Becky L. Conway-Campbell[1], Stafford L Lightman[1] (*[1]University of Bristol. United Kingdom, [2]University of Occupational and environmental Health. Japan, [3]University of Colorado. USA*)

Stress-related neuropsychiatric disorders represent a major worldwide problem. Hypothalamic-pituitary-adrenal (HPA) axis dysregulation and serotonergic dysfunction have both been implicated in the development of depression, however the relationship between the two is not fully understood. Here we show that expression of tryptophan hydroxylase (tph2) mRNA, which codes for the rate-limiting enzyme in the biosynthesis of serotonin in the raphe complex, fluctuates significantly over the 24 hour period. The lowest levels of tph2 expression coincided with the circadian nadir in adrenal glucocorticoid secretion, while the highest levels coincided with the initial rise in circulating glucocorticoids preceding the onset of the rats' active phase. Administration of the synthetic glucocorticoid methylprednisolone in drinking water suppressed adrenal glucocorticoid secretion, and induced a pronounced phase shift in glucocorticoid receptor (GR) activation in serotonergic neurons throughout the raphe complex. The circadian rhythm of tph2 mRNA expression was markedly altered in the MPL treated rats, particularly for nadir and peak expression levels throughout the dorsal (DRD), ventral (DRV), ventrolateral/ventrolateral periaqueductal grey (DRVL/VLPAG) and median (MnR) raphe nuclei. MPL treated rats exhibited a significantly reduced sucrose preference, consistent with anhedonic behaviour and symptomatic of a depressive phenotype. Taken together, our data demonstrate that the endogenous daily rhythm in tph2 expression throughout the raphe complex and the associated affective state of the individual are highly sensitive to HPA axis disruption, in particular shown here with synthetic glucocorticoid treatment. Moreover, our findings provide a potential mechanism underpinning the adverse side effects on mood and behaviour reported by patients treated with synthetic glucocorticoids. (COI:No)

# Oral Session 3

## Heart・Circulation 1

(March 18, 13:20～14:20, Hall 3)

### O03-1
#### Mitochondrial aerobic respiration in the fetal heart is activated just after initiation of the heartbeat

Tatsuya Sato, Nobutoshi Ichise, Takeshi Kobayashi, Hiroya Yamazaki, Yoshinori Terashima, Noritsugu Tohse (*Dept Cell Physiol, Sap Med Univ, Japan*)

**Backgrounds:** We demonstrated that the heartbeat in rat fetal heart initiates at around embryonic day 10.0 (E10.0) with a calcium transient via extracellular calcium influx being preceding to muscle contraction. We also showed that the pathway of gene expressions related to glucose metabolism assessed by transcriptome analysis in the heart just after initiation of the heartbeat was the secondly highest next to the pathway related to muscle contraction, suggesting that changes in energy metabolism play pivotal roles in initiation of the heartbeat. Although mitochondria are central site in energy metabolism in the adult heart, it remains unclear whether mitochondrial respiration is involved in energy metabolism in the fetal heart at the beginning of the heartbeat.
**Methods:** The embryos at E10.0 in Wistar rats were divided into two groups according to the hearts without (pre-) or with (post-) heartbeat. Comprehensive metabolites of the fetal heart were evaluated by metabolome analysis, and real time mitochondrial oxygen consumption rate (OCR) for enzymatically isolated fetal rat cardiomyocytes was assessed by an extracellular flux analyzer.
**Results:** According to the principal component analysis in the metabolomic analysis, fructose 6-phosphate, fructose 1, 6-bisphosphate, which are metabolites in glycolytic pathway, were major factor loading components in pre-heartbeat group, whereas GTP, reduced glutathione, and ATP, which are associated with mitochondrial respiration, were major determinants in post-heartbeat group. OCR in isolated cells at baseline was extremely low in both groups; however, maximal OCR induced by the addition of an uncoupler (FCCP) showed a significant increase only in cells from post-heartbeat group, suggesting the presence of proton gradient between the inner mitochondrial membrane and the matrix after initiation of the heartbeat.
**Conclusions:** The findings suggest that mitochondrial aerobic respiration is activated just after initiation of the heartbeat presumably in response to ATP demand in the fetal beating heart.
(COI:No)

### O03-2
#### Acute and Temporary Overstretch Destructed Inner Mitochondrial Membrane Without Subsequent Cardiac Dysfunction in Rat Hearts

Naritomo Nishioka[1,2], Hiroki Bochimoto[3], Ping Yu Xiong[4,5], Shunsuke Baba[1,6], Jun Tanihata[3], Susumu Minamisawa[1,3], Yoichiro Kusakari[1] (*[1]Dept Cell Physiol, Jikei Univ Sch Med, Tokyo, Japan, [2]Dept Cardiac Surgery, Jikei Univ Sch Med, Tokyo, Japan, [3]Division of Aerospace Med, Dept Cell Physiol, Jikei Univ Sch Med, Tokyo, Japan, [4]Dept Med, Queen's University, Kingston, ON, Canada, [5]Dept Biomed and Molecular Sciences, Queen's University, Kingston, ON, Canada, [6]Dept Pediatrics, Jikei Univ Sch Med, Tokyo, Japan*)

**Introduction:** We reported last year that acute overstretch destructed inner mitochondrial membrane with preserved sarcomere structure of rat papillary muscle. However, it is unclear whether acute overstretch-induced destruction of mitochondria would impair cardiac function or not.
**Purpose:** We made two different models for acute overstretch to investigate the changes in contractility and organelle structures in cardiac muscle.
**Methods:** 1) in vivo study: We ligated pulmonary artery (PA) of male SD-rats (BW>350g) for 30 seconds to make a model of right ventricle (RV) expansion, then released ligation and followed by 30 min. Cardiac function was monitored by transthoracic echocardiogram (TTE) and the intracellular ultrastructure of RV free wall was observed by transmission electron microscope (EM). 2) in vitro study: to mimic in vivo model, the RV papillary muscle of male SD-rats was overstretched within 2 seconds up to 120% of Lmax, and the overstretched status was kept for 5 minutes, then returned to Lmax (1Hz, 36℃).
**Results:** In in vivo study, TTE revealed PA ligation made the area of diastolic phase enlarged to 300%. However, there was no significant difference in RV ejection fraction and volume, and Tricuspid Annular Plane Systolic Excursion (TAPSE) between before and 30 min after ligation (n=9). In EM analysis, focal vacuolated mitochondria with decrease in electron density of matrix appeared, despite the sarcomere kept normal structure in cardiomyocytes of the RV expansion model. In in vitro study, the active tension was reduced to 34.6±6.3 % of Lmax during overstretching and recovered up to 101.0±21.7% of Lmax after returning to Lmax (n=5).
**Conclusions:** Acute and transient overstretch did not subsequently impair cardiac function, although it disrupted inner mitochondrial membrane. This study indicates the functional robustness of myofilaments and structural fragility of mitochondria to mechanical stress in cardiac muscles.
(COI:No)

### O03-3 (AP-5)
#### Drp1-mediated mitochondrial dynamics in cardiac remodeling

Akiyuki Nishimura[1], Kakeru Shimoda[2], Tomohiro Tanaka[2], Kazuhiro Nishiyama[1], Motohiro Nishida[1,2] (*[1]Grad Sch Pharm Sci, Kyushu Univ, Japan, [2]Dept cardiocirculatory Signal, NIPS, Japan*)

Mitochondria are dynamic organelles that interchanges their morphology between fusion (elongation) and fission (fragmentation) form. Proper mitochondrial quality control is indispensable for cardiac homeostasis and defects in mitochondrial dynamics are implicated in the development of cardiac diseases. Our group has investigated the molecular mechanism underlying the development of maladaptive cardiac remodeling, especially myocardial early senescence that is observed in cardiac disease patients and mice models and has been suggested as a major cause of cardiac dysfunction. We found that defective mitochondrial dynamics through aberrant interactions between mitochondria and actin cytoskeleton are a key determinant of cardiac remodeling and fragility. Dynamin-related protein 1 (Drp1), a mitochondrial fission-accelerating protein, was activated in myocardium after myocardial infarction, which induced mitochondrial fission-associated myocardial early senescence. We also found that the actin-binding protein filamin A acted as a guanine nucleotide exchange factor for Drp1. Hypoxic stress induced the interaction of filamin A with Drp1, leading Drp1 activation and mitochondrial fission-associated myocardial senescence in an actin binding-dependent manner in cardiomyocytes. We previously reported that polysulfidation of Drp1 at $Cys^{624}$, a redox-sensitive cysteine residue, negatively regulates its activity. Drp1-filamin A interaction was regulated by polysulfidation-depolysulfidation cycle of Drp1 $Cys^{624}$. Electrophile-mediated depolysulfidation of Drp1 promoted the interaction with filamin A and induced mitochondrial hyperfission. In addition, pharmacological perturbation of the Drp1-filamin A interaction by cilnidipine suppressed mitochondrial fission-associated myocardial senescence and improved chronic heart failure in mice. Our results suggest therapeutic potential targeting pathology-dependent Drp1-filamin A interaction for the treatment of chronic heart failure. (COI:No)

### O03-4
#### Roles of the TRPM4 channel in mitochondrial function, ROS generation, and calcium releasein myocardial ischemia–reperfusion injury

Chen Wang[1], Jian Chen[2], Keiji Naruse[1], Ken Takahashi[1] (*[1]Cardiovascular Physiol, Graduate Sch Med, Okayama Univ, Okayama, Japan, [2]Harbin Medical University*)

Ischemic heart disease is one of the most common causes of death in the current era. Mitochondrial dysfunction, reactive oxygen species (ROS) generation, and calcium (Ca2+) overload are three crucial factors that trigger myocardial ischemia–reperfusion (I/R) injury. Inhibition of TRPM4, a calcium-activated nonselective cation channel, protects the rat heartfrom I/R injury, but the specific mechanism is unclear.
In this study, we investigated the mechanism of cardioprotection against I/R injury via TRPM4, using two types of I/R injury models (500 $\mu$ mol-hydrogen peroxide[H2O2] and 24h of hypoxia [2% O2] followed by2h of reoxygenation[H/R]). We knocked out the TRPM4 gene in the rat cardiomyocyte cell line H9c2 using CRISPR/Cas9. With H2O2 treatment, both intracellular calcium levels and ROS production increased in wild-type (WT) cells, whereas these changes were mitigated in knock-out (KO) cells. Additionally, with this treatment, mitochondrial membrane potential and intracellular ATP levelsreduced in WT cells, whereas these changes were mitigated in KO cells. Furthermore, these effects of H2O2 in WT and KO cells were similarly observed underthe H/R condition.
The findings suggest that blockade of the TRPM4 channel might protect the myocardium from I/R injury by maintaining mitochondrial membrane potential and intracellular ATP levels, possibly owing to the prevention of aberrant increases in intracellular calcium and ROS.
Key words: Myocardial ischemia-reperfusion injury, TRPM4, Mitochondrial membrane potential, ATP, Reactive oxygen species, Calcium, CRIPSR/Cas9 (COI:No)

### O03-5
#### Heart rate variability analysis of parents and infants during a hug

Sachine Yoshida[1], Hiromasa Funato[1,2] (*[1]Dept Anatomy, Faculty Med, Toho Univ, Japan, [2]WPI-III, Univ Tsukuba, Japan*)

Parents often hold their infants firmly in their arms to express affection and joy. Such physical contact is well-known as a hug, but the physiological effects on infants and parents remain unclear. Here, we examined the heart rate variable of infants and their parents during the parent-infant hug with different month ages of the first-year infants. We found several parameters of infant heart rate variability changed around four months, indicating the pronounced parasympathetic activity. We examined changes in the R-R interval (RRI) in infants under and over four months old during being held, hugged, and hugged tightly by their parents. The RRI increase rate during a hug changed depending on the ages and activity levels of infants. Such context-dependent RRI changes were absent during holding and tight hugs. The parent-infant hug also decreased heart rates in the parents. A hug seems to function as one of the nonverbal communication methods between parents and infants after around four months. These findings will contribute to the understanding of typical and atypical development. (COI:No)

# Oral Session 4

## Neuron・Synapse

(March 18, 13:20～14:20, Hall 4)

### O04-1

#### A Novel Role of Thyroid Hormone Receptor in Synaptic Plasticity in Cerebellar Purkinje Cells

Ayane Ninomiya[1], Nobutake Hosoi[2], Michifumi Kokubo[1], Izuki Amano[1], Asahi Haijima[3], Wataru Miyazaki[4], Hirokazu Hirai[2], Noriyuki Koibuchi[1] (*[1]Dept. Integrative Physiol, Grad Sch Med, Gunma Univ, Japan, [2]Dept. Neurophysiology and Neural Repair, Grad Sch Med, Gunma Univ, [3]Sch Human Sciences, Waseda Univ, [4]Dept. Bioscience and Laboratory Medicine, Grad Sch Health Sci, Hirosaki Univ*)

Thyroid hormone (TH) is essential for the development and the maintenance of the brain function. TH action is mediated by TH receptor (TR). TR binds to a specific DNA sequence on TH-target genes and thus functions as a ligand-dependent transcription factor. In thyroid diseases such as congenital hypothyroidism or resistance to TH (RTH), TH-TR binding is dominantly disrupted, leading to the various symptoms such as motor deficits. However, the specific mechanism has not been cleared, besides that proper motor coordination is deeply related to long term depression (LTD) of synaptic transmission from parallel fiber (PF) to Purkinje cell (PC) in the cerebellum (Ito, 1989). Therefore, we examined the involvement of TR in synaptic plasticity at PF-PC synapses by using transgenic mice (Mf-1 mice) which express dominant-negative TR specifically in PCs. Since Mf-1 display the impairment of motor coordination and motor learning, decrease in TR signaling in PCs may alter synaptic plasticity and contribute to motor incoordination. A whole-cell patch clamp recording of Mf-1 PCs revealed the inhibition of LTD but instead the induction of long term potentiation (LTP) of the synaptic transmission at PF-PC synapses. This indicates that the intracellular calcium dynamics may be disrupted in Mf-1 PCs since LTD requires a higher elevation of the intracellular calcium concentration in PCs than LTP does. In addition, single-PC qPCR showed that the mRNA levels of some important molecules for the intracellular calcium dynamics in PCs (SERCA2, IP3R, and P/Q-type calcium channel) decreased in Mf-1 PCs, indicating the possible TH-target genes. Taken together, the present study suggested a novel possible role of TR in synaptic plasticity at PF-PC synapses by regulating the expression of some important genes for LTD occurrence in the cerebellum. This finding could give a new insight into the mechanism of motor deficits in thyroid diseases. (COI:No)

### O04-2

#### Presynaptic inhibition of GABA release from striatal medium spiny neurons onto cholinergic interneurons by M1 muscarine receptors

Toshihiko Momiyama, Etsuko Suzuki (*Dept Pharmacol, Jikei Univ Sch Med*)

We used transgenic mice with restricted expression of channelrhodopsin-2 (ChR2) in the striatal medium spiny neurons (MSNs). Whole-cell patch-clamp recordings were made from striatal cholinergic interneurons in P10-17 mice brain slices. Neurons were voltage clamped at -60 mV. Light stimulation (470 nm, 5 ms duration) evoked postsynaptic currents in the presence of glutamate and glycine receptor antagonists. These postsynaptic currents were blocked by GABAA receptor antagonist, bicuculline, suggesting they were GABAA receptor-mediated inhibitory postsynaptic currents (IPSCs). A muscarinic acetylcholine receptor agonist, carbachol (1 $\mu$M), suppressed IPSCs by 49.5 ± 7.8%, (n = 5). To examine the changes in GABA release probability, we calculated coefficient of variation (CV) at baseline, after application of bicuculline, after washout of bicuculline and after application of carbachol. CV at baseline and after application of bicuculline were 0.2 ± 0.02 and 0.2 ± 0.03, respectively. The CV was not increased after application of bicuculline, suggesting the action site of bicuculline was postsynaptic GABAA receptors. On the other hand, CV after application of carbachol was significantly increased (0.5 ± 0.06, p = 0.004), suggesting that GABA release probability was changed by carbachol. In addition, carbachol (10 $\mu$M), wich strongly inhibits light-evoked IPSCs, did not affect inward currents evoked by puff-applied GABA (100 $\mu$M). These results suggest that activation of M1 muscarine receptors presynaptically inhibits GABA release from MSNs onto cholinergic interneurons. (COI:No)

### O04-3

#### Excitability plasticity and behavioral modulation by acute inflammation of the cerebellum

Gen Ohtsuki[1,2] (*[1]Hakubi center, Kyoto Univ, Japan, [2]Dept Biophysics, Grad Sch Sci, Kyoto Univ, Japan*)

Cerebellar dysfunction is related to various psychiatric disorders, including autism-spectrum disorders and schizophrenia. However, the physiological mechanism has not been well understood. Recently, I have investigated the immune-triggered hyperexcitability in the cerebellum. Activated microglia via exposure to bacterial endotoxin lipopolysaccharide or heat-killed Gram-negative bacteria induced potentiation of the intrinsic excitability in Purkinje cells (PCs), which was suppressed by microglia-activity inhibitor or microglia-depletion. An inflammatory cytokine, tumor necrosis factor-$\alpha$ (TNF-$\alpha$) released from microglia via toll-like receptor 4 triggered this plasticity. Region-specific inflammation in the cerebellum in vivo showed depression- and autistic-like behaviors. Furthermore, both TNF-$\alpha$-inhibition and microglia-depletion reverted such behavioral abnormality. Resting-state functional MRI revealed overconnectivity between the inflamed cerebellum and prefrontal neocortical regions. Thus, immune activity in the cerebellum induces neuronal hyperexcitability and disruption of psychomotor behaviors in animals (Yamamoto et al./ *Ohtsuki, 2019 Cell Reports).

Excitability plasticity of the dendrites may be related to the selection of electro-conductivity of synaptic transmission along dendritic processes (Ohtsuki et al., 2012 Neuron; Ohtsuki and Hansel, 2018 iScience). In the later part of the talk, I will present the evidence of discordance of concurrent EPSCs between soma and dendrite of PCs. My results suggest that this discordance contributed to form a cluster of the synaptic transmission. Both of the induction of intrinsic plasticity and inhibition of SK channels reduced the number of clusters, implying that the heterogeneous excitability among PC dendrites may endow neurons with branch-specific computational power at dendrites (*Ohtsuki, *in revision*). I will also discuss the modulation of the electro-conductivity of PC dendrites by immune activation. Taken together, the plasticity of the intrinsic excitability of PCs may be associated with impaired neuronal information processing in a disease state, like as in the acute cerebellar inflammation. (COI:No)

### O04-4

#### Dendritic synapse clustering facilitates interaural time difference coding for low frequency sound

Rei Yamada, Hiroshi Kuba (*Dept Cell Physiol, Grad Sch Med, Nagoya Univ, Japan*)

Synaptic clustering at dendrites enhances nonlinearity of integration and hence has strong impact on the input-output relationship of neurons. However, how the synaptic distribution shapes specific brain functions are not well understood. Auditory coincidence detection is a neuronal basis of encoding the interaural time differences (ITDs) for sound localization. In birds, neurons in nucleus laminaris (NL) receive binaural excitatory inputs on separate dendrites and mediate auditory coincidence detection. The dendrtic structure of NL is differentiated tonotopically and neurons with low tuning frequency (low-CF neurons) have prominently long dendrites. In this study, we examined the dendritic location of synapses in low-CF neurons. We analyzed the distribution of excitatory synapses mainly using focal glutamate uncaging and found that synaptic terminals were clustered at distal dendrites. We recorded voltage responses at soma and found that responses generated at distal dendrites were strongly attenuated particularly at the strong stimulation. Model study revealed that the clustered inputs at distal dendrite generated large depolarization at the site, which decreased driving force of synaptic currents and increased shunting conductance of $K^+$ channels, then increased the extent of attenuation in an intensity-dependent manner. This sublinear summation prevented unilateral firing and increased the dynamic range of ITD coding. We concluded that the synaptic clustering at distal dendrite would be a cellular basis to accomplish the sound localization for wide intensity ranges of low frequency sound. (COI:No)

### O04-5

#### Sustained synaptic input regulates depotentiation and LTP suppression via the protein phosphorylation / dephosphorylation under the activation of NMDAR and mGluR-$IP_3$R

Jun-Ichi Goto[1], Satoshi Fujii[1], Kenya Kaneko[1], Hiroki Fujiwara[1], Yoshihiko Yamazaki[1], Katsuhiko Mikoshiba[2,3] (*[1]Department of Physiology, Yamagata University School of Medicine, Japan, [2]Laboratory for Developmental Neurobiology, Center for Brain Science, RIKEN, Japan, [3]Present address: Shanghai Institute for Advanced Immunochemical Studies, Shanghai Tech University, China*)

We examined the role for very low frequency (less than 0.1 Hz) firing of hippocampal CA1 pyramidal neurons under the *in vitro* slice preparations by examining the induction of depotentiation and LTP suppression. Electrophysiological recordings were obtained from CA1 region of guinea pig hippocampal slices. The monitoring stimuli were applied every 20 seconds to the Schaffer collateral - CA1 pyramidal cell pathway via a bipolar stimulating electrode to obtain field EPSPs and population spikes.

In the experiments of depotentiation, LTP was induced by tetanic stimulation (100 Hz, 100 pulses) and low frequency stimulation (2 Hz, 1000 pulses) was applied 30 minutes after the tetanus to induce depotentiation. The monitoring stimuli were halted for 20 minutes right after the tetanus or the low-frequency stimulation, then the induction of depotentiation was inhibited.

In the experiments of LTP suppression, low frequency stimulation (1 Hz, 1000 pulses) was applied 60 minutes before the tetanus (100 Hz, 100 pulses). This preconditioning normally suppresses the induction of LTP, while the monitoring stimuli were halted for 10 minutes right before the tetanus, the LTP suppression was almost totally blocked (normal amplitude of LTP was induced). Pharmacological studies indicated that the activation of NMDAR, mGluR, IP3R and calcineurin during not only the period of inductive stimuli but also the period of test pulse stimuli is critical for the induction of depotentiation and LTP suppression.

These results indicate that not only the inductive stimuli but also monitoring stimuli may contribute to the establishment of depotentiation and LTP suppression, probably via the regulation of postsynaptic molecular mechanisms such as calcium dependent protein phosphorylation / dephosphorylation events. The authors declare no conflict of interest regarding this presentation. (COI:No)

# Oral Session 5

## Behavior Science・Biorhythm・Neurochemistry

(March 18, 13:20〜14:20, Hall 5)

### O05-1

#### Respiratory phase transition modulates cognitive performance during the retrieval process

Nozomu Nakamura[1], Masaki Fukunaga[2], Yoshitaka Oku[1] (*[1]Dept Physiol, Hyogo Col Med, Japan, [2]Div Cereb Intg, Nat'l Inst Physiol Sci, Japan*)

Increasing evidence provides that the timing of respiration alters and shapes cognitive performance in the temporal resolution of sub-seconds. We previously demonstrated that, in healthy subjects performing a matching-to-sample visual recognition task, the accuracy was remarkably decreased when the retrieval process contained expiratory-to-inspiratory phase (EI) transition (or the onset of inspiration; Nakamura et al., PLoS ONE 13: e0204021, 2018). Meanwhile, immediately before the EI transition, the inspiratory activity in the brainstem starts and the expiratory activity has its peak; the period right before the EI transition might have another effect on performance even though the retrieval process does not contain the EI transition. Furthermore, it remains unclear about the neural basis of respiratory-dependent cognitive decline using fMRI. Here we modified the behavioral paradigm so as to be able to be extended to fMRI studies.

Our results showed that the accuracy was decreased in the retrieval process containing the EI transition, and unchanged in the retrieval process containing the inspiratory-to-expiratory phase transition. Moreover, the accuracy was significantly decreased when the retrieval process (or final choice) was completed immediately before the EI transition. We will discuss about the EI transition dependent effect on cognitive performance and its neural basis of the retrieval process (COI:No)

### O05-2

#### Dynamics of local cortical connectivity during sleep revealed by two-photon imaging and graphical modeling

Takeshi Kanda[1], Takehiro Miyazaki[1], Hideitsu Hino[2], Masashi Yanagisawa[1] (*[1]WPI-IIIS, Univ Tsukuba, Japan, [2]Institute of Statistical Mathematics*)

Sleep supports brain functions through its modulatory effects on the cerebral cortex. Whether sleep modulates functional connectivity in the cortical local circuits, however, is poorly understood. Here we investigated functional connectivity, that is, covarying activity between neurons, during spontaneous sleep/wake states using two-photon calcium imaging of identified excitatory/inhibitory neurons in the motor cortex. Functional connectivity was estimated with a statistical learning approach graphical lasso and quantified by "the probability of establishing connectivity (sparse/dense)" and "the strength of the established connectivity (weak/strong)". Local cortical connectivity was sparse in non-rapid eye movement sleep (NREM) sleep and dense in REM sleep, which was similar in both excitatory and inhibitory neurons. Sparse and dense connectivity during NREM and REM sleep could decrease and increase synaptic efficacy, respectively. Sleep deprivation is known to disrupt learning and memory. To understand the underlying mechanisms, we next examined if sleep deprivation and its recovery sleep affect local cortical connectivity. Sleep deprivation induced strong excitatory/inhibitory and dense inhibitory, but not excitatory, connectivity. Subsequent NREM sleep after sleep deprivation exhibited weak excitatory/inhibitory, sparse excitatory, and dense inhibitory connectivity. These findings indicate that local cortical connectivity becomes quite dense without NREM sleeps, which could cause saturation of learning ability such as synaptic potentiation. In addition, post-sleep deprivation NREM sleep might contribute to restoration of learning ability through depotentiation of synaptic efficacy. (COI:No)

### O05-3

#### The Protective Effect and Mechanism of CoA-Cl in acute phase after spinal cord injury

Issei Sakamoto[1, 2], Naoyuki Himi[1], Norito Hasyashi[1, 2], Emi Maruyama[1], Osamu Miyamoto[1] (*[1]Dept Physiol[2], Kawasaki Sch Med, Okayama, Japan, [2]Dept of Orthopedics Surgery, Kawasaki Sch Med, Okayama, Japan*)

Spinal cord injury (SCI) induces severe motor and sensory dysfunction. Previous studies in our laboratory showed the neuroprotective effects of COA-Cl, a novel synthesized adenosine analog, in a rat stroke model. We evaluated the neuroprotective effects of COA-Cl in acute and subacute phase of SCI in the present study. Male Sprague-Dawley rats aged 8 weeks were used. SCI model was produced at T8/9 level by using drop device (20 g x 25 mm). Rats were randomly divided into 4 groups. Acute group and subacute groups were administrated at a dose of 6 mg/kg COA-Cl in saline for 5 days from just and 4 days after SCI, respectively. As control groups, acute and subacute vehicle group were injected same volume of saline as COA-Cl groups. Motor function was evaluated using the Basso-Beattie-Bresnahan Locomotor Score (BBB score) and inclined plane test at 7, 14, 21, 28 and 31 days after SCI. The cavity volume of spinal cord was evaluated by HE stained tissue after the final motor assessment. At 7 days after SCI, apoptotic cells and pERK/ERK were also evaluated by TUNEL staining and Western blot, respectively. BBB score and inclined plane test were significantly improved in the acute group, while the subacute group did not show any improvement in motor functions. Furthermore, significant decreases in both the cavity volume and TUNEL positive cells in the spinal cord of the acute group were observed compared with the subacute group. In addition, the level of pERK/ERK was increased in acute group at 7days after SCI. Our findings suggest that COA-Cl exert neuroprotective effect via MAP kinase cascade at acute phase after SCI, resulting in the recovery of motor functions. COA-Cl could be a novel therapeutic agent for the acute phase of SCI. (COI:No)

### O05-4

#### Enriched environment affects neuronal dendrite morphology and oligodendrocyte differentiation in neonatal white matter injury model

Hideki Hida, Atsunori Hattori, Naoki Tajiri, Yoshitomo Ueda, Akimasa Ishida, Takeshi Shimizu (*Dept Neurophysiol & Brain Sci, Nagoya City Univ Grad Sch Med Sci, Japan*)

Hypoxia-ischemia (H-I) in preterm infants occasionally results in neonatal white matter injury (NWMI) associated with neurodevelopmental disabilities. Although we previously reported the improvement of disturbed motor function in NWMI by enriched environment (EE) that contains increased motor activity, social interaction and exploration, the cell dynamics in EE effect on NWMI model is still unclear. To investigate whether EE during the period of development can change microenvironment of NWMI model affecting on cell morphology and cell differentiation, a rat NWMI model made by H-I at P3 was grown in either condition of EE or standard environment (SE) from P25 to P70, followed by morphological and immunohistochemical assessments. In the NWMI model under SE, significantly complex dendrites with many branching were shown in H-I side of the cortex compared in the contralateral side. However, similar extension pattern was observed by Sholl analysis in both side of the cortex in the NWMI model under EE, which is comparable to the pattern in Sham groups. Interestingly, the dendrite complexity in NWMI-EE group was normalized to those in sham group. In addition to morphological changes, oligodendrocyte (0L) differentiation was induced by EE: significant increase of mature OL was shown on the upper area of ipsilateral motor cortex, and stronger MBP intensity was also detected in NWMI-EE group. These data suggested that EE during the period of development has significant effects on disturbed microenvironment in a rat NWMI model, affecting on neuronal dendrite morphology and OL differentiation. (COI:No)

### O05-5

#### Trapping of Mn ions in nerve vesicles by Ryanodine receptor antagonist Dantrolene for Mn-MRI method

Akio Inoue[1], Yuriko Inoue[2], Hiromitsu Ezure[2], Naruhito Ohtsuka[2], Akitoshi Inoue[3], Yoshinobu Manome[4], Koichi Shiraishi[5] (*[1]Human Brain Res. Cent., Grad Sch Med, Kyoto Univ, Japan, [2]Dep.Anat., Showa Univ.Sch.Med, [3]Med. Chem., Kansai Med. Sch, [4]Div.Mol. Cell.Biol., res.Cent.Med.sci., Jikei Uni.Med, [5]Dev. Med. Eng. Jikei Univ. Med*)

As nerve cells uptake Mn ions through Ca channel depending on nerve activity, and Mn ions induce the increase of T1 signal of MRI, Mn-MRI is used to monitor the brain activity in vivo. Then, we studied Ca and Mn ions inside the cultured Hippocampal neurons using fluorescent Ca indicator Fluo4, the fluorescence of which is increased bu Ca ions and is reduced by Mn ions. When nerve cells were activated by glutamate, Ca ions entered into nerve cells. And Mn ions also entered into nerve cells slowly. When Fluo4 was charged after Mn ion uptake, the glutamate activation induced Ca entry into the cells followed by reduction of fluorescence due to release of Mn ions from vesicles. This result indicates that Mn ions inside the cells were uptaken to the vesicles inside the cells and Mn ions were released from the vesicles by the Ca induced manner. When Mn ions charged cells were treated several timed with with glutamate, Mn ions inside the cells disappeared, and the flurescence of Fluo4 was not reduced by glutamate activation, Therefore, Mn ions inside the cells were released by nerve activation. Ca ions are considered to be released from vesicles through Ryanodine receptor, RyR, as. RyR activator, 4-Chloro-m-cresol, induced the release of Ca ions from the vesicles, while RyR antagonist, Dantrolene, reduced the increase of cellular Ca ions ater Glutamate activation. We prevented the release of Mn ions in the vesicles by Dantrolene, and measured the high quality Mn-MRI using Bruker 9.4T MRI machine with cryoprobe.

I have no COI with regard to the presentation. (COI:No)

# Oral Session 6

## Oral Physiology・Motor Function

(March 18, 13:20～14:20, Hall 6)

### O06-1

#### Simulation analysis of selective transport of cystatin D-fused HaloTag proteins to regulated and constitutive secretory pathways

Junko Fujita-Yoshigaki[1], Osamu Katsumata-Kato[1,2], Megumi Yokoyama[1,2] (*[1]Dept Physiol, Nihon Univ Dent Sch at Matsudo, Matsudo, Japan, [2]Res Inst Oral Sci, Nihon Univ Dent Sch at Matsudo, Japan*)

Exocrine glands such as salivary glands have two secretory pathways: regulated and constitutive pathways. The mechanism to separate secretory proteins to the two pathways in salivary gland cells remains to be clear. To analyse the proportion of secretory proteins transported into the two pathways in salivary acinar cells, we prepared expression system of the HaloTag proteins that fused with full-length cystatin D (fCst5H), which is one of salivary proteins, and its signal peptide sequence (ssCst5H). HaloTag is a reporter protein that is designed to form a stable covalent bond with fluorescent ligands, thus can be used for pulse-chase analysis. At 24 h after the transfection of the genes into primary culture of parotid acinar cells, HaloTag proteins were labeled with TMR ligand. After washout of unbound ligands, the cells were cultured for another 8 h. We collected culture medium and harvested cells to measure the amounts of HaloTag-fused proteins that were released to the medium and retained in the cells. After labeling samples with another HaloTag ligand, AlexaFluor 660 (AF660) ligand, samples were separated by SDS-PAGE and fluorescence intensities of the two ligands were measured. Although the ratios of released and retained TMR-labeled fCst5H and ssCst5H were comparable, the ratio of released and retained AF660-labeled fCst5H was lower than that of ssCst5H. To explain the difference between the two proteins, a mathematical model was formulated. We defined $k_c$ and 1-$k_c$ as the proportions of transport into constitutive and regulated pathways, respectively, and assumed that constitutive and regulated pathways take 60 min and 120 min to release secretory proteins. Simulation analysis revealed that $k_c$ of fCst5H was lower than that of ssCst5H, which indicates that fCst5H was transported into regulated secretory pathways more efficiently than ssCst5H. (COI:No)

### O06-2

#### Time Lapse Imaging of Mouse Ameloblasts

Masashi Shin[1], Aya Matsushima[2], Shihomi Mori[1,3], Fujio Okamoto[1], Hiroshi Kajiya[1], Hidemitsu Harada[4], John Bartlett D.[5], Koji Okabe[1] (*[1]Dept Physiol, Fukuoka Dent Col, Japan, [2]Fukuoka Dent Col, Japan, [3]Dept Oral Surg, Fukuoka Dent Col, Japan, [4]Dept Anat, Iwate Med Univ, Japan, [5]Div Biosci, Ohio Sta Univ, Col Dent, USA*)

**Purpose:** Tooth enamel is the hardest tissue in the body. Ameloblasts form enamel as they move away from the dentin enamel junction. Each ameloblast forms an enamel rod and each rod is the mineralized trail of the ameloblast as it migrates. However, how the ameloblasts move relative to each other to form the enamel rod pattern is unknown. We have generated a mouse model in which ameloblasts are labeled with a fluorescent protein (tdTomato) expressed by the amelogenin promoter. This mouse model enables us to monitor the movement of live ameloblasts and allows us to isolate stage specific ameloblasts.
**Materials & Methods:** The mandibular ameloblasts of AmelX-promoter-tdTomato (AT) mice were cleared of fluorescence by immersion in CUBIC reagents and the cleared tissue was observed by light-sheet microscopy. Time-lapse imaging was performed ex vivo by use of mandibular incisors from AT mice.
**Results:** tdTomato positive ameloblasts from AT mouse incisors were developmentally staged and were quenched of tdTomato fluorescence. Next, mandibular incisors were dissected from AT mice and live ameloblast movement was observed by time-lapse imaging ex vivo. tdTomato positive cells elongated, arranged radially, and showed collective migration toward the incisor tip.
**Conclusion:** For the first time, the dynamic movement of living mouse ameloblasts could be observed and analyzed. This method will provide new insights in understanding how ameloblast movement promotes enamel formation and calcification processes. (COI:No)

### O06-3

#### Distinct limb preference in forelimb-movement representations in the rat motor and parietal association cortices

Shogo Soma[1,2], Akiko Saiki[2], Junichi Yoshida[2], Shigeki Kato[4], Yukari Takahashi[5], Satoshi Nonomura[2,3], Yae Sugimura K.[5], Alain Rios[2,3], Masanori Kawabata[2,3], Kazuto Kobayashi[4], Fusao Kato[5], Yutaka Sakai[2], Yoshikazu Isomura[2,3] (*[1]Dept Mol Cell Physiol, Grad Sch Med, Kyoto Pref Univ Med, Japan, [2]Brain Sci Inst, Tamagawa Univ, Japan, [3]Dept Physiol and Cell Biol, Grad Sch Med Dent Sci, Tokyo Med Dent Univ, Japan, [4]Dept Mol Genet, Fukushima Med Univ, Japan, [5]Dept Neurosci, Jikei Univ Sch Med, Japan*)

The voluntary movements are expressed via motor information processing of distinct cortical areas. This processing are conducted not only in the primary and secondary motor cortices (M1 and M2) but also in the parietal association area (e.g., the posterior parietal cortex, PPC). To comprehensively understand the neuronal basis of controlling multiple limb movements, we developed a novel behavioral task to monitor movements of the right and left forelimbs separately and recorded the neuronal activity in the M1, M2, and PPC with cutting edge multi-neuronal recording techniques. We differentiated between intratelencephalic (IT) and pyramidal tract (PT) neurons in the motor cortices using optogenetically evoked spike collision in rats expressing channelrhodopsin-2 (Thy1-ChR2 transgenic rats) and found that M1-PT neurons exhibited a preferred spike activity during contralateral forelimb movement, whereas M2-IT neurons were associated with both contralateral and ipsilateral forelimb movements (bilateral spike activity). Surprisingly, the PPC neurons preferentially represented ipsilateral forelimb movements. To test the causality between neuronal activity and forelimb movements, we attempted to reproduce muscular movements by optogenetic activation of these cortical areas using Thy1-ChR2 transgenic rats. Consistent with the correlation between neuronal activity and forelimb movements, the optogenetic M1, M2 and PPC activation evoked contralaterally, bilaterally, and ipsilaterally biased forelimb movements, respectively. Finally, we examined the effects of optogenetic manipulation on task performance. In the VGAT-Cre rats, PPC or M1 inhibition by optogenetic GABA release shifted the behavioral limb preference contralaterally or ipsilaterally, respectively. In addition, weak optogenetic PPC activation, which was insufficient to evoke motor responses by itself, shifted the preference ipsilaterally; although similar M1 activation showed no effects on task performance. Our findings suggest that the motor information processing that controls forelimb movement rely on the orchestration of distinct cortical and projecting cell population. (COI:No)

### O06-4

#### Common coordinate of eye movements shared by saccadic and vestibulooculomotor systems

Mayu Takahashi, Yoshikazu Shinoda (*Dept Systems Neurophysiol, Grad Sch Med, Tokyo Medical and Dental Univ, Japan*)

Sensory signals for eye movements (visual and vestibular) are initially coded in different frames of reference but finally translated into common coordinate and share the same final common pathway, namely the same population of extraocular motoneurons. It is assumed that the saccade system uses the horizontal and vertical Cartesian coordinates, based on the findings that the lesions in the PPRF and the riMLF cause impaired horizontal and vertical saccades, respectively. Neural pathways for generating horizontal saccades from the superior colliculus (SC) were understood well, but those for generating vertical saccades have not been identified yet. This study analyzed synaptic connections from the SC to vertical ocular motoneurons and tectoreticular saccade neurons (TRNs) in the opposite SC in anesthetized cats. TRNs in the rostromedial SC activated superior rectus and inferior oblique motoneurons and TRNs in the rostrolateral SC activated inferior rectus and superior oblique motoneurons via the riMLF. These innervation patterns are similar to those in the VOR from the anterior canal and the posterior canal, respectively. Stimulation of the SC and recording postsynaptic potentials in TRNs in the opposite SC showed that the inhibitory commissural connection exists between the medial (lateral) SC representing upward (downward) oblique saccades on one side and the lateral (medial) SC representing downward (upward) oblique saccades on the other side. This pattern of reciprocal inhibition between the SCs is very similar to that seen between the bilateral vestibular nuclei in the oblique eye movements evoked from the anterior semicircular canal on one side and the posterior semicircular canal on the other side. These similarities of the motoneuronal innervation patterns and the commissural inhibitions in the VOR and saccade systems strongly suggest that both systems use the common semicircular canal coordinate. (COI:No)

### O06-5

#### Mechanism of intramuscular ectopic fat formation in sarcopenic obesity

Naoki Takada[1,2], Masaki Takasugi[1], Akiyoshi Uezumi[3], Hiroaki Nakamura[2], Naoko Ohtani[1] (*[1]Dept Pathophysiol, Grad Sch Med, Osaka City Univ, Japan, [2]Dept Orthop. Surg, Grad Sch Med, Osaka City Univ, Japan, [3]Tokyo Metropolitan Institute of Gerontology*)

Coexistence of sarcopenia and obesity, called sarcopenic obesity, is a major public health problem because its condition increases the risk of metabolic impairment and physical disability. In sarcopenic obesity, the formation of intramuscular fat has attracted attention as one of the causes of decreased skeletal muscle mass and quality. Platelet-derived growth factor receptor alpha (PDGFR $\alpha$ ) positive mesenchymal progenitors have recently been identified and studied as the origin of ectopic fat in skeletal muscle. PDGFR $\alpha$ -positive mesenchymal progenitors rapidly proliferate following muscle injury, and contribute to the differentiation of satellite cells, and promote skeletal muscle regeneration. Normally, PDGFR $\alpha$ -positive mesenchymal progenitors that proliferate during muscle regeneration eventually undergo apoptosis. On the other hand, in pathological conditions such as aging or neuromuscular diseases, it has been reported that some progenitors do not undergo apoptosis and differentiate into adipocytes. In this study, we aimed to establish a mouse model to elucidate the mechanism of obese sarcopenia. High fat diet-fed mice and genetically obese mice showed the ectopic fat cell formation, that should not be observed in normal situation, during muscle regeneration following muscle injury and loss of muscle mass. The intramuscular ectopic fat was confirmed to be derived from PDGFR $\alpha$ -positive mesenchymal progenitors by using PDGFR $\alpha$ -CreER mice for genetic lineage tracing. In addition, we compared the gene expression of PDGFR $\alpha$ -positive mesenchymal progenitors before and after muscle injury between high-fat-diet and normal diet-fed mice by using RNA-sequencing analysis. The analysis of the results indicated that the interaction with the extracellular matrix might be important for the differentiation of PDGFR $\alpha$ -positive mesenchymal progenitors into adipocytes. In this meeting, we will show the details of the results and the ideas about the mechanism of the ectopic fat formation in skeletal muscle. (COI:No)

# Oral Session 7

## Cell Physiology・Molecular Physiology 1

(March 18, 13:20～14:20, Hall 7)

### O07-1

#### Pathophysiological Roles of an Actin-Binding Protein Moesin in Primary Mouse Microglia

Tomonori Okazaki[1], Kotoku Kawaguchi[1], Takashi Nakahari[2], Shinji Asano[1] (*[1]Dept Mol Physiol, Col Pharm Sci, Ritsumeikan Univ, Japan, [2]Res Unit for Epithelial Physiol, Res Org of Sci and Tech, Ritsumeikan Univ, Japan*)

**Introduction:** Microglia (MG) are immune cells in the central nervous system. In resting state, MG with highly branched processes survey around cells whereas they retract their processes, migrate into injury sites and remove neuronal debris by phagocytosis in response to injury. They also secret many inflammatory cytokines and neuroprotective cytokines. Here, we focus on the roles of an actin-binding protein moesin which is involved in morphological changes through regulation of small GTPase; Rac, Rho and Cdc42 by using primary MG prepared from moesin-knockout (Msn-KO) mice.
**Methods:** Primary MG were prepared from whole brain of newborn mice by the shaker method. We examined phenotypes accompanying morphological changes and reorganization of the actin cytoskeleton. Process retraction induced by LPS stimulation was observed under microscope. Phagocytosis was evaluated by counting the numbers of FBS-coated fluorescence beads incorporated in the cells in absence or presence of UDP. Migration was evaluated by counting the number of migrated cells toward ADP by using Boyden chamber.
**Results:** The Msn-KO MG showed decreased rate of process retraction stimulated by LPS compared with the WT MG. The numbers of FBS-coated fluorescence beads were significantly decreased in the Msn-KO MG in the UDP-stimulated phagocytosis. The numbers of migrated cells were significantly decreased in the Msn-KO MG in the ADP-stimulated migration assay. The Msn-KO MG treated ADP or UDP showed decreased numbers of ruffle membrane compared with the WT MG. However, the Msn-KO MG retained their activity to synthesize and secrete tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and nitric oxide (NO) in response to LPS.
**Conclusions:** Moesin is not indispensable but involved in processes retraction, phagocytosis and migration accompanying with actin-remodeling and membrane ruffling. However, moesin is not involved in TNF-$\alpha$ nor NO secretion. (COI:No)

### O07-2

#### Glutamate modulates activities of microglia through JAK/STAT/IRF-dependent signaling pathway

Haruna Takeda, Kazuya Miyanishi, Kota Kanehisa, Kanta Mikami, Choudhury ME, Hajime Yano, Junya Tanaka (*Dept Mol Cel Physiol, Grad Sch Med, Ehime Univ, Japan*)

Glutamate is the most abundant excitatory amino acid neurotransmitter in the CNS. There have been literatures describing that glutamate affects morphology and functions of microglia, suggesting a possibility that glutamate mediates the interactions between neurons and microglia. Yet, the effect of glutamate on microglia has not been fully elucidated. Glutamate upregulated mRNA levels for several activation markers, such as F4/80, CX3CR1, MMP-2, Cathepsin S, MerTK and MFG-E8 in primary rat microglia culture. We investigated the effects of glutamate on phagocytic activity of microglia by incubating microglia cells (BV2; a murine microglial cell line and primary rat microglia) with fluorescent beads. The internalized bead fluorescence was evaluated by FACS. Glutamate enhanced uptake of florescent beads or microglial phagocytosis. When incubated with glutamate, enhanced phosphorylation of STAT1, STAT3 and expression of IRF1 protein was observed. Furthermore, siRNA-induced knockdown of JAK1, STAT1, or IRF1 abolished the stimulating effects of glutamate on mRNA expression that encoded CX3CR1, Cathepsin S, MFG-E8 and so on. However, glutamate did not affect the expression of mRNA for inflammatory mediators, such as IL-1$\beta$, IL-6. Thus it is suggested that glutamate-induced activation of JAK-STAT pathway is responsible for phagocytosis rather than proinflammatory reactions. We further examined the effects of compounds that suppress the JAK-STAT pathway on the actions of glutamate. Tofacitinib, a JAK1/3 inhibitor, suppressed glutamate-induced phosphorylation of STAT1, STAT3 and expression of IRF1. Taken together, glutamate may activate JAK1 in microglial cells, causing activation of the downstream signaling pathway. These results suggest a possibility that microglia change their activities in response to activities of glutamatergic excitatory neurons. It is necessary to determine whether the effects of glutamates are mediated by glutamate receptors expressed by microglia. (COI:No)

### O07-3

#### Chloride intracellular channel protein 2 prevents distant metastasis of malignant cells

Akihiro Umakoshi, Junya Tanaka (*Dept Physiol, Grad Sch Med, Ehime Univ, Japan*)

We have established a distant metastasis model using immunocompetent Wistar rats by transplanting rat glioma cell line C6 cells in the back of neonatals. The transplanted cells formed a visible tumor mass in the back within two weeks, and the cells metastasized into the lung by 5 weeks. The most transplanted rats died by 7 weeks due to the distant metastasis. Using this model, we transplanted C6 cells expressing enhanced green fluorescence protein (EGFP). Five weeks later, tumor masses were dissected from the back and the lung. Cells with EGFP fluorescence were isolated from the both tumor masses using a cell sorter 5 weeks after transplantation. Cells from the two tumor masses were transplanted again into the back of neonatal rats. Then, the cells from the lung tumor metastasized more frequently and earlier than the other ones. Total RNA purified from the cells and analyzed by RNA-seq. Consequently, chloride intracellular channel protein 2 (CLIC2) was found to be more highly expressed by the cells from the back tumor than those from the lung one. Then, C6 cells expressing CLIC2 at a higher level were established (C6-CLIC2 cells). C6-CLIC2 cells were not different from the control C6 cells in terms of proliferation and migration, while their invasive activity through Matrigel containing various extracellular matrix proteins was much weaker than the control cells. When transplanted into the back, the metastasis by C6-CLIC2 cells were much suppressed and the survival periods of animals were much prolonged. The mechanisms underlying the effect of CLIC2 expression are still to be elucidated, CLIC2 may strengthen the cell-cell contacts by increasing expression adhesion molecules, leading to prevention of intravasation of cells or hematogenous spread. (COI:No)

### O07-4

#### kinase activity of TRPM7 involvement in the regulation of lipid transfer

Chiaki Katagiri[1, 3], Taku Kaitsuka[2], Chigusa Shimizu O.[4], Kazuhiko Tomizawa[2], Chitoshi Takayama[4], Masayuki Matsushita[1] (*[1]Dept Molecul. Cell physiology, Grad Sch Med, Univ of Ryukyus, Japan, [2]Dept Molecul. Cell physiology, Grad Sch Med, Kumamoto Univ, Japan, [3]Dept Neurosurgery, Grad Sch Med, Univ of Ryukyus, Japan, [4]Dept Molecul anatomy, Grad Sch Med, Univ of Ryukyus, Japan*)

TRPM7, a member of TRP family of cation channels, is a unique bifunctional protein containing an ion channel and a C-terminus kinase domain. However, the physiological functions of TRPM7 and its kinase activity in vivo remain elusive. We generated kinase-inactive mutant mice and analyzed their phenotype. TRPM7 mutant mice show normal ion channel activity without noticeable kinase function in cells isolated from adult animals. These mice have normal body weight, food intake and general locomotor activity. Screening of serum clinical parameters showed that serum $Ca^{2+}$ and $Mg^{2+}$ levels were not altered, but serum triglyceride and total cholesterol were significantly decreased. TRPM7 kinase deficient mice fed high-fat diet increased the accumulation of fat and sclerosis of the liver compared to wild type mice. Our findings define TRPM7 kinase activity as a key cell signaling component that regulates lipid homeostasis in the liver. (COI:No)

### O07-5

#### Temporal regulation of IL-1$\beta$ induced JNK signaling dynamics

Taichiro Tomida, Kimitaka Yamaguchi, Masanori Ito, Yoshinori Mikami, Daisuke Ohshima, Satomi Adachi-Akahane (*Dept Physiol, Fac Med, Sch Med, Toho Univ, Japan*)

**Background:** JNK (c-jun N-terminal Kinase) is a multi-functional kinase mediating stress and inflammatory response involved in cell death, proliferation, and cytokine production in pathologies of a wide variety of inflammatory disorders. It has been known that the duration of JNK signaling determines the specificity of downstream cell functions that leads to either cell-death or survival, but how the dynamics of JNK are regulated in cells remains largely unknown.
**Aim:** We aimed to understand how inflammatory cytokine IL-1$\beta$ regulates JNK signaling dynamics in living cells and unveil the impact of JNK dynamics on downstream gene expression that determines cell fates.
**Methods:** JNK reporter based on FRET was engineered to analyze JNK activation dynamics in living HeLa cells. Periodic pulsatile IL-1$\beta$ stimuli to cells at variable frequencies were employed to analyze frequency-response of JNK activation, which is subjected to systems-analysis. Gene expression profiles were obtained by conducting microarray and qRT-PCR analyses.
**Results:** We succeeded in the quantitative evaluation of JNK dynamics at single cell resolution upon IL-1$\beta$ stimulation by FRET imaging. The frequency-response of JNK exhibited a typical negative-feedback-type regulation. We found that a phosphatase expressed downstream of p38 MAPK functions as a negative regulator. Such regulation forces JNK to be transiently activated even when cytokines are given continuously. In contrast, JNK activity was repeatedly re-activated when cytokines were repetitively applied as short pulsatile stimuli at interval >2hr. Consistently, we found that the expression of some JNK dependent genes are efficiently induced by the repetitive short stimuli depending on the frequency rather than the total duration of cytokine exposure.
**Conclusion:** Our novel approach combining FRET imaging and systems-analysis revealed a key mechanism that governs JNK dynamics in living cells. Such regulation may contribute to avoid excessive inflammatory response while properly inducing adequate response through gene expression in a temporally regulated manner. (COI:No)

# Oral Session 8

## Pathophysiology

(March 18, 13:20～14:20, Hall 8)

### O08-1

#### Preliminarily study on functional impairment of swallowing induce by scent stimulation

Yusuke Takatsuru[1], Shunichi Motegi[2], Keita Yonemochi[3], Noriaki Hattori[1] (*[1]Johmoh hospital, [2]Department of Radiology, Josai Clinic, [3]Gunma Prefectural College of Health Sciences*)

In recently, number of elder people is extremely increase in Japan compared to other country. Especially, number of "Kohki-kohreisha (over 75 years old people)" is increasing and patient who suffered with geriatric syndrome is most popular and problem case in clinical field. One of the geriatric syndromes, disfunction of swallowing is most difficult condition because of it inducing many diseases due to low nutrition. We actually have several supportive meals, but they sometimes induce farther health problem (Takatsuru et al., 2019. https://doi.org/10.6890/IJGE.201909_13(3).0017). Thus, to improve the swallowing function is important to keep elder people in healthy and good quality-of-life condition. We previously found the patient who improved the swallowing function by preference scent stimulation (data not published). In this study, we planning to confirm the effect of preference scent on brain function including swallowing. Healthy volunteer answered the questionnaire: preference scent (maple, peppermint, apple, coconuts, and Darjeeling tea. Both under blind and open manner question), food-intake-behavior (i.g., custom on meal intake, lively on food-intake such as visiting famous restaurant and cook the new dish after watching cooking program on TV), and knowledge-on-food (i.g., knowledge on seasonal food, recipe of cooking). Part of them also perform functional magnetic resonance imaging (fMRI) test with preference scent stimulation. We found that scent detection score was decreased dependent on age. On the other hands, preference scent could detect without depend on age. This result indicated that preference scent potentially suitable for stimulant on brain function without age. We also found that group of high score on the scent detection showed high score on the knowledge-on-food questionnaire. We next analysis the result of fMRI to confirm the relationship between the brain area which activated by preference scent and result of questionnaire on food-intake-behavior/knowledge-on-food. (COI:No)

### O08-2

#### Cellular mechanism of psychiatric symptom and behavioral change induced by thyroid dysfunction

Mami Noda, Tetsushi Niiyama, Kosuke Aoi (*Lab Pathophysiol, Grad Sch Phar Sci, Kyushu Univ, Japan*)

Thyroid hormones (THs) are essential for the development and function of the central nervous system (CNS). In the CNS, circulating thyroxine (T4) crosses blood-brain barrier via specific transporters and is taken up to astrocytes, becomes L-tri-iodothyronine (T3), an active form of TH, by type 2 de-iodinase (D2). T3 is released to the brain parenchyma from astrocytes (glioendocrine system). In adult CNS, both hypo- and hyper-thyroidism, the prevalence in female being 10 times higher than that in male, may affect psychological condition and potentially increase the risk of cognitive impairment and neurodegeneration including Alzheimers disease (AD). We have reported that non-genomic effects of T3 on microglial functions and its signaling and sex- and age-dependent effects of THs on glial morphology in the mouse brains of hypo- and hyper-thyroidism. Behavioral changes also showed sex-dependence. The significant effect of THs on synaptic spine in male and females hyperthyroidism was analyzed as well. These results may help to understand physiological and pathophysiological functions of THs in the CNS and how hypo- and hyper-thyroidism affect psychological condition and cognition. (COI:No)

### O08-3

#### Mitochondrial antidotal machinery against N6-isopentenyladenosine is essential for sustaining glioma-initiating cells

Atsushi Fujimura[1,2], Takahiro Yamamoto[3], Fanyan Wei[4], Kazuhito Tomizawa[4] (*[1]Dept Physiol, Grad Sch Med, Dent, Pharma, Okayama Univ, Japan, [2]Neutron Therapy Research Center, Okayama Univ, Japan, [3]Dept Neurosurgery, Faculty Life Sci, Kumamoto Univ, Japan, [4]Dept Mol Physiol, Faculty Life Sci, Kumamoto Univ, Japan*)

Mitochondria are pivot points in various biological phenomena. In glioma, mitochondria control energy production and cellular metabolism to adapt to tumor microenvironment such as hypoxia, and thus contribute to sustaining cell viability and stemness, which further resulted in poor prognosis. Mitochondrial function is sustained by genomic DNA-encoded proteins as well as mitochondrial DNA-encoded proteins, whose expressions are tightly regulated by intra-mitochondrial translational machinery. Previously, we identified CDK5RAP1 as one of the key regulators of intra-mitochondrial translation in skeletal and cardiac muscles. CDK5RAP1 converts N6-isopentenyladenosine to 2-methylthio-N6-isopentenyladenosine at the anticodon-loop of the several mitochondrial tRNAs, and consequently potentiates the accuracy and efficiency of the intra-mitochondrial translation. Therefore, our starting hypothesis in this study was: CDK5RAP1 regulated cell viability and stemness by controlling intra-mitochondrial translation. To demonstrate this, we performed loss-of- and gain-of-function study with patient-derived glioma-initiating cell (GICs). As we expected, CDK5RAP1 was required to sustain GIC-related traits such as self-renewal capacity, tumor-propagating potential, undifferentiated markers. However, to our great surprise, these phenomena were independent from CDK5RAP1 regulation of intra-mitochondrial translation. In GICs, loss-of-CDK5RAP1 did not induce the translational deficiency and respiratory deficit, but attenuate the GIC-related characteristics. We found that CDK5RAP1 deficit resulted in the accumulation of N6-isopentenyladenosine, which acted as cytotoxic agent in GICs. Mechanistically, the accumulated N6-isopentenyladenosine triggered autophagic programs as validated by autophagosome formation, AMPK activation, and mTOR signaling pathway inhibition, which further resulted in loss-of-GIC related traits. By conversion of N6-isopentenyladenosine to 2-methylthio-N6-isopentenyladenosine in mitochondria, CDK5RAP1 abrogated the antitumor effect of N6-isopentenyladenosine. Moreover, we demonstrated that hypoxic condition activated CDK5RAP1 to promote amelioration of the tumor-suppressive effect of N6-isopentenyladenosine. Our work shows that GICs utilize the detoxification mechanism to abrogate endogenous N6-isopentenyladenosine, and indicated that the mechanism might be a good target to develop anti-tumor strategy. (COI:No)

### O08-4

#### Segmental or unilateral hyperhidrosis accompanied by anhidrosis in another area may be compensatory: estimated based on the mechanism of the similar efferent phase of the physiological skin pressure−sweating reflex

Youko Inukai, Satoshi Iwase, Motohiko Satou (*Dept Physiol, Sch Med, Aichi Med Univ, Japan*)

**Background:** Segmental or unilateral hyperhidrosis is a form of the sweating disorder. Most cases with localized unilateral hyperhidrosis are considered idiopathic. Some of the secondary localized segmental or unilateral hyperhidrosis are caused by direct overactivity of sympathetic neurons. While, the other cases of these disorders which are accompanied by anhidrosis or hypohidrosis in another area may be compensatory; it is likely caused by underlying lesions in the areas with anhidrosis, but the precise mechanism remains unclear. This hyperhidrosis often occurs on contralateral to the same dermatomes with anhidrosis, and ipsilateral rostral and caudal dermatomes adjacent to those of anhidrosis. The similar efferent phase of the physiological
**skin pressure−sweating reflex:** might be associated with these mechanisms. This reflex is primarily due to inhibition of ipsilateral sweating by unilateral skin pressure; secondarily sweating is increased on the contralateral same dermatome and ipsilateral adjacent other dermatomes.
**Objective:** Pathophysiology of unilateral or segmental hyperhidrosis was estimated based on experimental findings of the skin pressure−sweating reflex.
**Study 1:** Microneurography indicated that unilateral skin pressure reduced the amplitude of sudomotor nerve activities ipsilaterally and increased this contralaterally. But those synchronisms were not changed.
**Study 2:** Studies using the ventilated capsule method during heating showed that sweating decreased on the upper body and increased on the lower body by pressure on the skin of bilateral back by lying supine. Central sudomotor sympathetic outflow in response to body temperature was simultaneously hyperactivated, indicating that sweating is enhanced in compensation to maintain a constant total sweating rate.
**Conclusion:** Segmental or unilateral hyperhidrosis in segments not directly affected may be compensatory. (COI:No)

### O08-5

#### Contributions of mitochondrial dysfunction to baroreflex dysregulation in hepatic encephalopathy

Ching-Yi Tsai (*Institute for Translational Research in Biomedicine, Chang Gung Memorial Hospital, Kaohsiung, Taiwan*)

Acute hepatic failure is a devastating consequence of hepatotoxic liver injury that can lead to the development of neurological complication called hepatic encephalopathy (HE) and is associated with 50-90% mortality without liver transplantation. Despite a highly challenging clinical problem, systematic evaluations of the cellular mechanisms of HE-related mortality are still lacking. Clinical studies showed that the degree of baroreflex dysregulation is related to the severity of HE. At the cellular level, oxidative stress resulting from mitochondrial dysfunction in brainstem nuclei in the baroreflex circuit, including nucleus tractus solitarii (NTS) and rostral ventrolateral medulla (RVLM) are known to result baroreflex dysregulation. This study investigated whether the same mechanism underlies the high mortality in HE. An azoxymethane (AOM)-induced acute liver failure model of HE employing C57BL/6 mouse was used. Diffusion tensor imaging (DTI) of the brainstem was performed, together with blood pressure, heart rate and indices of baroreflex recorded by radiotelemetry. Animals died within 20-36 h after AOM (100 $\mu$g/g, ip) injection. DTI further revealed that the connectivity between the NTS and nucleus ambiguus (NA), the origin of the vagal innervation of the heart, was progressively disrupted though sustained, concurrent with impaired but persistent cardiac vagal baroreflex. On the other hand, the connectivity between NTS and RVLM was progressively disrupted until its disappearance, coincidental with the abolition of baroreflex-mediated sympathetic vasomotor tone that signifies brain death clinically. Results from JC-1 staining of the ventrolateral medulla showed a decrease of mitochondrial membrane potential during HE, alongside an elevation of reactive oxygen species and necrotic cell death and a reduction in ATP level in the NTS or RVLM. Our results suggested that impairment of baroreflex takes place during the progression towards death in HE, and mitochondrial dysfunction induces bioenergetic failure, oxidative stress and necrotic cell death are the culprits. (COI:No)

# Oral Session 9

## Ion Channel・Receptor 1

(March 18, 13:20〜14:20, Hall 9)

## O09-1

### Regulation of phosphoinositide distribution by voltage-sensing phosphatase in mouse spermatozoa

Takafumi Kawai, Yasushi Okamura (*Grad. Sch. of Med., Osaka Univ, Japan*)

Voltage-sensing phosphatase (VSP) shows phosphoinositides phosphatase activity that is coupled to membrane potential. In the present study, we report that VSP is activated in mice sperm flagellum and generates unique subcellular distribution pattern of PtdIns(4, 5)P2. We already found that VSP protein is expressed in matured sperm and the VSP convert PtdIns(4, 5)P2 into PtdIns(4)P by MS/MS analysis. VSP-deficient sperm show severe defect in their motility after capacitation, but not before capacitation, resulting in significant reduction in success rate of fertilization in in vitro fertilization experiment. This was because abnormal Ca2+ influx occur in tail of VSP-KO sperm. The abnormal Ca2+ signal appears to be caused by the enhanced K+ conductance. Electrophysiological analysis indicates that K+ current that would be derived from Slo3, sperm specific K+ channel is enhanced in VSP-/- sperm. However, the high PtdIns(4, 5)P2 affinity of Slo3 may not account for the altered K+ channel activity in sperm flagellum, assuming that PtdIns(4, 5)P2 level in sperm flagellum is similar to other cell types, such as neurons. Most interestingly, freeze-fracture electron microscopy analysis indicates that normal sperm have much less PtdIns(4, 5)P2 in the principal piece than in the midpiece of the flagellum, and this polarized PtdIns(4, 5)P2 distribution disappeared in VSP-deficient sperm. Thus, VSP appears to optimize PtdIns(4, 5)P2 distribution of the principal piece. Here we discuss how such specialized PtdIns(4, 5)P2 distribution regulate the Slo3 activity. (COI:No)

## O09-2

### Involvement of Pannexin in the trigeminal ganglion in trigeminal neuropathic pain

Ryoko Kurisu[1, 2, 3], Masamichi Shinoda[3], Yoko Yamazaki[2], Masahiko Shimada[1, 2], Koichi Iwata[3] (*[1]Dept Orofacial pain management, Grad Sch Med Dent Sci, Tokyo Med Dent Univ, Tokyo, Japan, [2]Dept Orofacial Pain Clinic, Dent Hosp, Tokyo Med Dent Univ, Tokyo, Japan, [3]Dept Physiol, Nihon Univ Sch Dent, Tokyo, Japan*)

Trigeminal nerve injury occasionally causes orofacial neuropathic pain, which is difficult to diagnose and treat. It is essential to know the mechanisms underlying orofacial neuropathic pain to develop the appropriate treatment of these patients. The pannexin (Panx) which facts predominantly as a transmembrane channel connecting the intracellular and extracellular space is reported to be involved in the regulation of the excitability of ganglionic cells. In this study, we examined the involvement of Panx in the trigeminal ganglion (TG) in orofacial neuropathic pain following partial infraorbital nerve ligation (pIONL) in rats.

Male Sprague-Dawley rats (200g-260g) were used in this study. We established pIONL model rats by tight ligation with 6-0 silk of one-third thickness of the left infraorbital nerve under deep anesthesia. Mechanical head-withdrawal threshold (MHWT) of left whisker pad skin was measured using von Frey filament every other day before and 14 days after pIONL. On day 14, Panx expression in TG was also assessed immunohistochemistry. Moreover, MHWTs were measured after pIONL with the continuous intra-TG administration of pannexin inhibitor (10Panx; 1 microl, 100 mM).

The MHWT of the whisker pad skin ipsilateral to pIONL was significantly decreased on day 1 after pIONL, and the decrease of MHWT persisted until day 14. Panx was expressed in TG neurons and satellite glial cells in TG, and the number of Panx-immunoreactive TG neurons innervating the whisker pad skin was increased on day 14. The intra-TG 10Panx administration significantly recovered the decreased MHWT after pIONL.

The present findings suggest that Panx expressed in TG neurons and satellite glial cells in TG is involved in orofacial mechanical allodynia associated with pIONL. (COI:No)

## O09-3

### Molecular mechanism of ion selectivity through the KcsA K+ channel

Takashi Sumikama[1], Kenichiro Mita[2], Shigetoshi Oiki[2] (*[1]NanoLSI, Kanazawa Univ, [2]Facult Med Sci, Univ Fukui*)

Selectivity of the $K^+$ channels is critical to maintain the resting potential. Conventional explanation for the selectivity is usually based on the difference in affinity: the affinity of $K^+$ to the $K^+$ channels is higher than that of $Na^+$, so $K^+$ preferentially permeates the $K^+$ channels compared with $Na^+$. However, our recent study (Sumikama and Oiki, JPS 2019) showed that the affinity of $K^+$ to the KcsA $K^+$ channel is low ($K_d$ of a third $K^+$ ion to the channel holding two ions is approximately 50 mM), posing a question for the explanation. Here, we examined $Na^+$ permeation through the KcsA $K^+$ channel by molecular dynamics simulations and electrophysiological measurements. The measured $Na^+$ current through the $K^+$ channel at 350 mV was 1.2 pA (by experiments at 2 M) and 0.14 pA (by simulations at 1 M), and the conductance ratio ($\gamma_K/\gamma_{Na}$) was 78.3 (by experiments) and 38.5 (by simulations). That is, the selectivity is not so high as expected, and $Na^+$ ions really but slowly permeate the $K^+$ channel. The analysis on the ion trajectories in the simulations indicates that the difference in the free energy barriers at both intracellular and extracellular entrances causes the difference in the permeation rates between $K^+$ and $Na^+$. Especially, the barrier at the extracellular side is high for $Na^+$, blocking the influx of $Na^+$. Thus, it was revealed that the selectivity is not originated by affinity, but by kinetics. (COI:No)

## O09-4

### Effects of removing mucus on $HCO_3^-$ and $CO_2$ transport in guinea-pig pancreatic duct

Libin Liu (*Dept Human Nutrition, Grad Sch Med, Nagoya Univ, Japan*)

In the exocrine pancreas, goblet cells and some duct cells produce mucin. When mucin is secreted out of the cells and hydrated, mucin forms mucus. Mucus layer is thought to protect ductal cells from the harmful contents of the pancreatic juice. However, the physiological role of mucus in pancreatic duct is largely unknown.

we examined the effects of removing mucus on $HCO_3^-$ and $CO_2$ transport in guinea-pig pancreatic duct cell. Interlobular pancreatic duct segments were isolated and the lumen was microperfused separately from the bath. We tried to remove luminal mucus by applying N-acetyl-L-cysteine (acetylcysteine, 6 or 12 mg/ml) for 6 min to the luminal perfusate. Intracellular pH (pHi) was measured in duct cells loaded with BCECF.

When ducts were luminally perfused with 125mM $HCO_3^-$-5% $CO_2$ solution, luminal application of acetylcysteine caused ($p<0.05$) pHi increase by $0.14 \pm 0.02$ (n=6). To examine $HCO_3^-$ and $CO_2$ transport across the apical membrane, bath and lumen were first perfused with $HCO_3^-$- $CO_2$-free Hepes-buffered solution and the luminal perfusate was switched to 125mM $HCO_3^-$-5% $CO_2$ solution. pHi rapidly decreased due to $CO_2$ diffusion into the cells and cells remained acidified. Initial pHi decline was ~26% faster ($p<0.05$) after luminal treatment with acetylcysteine (n=6).

To examine $HCO_3^-$ permeability of the apical membrane, duct cells were stimulated by forskolin (1 $\mu$M) and depolarized by applying 70mM $K^+$ to the bath. When the luminal perfusate was switched to 125mM $HCO_3^-$-5% $CO_2$ solution, pHi transiently dropped and then increased due to $HCO_3^-$ entry into the cells via CFTR. The rate of pHi increase was ~43% faster ($p<0.05$) after treatment with acetylcysteine (n=6).

These data suggest that removal of luminal mucus enhances $HCO_3^-$ and $CO_2$ permeability of the apical membrane in guinea-pig pancreatic duct cell. (COI:No)

## O09-5

### Secretory reflex pathway of short chain fatty acids in the rat distal colon

Daiki Harata[1], Shinji Asano[2], Atsukazu Kuwahara[3], Yuko Kuwahara[3], Ikuo Kato[4], Toshio Inui[5], Yoshinori Marunaka[3, 6, 7] (*[1]Dept Mol Physiol, Grad Sch Life Sci, Ritsumeikan Univ, Japan, [2]Dept Mol Physiol, Col Pharm Sci, Ritsumeikan Univ, Japan, [3]Res Unit for Epithelial Physiol, Res Org of Sci and Tech, Ritsumeikan Univ, Japan, [4]Dept Med Biochem,Col Pharm Sci, Kobe Pharm Univ, Japan, [5]Saisei Mirai Clinics, Japan, [6]Res Inst for Clin Physiol, Kyoto Ind Health Assoc, Japan, [7]Dept Mol Cell Physiol, Grad Sch Med Sci, Kyoto Pref Univ Med, Japan*)

Propionate, a short chain fatty acid, induces biphasic ion transport, $K^+$ secretion followed by $Cl^-/HCO_3^-$ secretion in rat distal colon. These secretory responses are mediated via activation of an enteric neural reflex circuit constituted by afferent, inter- and efferent neurons. Previous study showed that efferent neurons stimulated by propionate is cholinergic, however, afferent neurons stimulated by propionate still remained uncertain. Propionate stimulates free fatty acid receptor 2 and 3 (FFA2/3) expressed in enteroendocrine L cells. Stimulation of these receptors leads to release of neuropeptides such as GLP-2 from L cells. As gastrointestinal tract is innervated by intrinsic and extrinsic neurons, we hypothesized that released GLP-2 stimulates intrinsic and extrinsic afferent neurons, and the stimulated afferent neurons release tachykinins. Therefore, in the present study, the contribution of these neuropeptides to afferent neuronal reflex pathway stimulated by propionate, and in addition, the involvement of intrinsic and extrinsic neurons to the neural reflex circuit were studied. We prepared muscle-stripped mucosa-submucosa preparation of rat distal colon and measured short-circuit current (Isc) as an indicator of net ion transport using Ussing chamber. Pretreatment with tetrodotoxin (TTX) or a blocker of Nav1.8, TTX-insensitive voltage-dependent $Na^+$ channel, A803467, alone had no effect on ion transport stimulated by propionate, however, simultaneous pretreatment with TTX and A803467 suppressed $Cl^-/HCO_3^-$ secretion, suggesting a synergistic contribution of intrinsic and extrinsic neurons within the neural reflex circuit. GLP-2(3-33) and GLP-2(11-33), GLP-2 antagonists, suppressed $K^+$ secretion stimulated by propionate. Pretreatment with CP96345, an NK1 antagonist, suppressed $Cl^-/HCO_3^-$secretion, and on the contrary, osanetant, an NK3 antagonist, enhanced $Cl^-/HCO_3^-$ secretion stimulated by propionate. In conclusion, it is considered that propionate-induced colonic ion transport is mediated via activation of neural reflex circuit constituted by intrinsic and extrinsic neurons and the afferent pathway is mediated via GLP-2 and tachykinins. (COI:No)

# Oral Session 10

## Autonomic Nervous・Endocrinology

(March 18, 13:20〜14:20, Hall 10)

### O10-1

#### Nicotinic cholinergic modulation of olfactory bulb function

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

Olfaction is known to severely decline in the preclinical stage of Alzheimer's disease, in addition to the age-related impairment. The olfactory bulb, the first olfactory center, receives cholinergic basal forebrain input, as does the neocortex. Our previous study showed that nicotinic acetylcholine receptors (nAChRs) in the brain play a crucial role in vasodilation in the neocortex that is induced by basal forebrain cholinergic activation or nicotine injection. In this study, we examined cholinergic regulation of blood flow in the olfactory bulb, using adult rats anesthetized with urethane.

Focal chemical stimulation by microinjection of L-glutamate into the horizontal limb of the diagonal band of Broca (HDB) in the basal forebrain which is the main source of cholinergic input to the olfactory bulb increased extracellular ACh release in the ipsilateral olfactory bulb. When the regional cerebral blood flow was measured using laser speckle contrast imaging, the focal chemical stimulation of the HDB did not significantly alter the blood flow in the olfactory bulb, while increases were observed in the neocortex.

Next, we investigated the effect of nAChR activation on the odor-induced olfactory bulb blood flow response. Odor stimulation increased olfactory bulb blood flow, without changes in neocortical blood flow and systemic blood pressure. Intravenous injection of nicotine (30 $\mu$g/kg), a nAChR agonist, significantly augmented the odor-induced increase response of olfactory bulb blood flow. This nicotine-induced augmentation was negated by $\alpha 4\beta 2$-preferring nAChR antagonist.

We conclude that basal forebrain cholinergic activation increases extracellular ACh release in the olfactory bulb. The increased ACh does not produce vasodilation at rest in the olfactory bulb. Activation of nAChRs in the brain potentiates the odor-induced blood flow response in the olfactory bulb. (COI:No)

### O10-2

#### Effect of ionotropic glutamate receptor blockade in rat RVLM on sympathomotor activation by mesencephalic locomotor neurons projecting to the RVLM

Satoshi Koba, Nao Kumada, Tatsuo Watanabe (*Div Integr Physiol, Tottori Univ Fac Med, Japan*)

Our preliminary data showed that mesencephalic locomotor neurons sending axonal projections to the rostral ventrolateral medulla (RVLM) (MLR-RVLM pathway) are capable of eliciting sympathomotor activation in rats, suggesting that the MLR-RVLM pathway is a part of brain circuitries for central command. This study examined the effect of ionotropic glutamate receptor blockade in rat RVLM on sympathomotor activation by the MLR-RVLM pathway. Male rats received microinjections bilaterally in the RVLM with a retrograde adeno-associated virus that encoded a channelrhodopsin with green fluorescence protein. Under anesthesia, 473 nm wavelength laser illumination of the MLR after administration in the RVLM with a cocktail of AP5 and CNQX, NMDA and AMPA glutamate receptor blockers, respectively, elicited 29% less ($P<0.05$) renal sympathoexcitation than that after saline administration ($n = 6$). Likewise, ventral root excitation in response to laser illumination of the MLR seen after saline administration in the RVLM of decerebrate, nonanesthetized rats was suppressed to a 65% extent ($P<0.05$) by glutamate receptor blockers administered preliminarily ($n = 6$). These results indicate that ionotropic glutamate receptor blockade in rat RVLM reduced sympathomotor activation caused by excitation of the MLR-RVLM pathway. Glutamatergic neurons in the MLR-RVLM pathway likely play a significant role in generating central command function. (COI:No)

### O10-3

#### Protective role of orphan nuclear hormone receptor COUP-TFII in acute kidney injury

Sumiyasu Ishii, Noriyuki Koibuchi (*Dept. Integr. Physiol, Gunma Univ. Grad. Sch. Med, Japan*)

**Background and Aims:** An orphan nuclear hormone receptor chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) plays essential roles in organogenesis of embryos. Recently COUP-TFII is also implicated in several diseases in adults. Here we focus on the role of COUP-TFII in cisplatin-induced acute kidney injury (AKI).

**Methods:** Tissue distribution of COUP-TFII was analyzed by RT-qPCR. Male tamoxifen-inducible COUP-TFII-knockout mice or control mice were intraperitoneally treated with 30 mg/kg body weight of cisplatin at 12 weeks old to induce AKI. The kidney samples were subject to morphological studies, TUNEL assay, immunohistochemistry and RT-qPCR. Serum levels of creatinine, blood urea nitrogen and tumor necrosis factor alpha (TNF-$\alpha$) were measured. Depletion of COUP-TFII using siRNA followed by RT-qPCR and chromatin immunoprecipitation were done in VA-13 cells.

**Results:** COUP-TFII was the most abundantly expressed in the kidney among organs. Administration of cisplatin induced a more severe AKI in adult COUP-TFII-knockout mice. An increase in dead cells in both proximal tubules and thick ascending limb of Henle's loop (TAL) was observed in the knockout mouse kidney. The expression levels of COUP-TFII decreased in the TAL by cisplatin administration. There was no difference in the expression levels of transporter mRNAs responsible for cellular cisplatin uptake between control and knockout mouse kidney. COUP-TFII-knockout mice and COUP-TFII-depleted cells exhibited an elevation in TNF-$\alpha$ levels, suggesting the involvement of the TNF-$\alpha$ pathway. Chromatin immunoprecipitation showed that COUP-TFII was enriched in the potential binding site, suggesting that COUP-TFII might directly suppress the TNF-$\alpha$ gene at transcriptional level.

**Conclusions:** These results indicate the involvement of COUP-TFII in the pathophysiology of AKI and COUP-TFII may be a potential therapeutic target for AKI. (COI:No)

### O10-4

#### Effects of *Aquilaria subintegra* leaves extract on learning and memory of ovariectomized rats

Khachanan Inthiwong, Onrawee Khongsombat, Pornnarin Taepavarapruk (*Department of Physiology, Faculty of Medical Science, Naresuan University*)

*Aquilaria subintegra* (AS) is one of Aquilaria species which able to produce agarwood. Most of the agarwood is processed into oil which is used in perfumes and in the production of traditional medicine as anti-asthma, anti-stress, painkiller, and many other diseases. AS leaf extracts are also known to possess antipyretic, laxative and antimicrobial activities. Data from an *in vitro* study suggested the potential of AS to treat neurological related disease such as Alzheimer's disease (AD) due to its ability to inhibit acetylcholinesterase and its anti-oxidant properties.

**Aim of the study:** This study aimed to investigate the effect of AS leaves extract on learning and memory of ovariectomized rats that has been widely used as an animal model of AD.

**Materials and method:** Female Sprague Dawley rats were subjected to bilateral ovariectomy (OVX). Following a full recovery, AS leaves extracts (10, 100, 1000 mg/kg B.W.) were orally administered for 60 days. The negative control group received RO water and the positive control group received donepezil. Novel object recognition (NOR) and Morris water maze (MWM) tests were employed to evaluate their cognitive functions. The levels of superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), as well as acetylcholinesterase (AChE) activities in the hippocampus were measured.

**Results:** OVX rats received AS leaves extract for 60 days showed a significant improvement in both object recognition and spatial memories when compared to the OVX control group. Treatment with AS leaves extract also induced significant increases in the activities of SOD, CAT and AChE and reduced the level of MDA in the hippocampus when compared to the OVX group.

**Conclusions:** AS leaves extracts could improve cognitive and memory impairments in female OVX rats by the antioxidant activities and acetylcholinesterase inhibition of the leaves, therefore AS may be useful in reducing the risk of AD in postmenopausal women. (COI:No)

### O10-5

#### Elevated Blood Pressure induced by High-Salt-Diet Consumption Caused Negative Calcium Balance and Bone Loss in Rats

Sirion Aksornthong[1,2], Wacharaporn Tiyasatkulkovit[2,3], Nattapon Panupinthu[1,2], Narattaphol Charoenphandhu[1,2,4,5] (*[1]Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand, [2]Center of Calcium and Bone research, Faculty of Science, Mahidol University, Bangkok 10400, Thailand, [3]Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand, [4]Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom 73170, Thailand, [5]The Academy of Science, The Royal Society of Thailand, Bangkok 10300, Thailand*)

With the change in dietary consumption in the modern lifestyle especially the exceeding amount of salt, high-salt-diet (HSD)-induced hypertension will become worldwide problem. Beside the risk of cardiovascular diseases, clinical studies demonstrated low bone mass and high risk for bone fracture in hypertensive patients. However, investigation of association between hypertension and bone loss is still controversy. In vitro study also showed direct effect of high NaCl environment on enhancing osteoclast differentiation and bone resorption. Nevertheless, data from in vivo model of HSD-induced hypertensive model have been limited. Therefore, this study aims to investigate whole-body calcium balance and bone micro-structure in HSD-induced hypertensive rats. The male Sprague-Dawley rats received HSD (HSD; 8% NaCl) or normal diet (Control; 0.8% of NaCl) for 5 months. As expected, rats that received HSD showed significant elevation of blood pressure with the onset at first month and remained hypertensive throughout the study (SBP 132.22 ± 18.5 mmHg and DBP 115.04 ± 2.96 mmHg) with marked hypertrophy of cardiac myocytes shown by histology. Next, calcium balance study was performed monthly. We found drastically calcium loss via urine and feces, together with decreased fractional calcium absorption leading to negative calcium balance, especially 3-month after HSD induction. Micro-computed tomography analysis demonstrated significant decreased trabecular bone mineral density (BMD) of tibia and the increasing medullary area of tibia diaphysis at 5 month of HSD induction. Consistent with the negative calcium balance and impaired bone microstructures, bone histomorphometric analyses showed a significant decrease in osteoblast number and osteoid volume in rats receiving HSD for 5 months. These findings suggest HSD-induced hypertension caused negative calcium balance and reduction in BMD via impaired bone formation. (COI:No)

# Oral Session 11

## Ion Channel・Receptor 2

(March 19, 11:00～12:00, Hall 4)

### O11-1

#### Aluminum ion blocks hTRPV1 and hTRPA1 activities

Fumitaka Fujita[1,2,3,4], Yoshiro Suzuki[7], Kaori Saito[1,2,3], Masayuki Takaishi[1,4], Fumihiro Okada[1,2], Makoto Tominaga[4,5], Ken J. Ishii[3,6] (*[1]Mandom corporation, [2]Advanced Cosmetic Science, Graduate School of Pharmaceutical Sciences, Osaka University, [3]Center for Vaccine and Adjuvant Research (CVAR), National Institutes of Biomedical Innovation, Health and Nutrition (NBIOHN), [4]Thermal Biology Group, Exploratory Research Center on Life and Living Systems National Institutes of Natural Sciences, [5]Department of Physiological Sciences, SOKENDAI, [6]The Institute of Medical Science, The University of Tokyo, [7]Iwate Medical University*)

Aluminum is utilized in human life from ancient time. Aluminum potassium sulfate has been used as an antiperspirant from the Roman period. In modern medicine, aluminum compounds are formulated in several vaccines as adjuvants which enhance immune responses at vaccination. On the other hand, aluminum pottassium sulfate shows the anti-inflammatory effect in Japanese spa. Putting all this together, aluminum compounds have several effects on human body including opposite one. We hypothesize that aluminum shows different effects in the different states. For clarification of the aluminum effect, we focused on thermo-sensitive Transient Receptor Potential (TRP) channels because we already found alumminum ion activates hTRPM4. TRP channels expressed in sensory neuron were evaluated whether aluminum ion affects their activities. In calcium imaging experiments, aluminum ion blocked hTRPV1 and hTRPA1 activities, but not hTRPM8, hTRPV3 or hTRPV4 ones. $IC_{50}$ values of aluminum potassium sulfate at pH 5.0 on hTRPV1 and hTRPA1 were 103 $\mu$M and 246 $\mu$M, respectively. Futhermore, aluminum potassium sulfate inhibited hTRPV1 and hTRPA1 even at the neutral pH 7.4. These results indicated that aluminum plays a role as an analgesic agent at the ionic state, suggesting aluminum compounda used as vaccine adjuvant might have the analgesic role at vaccination. (COI:No)

### O11-2

#### A theoretical approach to investigating the arrhythmogenecity of TRPM4 channel overactivation via CaMKII

Yaopeng Hu[1], Daniela Ross Kaschitza[2], Maria Essers[2], Prakash Arullampalam[2], Yuanyuan Cui[1], Hugues Abriel[2], Ryuji Inoue[1] (*[1]Dept Physiol, Sch Med, Fukuoka Univ, Japan, [2]Biochem Mol Med, Bern Univ, Switzerland*)

Recent evidence indicates that enhanced activity of TRPM4 contributes to acquired arrhythmic changes under stressed conditions. It is reported that TRPM4 channel activity is strongly modified by $Ca^{2+}$/calmodulin, but the mechanism underlying it remains unknown. We therefore investigated it by noting a potential significance of CaMKII-mediated TRPM4 channel overactivation under disrupted $Ca^{2+}$ homeostasis by both cellular experiments and numerical simulations.

All experiments were performed in TRPM4 expressing HEK293 cells and HL-1 cardiomyocytes. The possible protein-protein interaction was tested by the Duolink immunoassay. In order to quantitatively evaluate how CaMKII modifies TRPM4 activation, the average relationships of steady state open probability potential and time constant for voltage-dependent activation versus membrane potential at different $[Ca^{2+}]_i$ were reconstructed. Numerical descriptions for TRPM4 channel gating both with and without treatment of a CaMKII inhibitor KN-62 were obtained as well. In TRPM4 expressed HL-1 cells, the incidence of early afterdepolarizations (EADs) was increased after incubation with AngII which also activated the CaMKII signaling. Both TRPM4 channel blocker 9-phenanthrol and KN-62 could suppress this arrhythmic change. Mathematical simulation indicated that a prominent increase in TRPM4 current density induced EADs at the late repolarization phase. Intriguingly, incorporation of altered rate constants after treatment with KN-62 could significantly alleviate these abnormal excitations.

In this study, an important mechanism underlying CaMKII-mediated TRPM4 channel regulation under stressed condition was displayed. The simulation described here could facilitate our understanding about electrophysiological changes induced in remodeled cardiomyocytes. (COI:No)

### O11-3

#### Functional Coupling of Metabolic Sensors, TRPM2 and Sirtuin

Makiko Kashio[1], Makoto Tominaga[2,3], Satoru Masubuchi[1] (*[1]Dep Physiol, Aichi Med Univ, Aichi, Japan, [2]Div Cell Signal, NIPS, Okazaki, Aichi, Japan, [3]Thermal Biology Group, ExCELLS, Okazaki, Aichi, Japan*)

TRPM2 is a thermosensitive non-selective cation channel expressed in various tissues including brain, spleen and pancreatic *b*-cells where TRPM2 is continuously affected by core body temperature. TRPM2 activity at body temperature could be regulated along with metabolic state because its activity is affected by intracellular factors reflecting cellular metabolism such as $NAD^+$ metabolites, $Ca^{2+}$ and redox signal. Therefore, TRPM2 is suggested to function as body temperature/metabolic sensor.

Additional metabolic sensors, sirtuins, are a group of $NAD^+$-dependent enzymes to regulate energy homeostasis, circadian rhythm and longevity, etc. Because sirtuins generate o-acetyl ADPR (OAADPR), a TRPM2 activator, along with its enzymatic activity, we are interested in functional coupling of TRPM2 and sirtuins, especially in SIRT1 which is present in cytoplasm to interact with TRPM2 in plasma membrane.

Intracellular $Ca^{2+}$-imaging of TRPM2/SIRT1-expressing HEK293T cells has revealed that a SIRT1 activator leads TRPM2 activation and intracellular $Ca^{2+}$-elevation. Moreover, immunoprecipitation studies have clarified physical interaction between TRPM2 and SIRT1 which is enhanced by phorbol ester (PMA)-treatment. Analysis of phosphoproteins using phos-tag SDS-PAGE has shown that PMA-treatment also increases TRPM2 phosphorylation, suggesting an PKC-mediated effective coupling of TRPM2 and SIRT1.

We'd like to discuss possible regulatory mechanisms and physiological functions of metabolic sensors, TRPM2 and SIRT1. (COI:No)

### O11-4

#### Deletion of Trpm4 alters the function and expression of NaV1.5 channel in mouse cardiac myocytes

Prakash Arullampalm, Jean-Sébastien Rougier, Lijo Cherian, Hugues Abriel (*University of Bern, Institute of Biochemistry and Molecular Medicine*)

Transient receptor potential melastatin member 4 (TRPM4) encodes a Ca+-activated non-selective cation channel and expressed in several tissues including the heart. Pathogenic mutants in the TRPM4 gene have been reported in patients with inherited cardiac diseases including conduction blocks and Brugada syndrome but its role in cardiac channelopathy is still unclear. To study the functional consequences on cardiac electrical activity due to the deletion of Trpm4 (Trpm4-/-) in mice, we performed perforated patch-clamp and immunohistochemistry studies on isolated atrial and ventricular cardiac myocytes. We demonstrate that Trpm4 is expressed in atrial and ventricular cardiac myocytes and Trpm4 deletion reduces the peak Na+ current in the myocytes.

Furthermore, we performed ex-vivo pseudo-surface (sECG) and intracardiac (iECG) electrocardiogram on a Langendorff setup. The heart is perfused retrogradely by the coronary arteries. We used two silver electrodes on the heart for pseudo surface ECG (sECG) and inserted an octapolar intracardiac catheter via the right atrium to the apex of right ventricle to record ex-vivo intracardiac ECG (iECG).

In iECGs four out of five Trpm4-/- mice hearts show intraventricular conduction delay to reach impulse from apex of right ventricle to 3mm proximal part of right ventricle. We did not see any delay in Trpm4 +/+ mouse heart. Moreover, we challenged those hearts with mexiletine (Sodium channel blocker). Surprisingly Trpm4-/- mice hearts are less sensitive to mexiletine.

This study provides the first evidence that Trpm4 shows directly impact on Na+ current in mouse cardiac myocytes and Trpm4 prevents sensitivity of mexiletine. In the further, we will apply our newly identified mouse-specific Trpm4 blockers and some other INa blockers to understand more about the role of Trpm4 regulating Na+ current in mouse heart. (COI:No)

### O11-5

#### BARP is a key regulator of the localization and dynamics of voltage-dependent $Ca^{2+}$ channel complexes

Akito Nakao[1], Yoshihiro Matsunaga[1], Takafumi Miki[2], Yasuo Mori[1] (*[1]Dept Synthetic Chemistry and Biological Chemistry, Grad Sch Engineering, Kyoto Univ, Japan, [2]Grad Sch Brain Science, Doshisha University, Japan*)

The dynamics and localization of voltage-dependent $Ca^{2+}$ channel (VDCC) complexes are crucial for neuronal excitability, neurotransmitter release, and $Ca^{2+}$-induced gene regulation. Recently, VDCC beta-anchoring and -regulatory protein (BARP) was identified as a novel regulator of VDCC activity via the interaction with VDCC $\beta$ subunits. However, its molecular mechanism and physiological significance have been largely unknown. In this study, we demonstrated that BARP reduced the surface expression level of $\alpha_1$ subunit, resulting in the decrease of current density of VDCC. Fluorescence recovery after photobleaching assay revealed that BARP accelerated the fluorescence recovery of $\alpha_1$ and $\beta$ subunits, presumably because BARP increases the dynamics of VDCC subunits for the switching and targeting of VDCC complexes. In cultured hippocampal neurons, BARP targeted $\alpha_1$ and $\beta$ subunits to axonal and dendritic protrusions. Electron microscopic study showed that hippocampal neurons of BARP knockout mice had a lower density of spines and shorter-necked spines. These results suggest that BARP is a key regulator of the localization and dynamics of VDCC complexes for various physiological events such as proper formation of dendritic spines. (COI:No)

# Oral Session 12

## Sensory Function

(March 19, 11:00～12:00, Hall 5)

### O12-1

#### Effect of light isoflurane anesthesia on orientation selectivity in mouse superior colliculus

Masatoshi Kasai[1], Tadashi Isa[1,2] ([1]*Dept Neurosci, Grad Schl Med, Kyoto Univ, Japan,* [2]*ASHBi, Kyoto Univ, Japan*)

Superior colliculus (SC) is a brain stem center which plays key roles in generating spatial attention and mediating the signal for sensory-motor translation. Recent studies wide filed or two-photon calcium imaging techniques reported that the superficial layer of the SC (sSC) has orientation columnar like functional structures (Feinberg & Meister, 2014) or the global spatial segregation of direction selective (DS) or orientation selective (OS) cells in accordance with its retinotopic locations (Malmazet et al., 2018). But it remains unclear what kind of rules regulate this functional structure.

Here we investigated effects of anesthesia on DS and OS in the mice sSC. We delivered GCaMP6f calcium sensor to the sSC neurons by injecting Adeno-associated virus (AAV) vector (AAV1-hSyn-GCaMP6f) after making a small suction in caudo-medial part of the cortex. To achieve long-term optical access to the SC, we implanted a small glass cube on the sSC. The Neuronal population responses were recorded by in vivo two photon calcium imaging. Direction and orientation selectivities of the sSC neurons were tested by applying moving bar stimuli (width: 3–5 deg, speed: 10–20 deg/s, 12 directions) and calculating global direction or orientation selectivity index.

First, we test the effect of isoflurane. At normal maintenance level of isoflurane administration (2–3%) caused strong response suppression as seen in the sensory cortices. But during the combination of light isoflurane (0.8–1%) with chlorprothixene change the preferred orientation or direction in similar angles. On the other hand, the ketamine-xylazine anesthesia did not change the selectivity at the population level. These results suggest that the DS and OS could modulated by GABAergic inhibitory network, which is activated by isoflurane. NMDA receptor mediated excitatory network, which suppressed by ketamine, have little effect on DS and OS modulation in the sSC. (COI:No)

### O12-2

#### Inhibitory effect of oxytocin on the regulation of colorectal motility via descending pain inhibitory pathways in hyperalgesic rats

Kazuhiro Horii[1], Hiroki Shimaoka[1], Yuuki Horii[1], Takahiko Shiina[1], Yasutake Shimizu[1,2] ([1]*Lab Vet Physiol, Unit Grad Sch Vet Sci, Gifu Univ, Gifu, Japan,* [2]*Center for Highly Advanced Integration of Nano and Life Sciences, Gifu University (G-CHAIN)*)

We have previously demonstrated activation of the descending pain inhibitory pathways by noxious stimuli in the colorectum enhances colorectal motility in rats. It has been known that the descending pain inhibitory pathways are modulated during development of hyperalgesia. However, it remains unclear whether the development of hyperalgesia also impacts on the pathways regulating colorectal motility. Thus, the purpose of this study is to examine whether the regulation of colorectal motility via the descending pain inhibitory pathways are modulated in an experimental model of hyperalgesia. To induce hyperalgesia in rats, we injected complete Freund's adjuvant into left hind paw two days before experiments. Anesthetized rats were cannulated in the colorectum, and changes of intraluminal pressure and expelled volume were measured. Intrathecal administration was performed by a spinal cannula located at the L6-S1 level of the spinal cord. Administration of a noxious stimulant capsaicin into the colorectal lumen enhanced colorectal motility in male rats. However, in hyperalgesic male rats, capsaicin administration failed to enhance colorectal motility. In the case of female rats, administration of capsaicin had no effects on colorectal motility not only in hyperalgesic but also intact conditions. When an inhibitor of oxytocin receptor was intrathecally injected into the spinal cord, capsaicin enhanced colorectal motility both in hyperalgesic male and female rats. The oxytocinergic inhibitor had no effects on colorectal motility in intact males and females. Our findings suggest that oxytocin in the lumbosacral spinal cord negatively impacts the colorectal motility in both sexes. Considering that the inhibitory effects of oxytocin on the colorectal motility are manifested exclusively in the hyperalgesic condition, oxytocinergic descending pathway may be activated only when potent painful stimuli are applied. The oxytocinergic pathways would be involved in pathological mechanisms of defecation disorder associated with inflammatory diseases such as colonic and bladder inflammation. (COI:No)

### O12-3

#### Presynaptic feedback system of oxytocin-ergic neurons in the hypothalamus of adjuvant arthritic rats

Takanori Matsuura[1,2], Teruaki Fujitani[1], Makoto Kawasaki[1], Hitoshi Suzuki[1], Haruki Nishimura[1,2], Kazuhiko Baba[1,2], Yoichi Ueta[2], Akinori Sakai[1] ([1]*Department of Orthopedics, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan,* [2]*Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan*)

**Background and aims:** Oxytocin (OXT) is a neurohypophysial hormone that is synthesized in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus. Recently, it has been suggested that OXT plays a role in sensory modulation. It was shown that OXT was up-regulated by acute and chronic nociception. However, the up-regulated mechanism is little known.

**Methods**: In the present study, we examined excitatory postsynaptic currents (EPSCs) in OXT-ergic neurons in the PVN after chronic inflammation from an adjuvant arthritis (AA) model rat using the whole cell patch clamp recording. We used transgenic rats that expressed OXT and the monomeric red fluorescent protein 1 (mRFP1) fusion gene to visualize the OXT-ergic neurons. To induce AA, OXT-mRFP1 transgenic rats were intracutaneously injected heat-killed Mycobacterium butyricum (1 mg/rat) in paraffin liquid at the base of their tails. Furthermore, the feedback system of synthesized OXT was also examined by OXT receptor antagonist (L-368,899).

**Results:** We found that the frequency of miniature (m)EPSCs and spontaneous (s)EPSCs in OXT-mRFP1 neurons in the PVN has significantly increased in AA rats. Further, L-368,899 dose-dependently even more increased the frequency of sEPSCs and mEPSCs in the neurons in AA rats. In bath application of GABAA receptor antagonist (picrotoxin) and cannabinoid receptor 1 antagonist (AM 251), L-368,899 still increased the frequency of mEPSCs. However, under the bath application of NOS inhibitor (L-NAME), L-368,899 did not change the frequency in AA rats.

**Conclusions:** It is suggested that OXT-ergic neurons are up-regulated by increasing glutamate release in the AA rats, and up-regulated OXT neurons have a feedback system by released OXT. It is possible that NO but not GABA may contributes to the feedback system of up-regulated OXT neurons. (COI:No)

### O12-4

#### Activation of astrocytes in the trigeminal nucleus associated with ocular neuropathic pain in a rat dry eye model

Yuto Tei[1,2], Yoshinori Mikami[1], Masanori Ito[1], Taichiro Tomida[1], Daisuke Ohshima[1], Yuichi Hori[2], Satomi Adachi-Akahane[1] ([1]*Department of Physiology, Faculty of Medicine, Toho University,* [2]*Department of Ophthalmology, School of Medicine, Toho University*)

Chronic dry eye symptom is associated with not only corneal epithelial disorder but also ocular neuropathic pain. However, its molecular mechanism remains to be elucidated. The aim of this study was to elucidate the molecular mechanism underlying the progression of dry eye-induced ocular pain by focusing on the involvement of glial cells in the trigeminal nucleus. The unilateral dry eye was induced by left extraorbital and intraorbital lacrimal gland excision (LGE) in 6-week-old SD rats. For sham surgery, incisions were made and glands were partially exposed on the right side. Spontaneous tear volume was reduced from the day after LGE. One week after the LGE operation, the spontaneous eye blink rates and the corneal epithelial injury score evaluated by fluorescein staining were significantly higher on the LGE side than the sham side. The sensitivity of the cornea to hypertonic stimulation was significantly higher on the LGE side compared with the sham side, indicating that the LGE-induced corneal epithelial defects progressed to neuropathy. Based on these results, we performed experiments at two weeks and eight weeks after LGE as acute phase and chronic phase of dry eye-induced neuropathic pain, respectively. The glial cell activation in subnucleus interpolalis/subnucleus caudalis of the trigeminal nucleus (Vi/Vc) was analyzed by immunostaining. The density of Nissl staining and immunostaining indicated that the density of neurons was significantly lower on the LGE side than the sham side. The intensity of GFAP-immunopositive regions in the Vi/Vc on the LGE side was enhanced in both two and eight weeks after surgery. These results suggest that reactive astrocytes are already increased in the acute phase in the trigeminal nucleus of the LGE side. The dry eye-induced corneal epithelial damage may develop ocular neuropathic pain by activation of astrocytes. (COI:No)

### O12-5

#### Widespread inhibition and modulation in the mouse olfactory sensory neurons in vivo

Takeshi Imai[1,2], Shigenori Inagaki[1], Ryo Iwata[2] ([1]*Dept Dev Neurophysiol, Grad Sch Med, Kyushu Univ, Japan,* [2]*RIKEN CDB*)

Odor recognition starts from olfactory sensory neurons (OSNs), but this process is yet to be fully understood in physiological conditions in vivo. Here, we performed two-photon calcium imaging of mouse olfactory sensory neurons in vivo and found that odors produce not only excitatory, but also inhibitory responses at their axon terminals. Robust inhibitory responses at OSN axon terminals remained in two independent mutant mice, in which all possible sources of presynaptic lateral inhibition were eliminated. Therefore, we examined the responses in the olfactory epithelium in vivo, and found widespread inhibitory responses at the level of OSN somata. Moreover, responses to odor mixtures demonstrated extensive mutual modulation (both suppression and enhancement) in OSNs. An in vitro assay demonstrated that some odorants act as inverse agonists to some odorant receptors. The bidirectional nature of OSN responses may be useful for robust odor coding under noisy sensory environment. (COI:No)

# Oral Session 13

## Muscle Physiology

(March 19, 11:00～12:00, Hall 6)

### O13-1
#### Roles of extracellular vesicles secreted from mouse muscle cells in muscle-bone interactions

Yoshimasa Takafuji, Kohei Tatsumi, Masayoshi Ishida, Naoyuki Kawao, Kiyotaka Okada, Hiroshi Kaji (*Dept Physiol and Regene, Med, Kindai Univ, Japan*)

**Purpose:** The interactions between muscle and bone via humoral factors have been recently noted. It is known that various muscle-derived humoral factors (myokines), such as myostatin, irisin, follistatin and osteoglycin, affect bone metabolism. Extracellular vesicles (EVs) contain various proteins or micro RNA, and play a vital role in physiological and pathophysiological processes, such as cell proliferation, differentiation and metastasis, by transferring their contents to the distant tissues. Recently, several evidences suggest that EVs are crucial mediators which is responsible for the cell-cell communications in bone tissue. However, roles of EVs in the muscle-bone interactions have still remained unknown. In the present study, we investigated the effects of EVs secreted from mouse C2C12 myoblasts (Myo-EVs) on osteoclasts and osteoblasts in mice. In addition, the effects of loading of mechanical stress or treatment with endocrine factors on C2C12 myoblasts on the bioactivity of Myo-EVs were investigated.
**Results:** Myo-EVs were isolated from the conditioned medium of C2C12 cells by ultracentrifugation. Myo-EVs suppressed osteoclast formation and mRNA expressions of osteoclast-related genes induced by receptor activator of nuclear factor $\kappa$B ligand (RANKL) in mouse bone marrow cells. Moreover, Myo-EVs suppressed oxygen consumption and mRNA expressions of mitochondrial biogenesis markers induced by RANKL in mouse bone marrow cells. Fluid flow shear stress on C2C12 myoblasts enhanced the suppressive effects of Myo-EVs on osteoclast formation and the mitochondrial biogenesis, but treatment with 1, 25-dihydroxyvitamin-$D_3$ or dexamethasone on C2C12 myoblasts didn't enhanced these effects of Myo-EVs. On the other hands, Myo-EVs didn't affect the phenotypes and the mitochondrial biogenesis of mouse primary osteoblasts.
**Conclusion:** The present study first showed that Myo-EVs suppress osteoclast formation and the mitochondrial energy metabolism in mouse bone marrow cells. EVs secreted from skeletal muscles might be a crucial mediator for the muscle-bone interactions. (COI:No)

### O13-2
#### Is N-terminal titin fragment a new urinary biomarker to detect muscle atrophy?

Jun Tanihata, Susumu Minamisawa (*Div. Aerospace Med, Dept Cell Physiol, The Jikei Univ, Japan*)

**Introduction:** Many studies have attempted to determine the associations between blood and urine biomarkers and muscle damage and atrophy. However, poor correlations between the changes in biomarker levels and the magnitude of muscle damage and atrophy have been reported. Recently, the N-terminal fragment of titin(N-titin), a giant sarcomeric protein that is involved in muscular passive tension and viscoelasticity, has been reported to detect muscle damage in patients with skeletal muscle dystrophy and in healthy volunteers with endurance exercise.
**Purpose:** To investigate whether urinary N-titin is changed during the muscle atrophic period and whether its increase reflects muscle atrophy.
**Methods and Results:** C57BL/6J mice (male, 10 weeks of age) were used of this study. Urine samples were obtained after sciatic nerve removal surgery (denervation) to induce muscle atrophy. We measured the urinary levels of N-titin with a highly sensitive ELISA system. 11 days after denervation, dissection was performed, and the limb muscle was weighed. The weight of 4 types of limb muscles were significantly decreased at 11 days after denervation. Although skeletal muscle weights were predominantly reduced and mRNA levels of Atrogin-1 and MuRF-1 such as markers of muscle atrophy, were increased, there was no significant change in urinary titin level. On the other hand, mRNA levels of titin in soleus muscles, a typical slow twitch muscle, were increased by muscle atrophy.
**Conclusion:** These results suggest that it is difficult to identify the muscle atrophy with urinary titin levels. We would like to identify a urinary biomarker that detect muscle atrophy other than titin. (COI:No)

### O13-3
#### Increase in phospholamban content in mouse skeletal muscle after denervation

Tsutomu Nakada[1,2], Masatoshi Komatsu[2], Mitsuhiko Yamada[2] ([1]*Dept Inst Analysis, RCSAS, Shinshu Univ, Japan,* [2]*Dept Mol Pharmacol, Shinshu Univ Sch Med, Japan*)

It is well-known that denervation of motor nerves induces atrophy and decreases contractile force of the skeletal muscle. However, it is not completely understood how denervation alters calcium handling in the skeletal muscle. We investigated the effect of denervation on the expression and function of proteins involved in calcium handling. Two weeks after denervation of the right sciatic nerve in mice, we observed a significant decrease in mass and cross-sectional area of the ipsilateral tibialis anterior (TA) and flexor digitorum brevis (FDB) muscles. Also, we observed a significant decrease in the specific tetanus contractile force in the ipsilateral TA muscle. Calcium imaging of the ipsilateral FDB showed that the peak twitch and tetanus calcium concentrations were significantly decreased due to a decrease in calcium content of the sarcoplasmic reticulum (SR). Denervation reduced the maximum rate of sarcoplasmic/endoplasmic calcium ATPase (SERCA) activity. Interestingly, the amount of phospholamban (PLN), but not its transcripts, was increased in the ipsilateral vs. contralateral side after denervation, suggesting that denervation impairs constitutive regulation of PLN. Immunohistochemical analysis revealed increased PLN in all major fiber types in TA with IIx fibers showing a threefold higher expression than the contralateral side. These results suggest that the abnormal increase in PLN in the ipsilateral fast-twitch fibers may be involved in decreased SERCA activity, SR calcium content, peak calcium transients, and contractile forces of denervated muscles. (COI:No)

### O13-4
#### The role of calpain activation and vimentin cleavage in the signal transduction of abnormal vascular smooth muscle contraction meditated by SPC/Fyn/ROK pathway

Hiroko Kishi, Qian Lu, Tomoka Morita, Ying Zhang, Bochao Lyu, Min Zhang, Nan Li, Minhui Xu, Sei Kobayashi (*Dept Mol Cell Physiol, Grad Sch Med, Yamaguchi Univ, Japan*)

Rho-kinase (ROK)-mediated $Ca^{2+}$-sensitization of vascular smooth muscle (VSM) plays a critical role in abnormal VSM contractions such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC)/Fyn/ROK pathway as a novel signaling pathway for abnormal VSM contraction. As possible downstream targets of Fyn tyrosine kinase, we identified vimentin by focused proteomics in which tyrosine-phosphorylated proteins were concentrated using 4G10 antibody and identified by tandem mass spectrometry. Interestingly, western blot analysis revealed that SPC induced limited proteolysis of vimentin not only in human coronary artery smooth muscle cells (CASMCs) but also in VSM strips of the porcine coronary artery. Since vimentin is reported as the target of calpain, we examined the possible involvement of calpain. In CASMCs, SPC increased calpain activity, which was blocked by PD150606, a calpain inhibitor. Furthermore, PD150606 inhibited the SPC-induced abnormal VSM contraction both in porcine coronary and mouse basilar arteries. PD150606 also inhibited U46619-induced VSM contraction in porcine coronary arteries. PD150606 did not inhibit $Ca^{2+}$-dependent contraction induced by high K+ depolarization. Mass spectrometric analysis showed that calpain cleaved vimentin at its N-terminus. These findings suggest the possible involvement of calpain in the signal transduction of $Ca^{2+}$-sensitization of VSM contraction induced by SPC and U46619. (COI:No)

### O13-5
#### Role of paxillin in sphingosylphosphorylcholine (SPC)-induced $Ca^{2+}$-sensitization contraction of vascular smooth muscle

Ying Zhang[1], Bochao Lyu[1], Tomoka Morita[1], Dan Cui[2], Hiroko Kishi[1], Min Zhang[1], Qian Lu[1], Nan Li[1], Eiji Ikeda[2], Sei Kobayashi[1] ([1]*Dept Mol Cell Physiol, Grad Sch Med, Yamaguchi Univ, Ube, Japan,* [2]*Dept Pathol, Grad Sch Med, Yamaguchi Univ, Ube, Japan*)

Rho-kinase (ROK)-induced $Ca^{2+}$-sensitization contraction of vascular smooth muscle (VSM) is a major cause of cardiovascular and cerebrovascular vasospasm. We previously demonstrated that sphingosylphosphorylcholine (SPC) induced this abnormal contraction via a SPC/Fyn/ROK pathway. However, Fyn could not directly activate ROK in vitro/in vivo experiments. The combination of pull down assay and MALDI-TOF mass spectrometry made us identify paxillin as the possible downstream molecule of Fyn. Until now, no direct evidence is provided to prove paxillin involved in SPC-induced contraction. In the present study, we examined the role of paxillin in SPC-induced $Ca^{2+}$-sensitization contraction of VSM. First, paxillin knockdown inhibited SPC-induced contraction in human coronary artery smooth muscle cells. Then we used Cre-loxP system to generate tamoxifen-inducible and smooth muscle-specific paxillin knockout mouse and showed that targeted deletion of paxillin in VSM inhibits SPC-induced $Ca^{2+}$-sensitization of VSM contraction in thoracic aorta. Paxillin knockout also inhibited SPC-induced myosin light chain phosphorylation. These results indicate that paxillin plays an important role in SPC-induced $Ca^{2+}$-sensitization contraction of VSM. (COI:No)

# Oral Session 14

## Others

(March 19, 11:00～12:00, Hall 7)

### O14-1

#### Aerobic capacity is associated with blood volume or total hemoglobin mass in female runners

Kazunobu Okazaki[1,2], Nanae Shirai[3], Yasushi Enomoto[3] (*[1]Res Ctr for Urban Health & Sports, Osaka City Univ, Osaka, Japan, [2]Dept of Environ Physiol for Exerc, Osaka City Univ Grad Sch of Med, Osaka, Japan, [3]ARIHHP, Univ of Tsukuba, Tsukuba, Japan*)

High aerobic capacity (maximal oxygen uptake, $VO_{2max}$; and lactate threshold, LT) and low oxygen cost ($O_2$ cost) are the fundamental physiological factors for the high endurance athletic performance. It has been well known that $VO_{2max}$ is closely correlated with blood volume (BV) and total hemoglobin mass (THb) in endurance athletes. On the other hand, the increased venous return to the heart with the increased BV would enhance muscle blood flow and therefore reduce lactic acid accumulation and enhance LT during exercise. Therefore, it is thought that LT is also associated with BV and THb in endurance athletes. In this study, we investigated that relationships between aerobic capacity and blood volumes or blood constituents in female runners. In fifteen female collegiate middle and long distance runners, BV, THb (optimized CO rebreathing method) and hematological values were determined at rest, and $VO_{2max}$, velocity at $VO_{2max}$, LT (velocity at 3 mM/L of lactic acid concentration in blood), and $O_2$ cost were determined during a graded maximal treadmill running test. We found that there are significant correlations between $VO_{2max}$ and BV ($R = 0.72$, $P = 0.002$) or THb ($R = 0.75$, $P = 0.001$), and between LT and BV ($R = 0.66$, $P = 0.008$) or THb ($R = 0.71$, $P = 0.003$). On the other hand, there were no significant correlations between $O_2$ cost and BV or THb, and between $VO_{2max}$ or LT and hematological parameters (red blood cell counts, hemoglobin concentration, hematocrit). These results suggest that the increase in BV and THb are required to achieve high endurance athletic performance through the increase in $VO_{2max}$ and LT in female runners. (COI:No)

### O14-2

#### Evaluation of physical activity in wheelchair propulsion by one hand one foot using ActiGraph accelerator

Tomoyuki Ito (*Dept Rehab Med, Tanabe Memorial Hosipital, Kyo-Tanabe, Japan*)

ActiGraph is a clock-type accelerator that is attached to the wrist or trunk and can measure physical activity during walking from three-dimensional acceleration. Recently, physical activity during propulsion of a wheelchair by bilateral arms has been reported using ActiGraph attached to the wrist and/or trunk. However, there is no report of physical activity of one-sided upper and lower limb propulsion by ActiGraph seen in hemiplegic patients. In this study, we compared the relationship between the oxygen intake (VO2) and the vector magnitude (VM) measured by ActiGraph when the wheelchair propelled by one hand and one foot.

Twelve healthy individuals (7 male, 5 female, age 35.2 ± 9.4 years, weight 61.4 ± 12.0 kg; mean ± SD) propelled wheelchair around one lap of 40m counterclockwise. After 3 minutes of rest, 3 minutes of slow wheelchair propulsion (3 km / h for male and 2.5 km / h for female) was made followed by of fast wheelchair propulsion (5 km / h for male and 4 km / h for female). ActiGraph GT3X-BT were worn on the right wrist and the right ankle.

VO2 at rest, slow, fast wheelchair propulsion were 3.6 ± 0.1, 7.7 ± 1.5, 12.0 ± 2.8 ml/min/kg, respectively, upper limb VM at slow, fast wheelchair propulsion were 11264.6 ± 543.8, 2068.5 ± 750.7 counts, respectively, and lower limb VM: 820.4 ± 187.6, 1134.0 ± 372.4 counts, respectively, and total VM at slow, fast wheelchair propulsion were 2085.0 ± 489.3, 3202.5 ± 1079.7 counts, respectively. There was a significant positive correlation between VO2 and total VM ($r = 0.95$).

Since there was a positive correlation between VO2 and total VM, the physical activity of wheelchair propulsion by one hand and one foot can be measured using ActiGraph. (COI:No)

### O14-3

#### A dual orexin receptor blocker suvorexant inhibits hypercapnic ventilatory augmentation

Isato Fukushi[1,2], Shigefumi Yokota[3], Kotaro Takeda[1,4], Jiro Terada[1,5], Yasumasa Okada[1] (*[1]Clin Res Ctr, Murayama Med Ctr, Musashimurayama, Japan, [2]Fac Hlth Sci, Iryo Sosei Univ, Iwaki, Japan, [3]Dept Anat and Neurosci, Shimane Univ, Izumo, Japan, [4]Fac Rehabili, Sch Healthcare, Fujita Hlth Univ, Toyoake, Japan, [5]Dept Respirology, Grad Sch Med, Chiba Univ, Chiba, Japan*)

Suvorexant (Belsomra ®), a dual orexin receptor antagonist, inhibits the arousal system in the central nervous system, and has been widely used for the treatment of insomnia. Because many of sleep-inducing drugs suppress ventilation, concerns could be raised whether suvorexant affects breathing. However, the effects of suvorexant on ventilation have not been well clarified. To address this issue, we firstly analyzed orexin receptor (OX2R) expression in neurons in the brainstem respiratory regions with putative respiratory neuron markers, i.e., with Phox2b in the parafacial respiratory group/retrotrapezoid nucleus (pFRG/RTN), and NK1R/somatostatin in the preBötzinger complex (preBötC) of the mouse, respectively. Secondly, we analyzed ventilatory parameters in room air, hypercapnic (5% $CO_2$) and hypoxic (10% $O_2$) conditions before and after injection of two doses of suvorexant (10 mg/kg and additional 90 mg/kg). Experiments were performed in unanesthetized adult male mice. Respiratory flow was non-invasively measured by whole body plethysmography. Respiratory parameters (tidal volume, respiratory rate, and minute ventilation) were calculated from the measured respiratory flow. The oxygen concentration in the chamber was monitored with an oxygen concentration analyzer. In results, immunohistological analysis demonstrated that OX2R is expressed in Phox2b and NK1R/somatostatin immunoreactive neurons in the pFRG/RTN and preBötC, respectively, suggesting the involvement of orexin in respiratory control. Either dose of suvorexant did not affect ventilation in room air or hypoxic ventilatory responses. However, hypercapnic ventilatory augmentation was significantly attenuated in the suvorexant-administered conditions as compared to the control (before suvorexant-administered) condition (two factor within-subject ANOVA). None of the mice died in even after high dose (total cumulative dose 100 mg/kg) of suvorexant administration. We conclude that suvorexant inhibits hypercapnic ventilatory augmentation. Suvorexant may have to be carefully used in patients with hypercapnic respiratory failure or sleep related hypoventilation. (COI:No)

### O14-4

#### Bidirectional remote control of deep neuron activities using X-rays

Takanori Matsubara[1], Takayuki Yanagida[2], Noriaki Kawaguchi[2], Takashi Nakano[2], Junichiro Yoshimoto[2], Shin-ichiro Horigane[3], Shuhei Ueda[3], Sayaka Takemoto-Kimura[3], Akihiro Yamanaka[1], Takayuki Yamashita[1] (*[1]Dept Neurosci II, RIEM, Nagoya Univ, Japan, [2]NAIST, [3]Dept Neurosci I, RIEM, Nagoya Univ, Japan*)

Recent advances in material science in close conjunction with neuroscience have made it possible to achieve remote/wireless optogenetic control of neuronal activities in living animals. Light-sensitive proteins called opsins are activated only by visible light, which has a low tissue penetration depth. Therefore, for stimulating a large population of neurons in the deep brain, it would be advantageous to employ up-converting phosphors which emit visible light in response to more tissue-penetrating near-infrared (NIR) light (Chen et al., Science 359: 679–684, 2018; Miyazaki et al., Cell Reports, 26: 1033-1043, 2019.). However, the tissue penetration depth of NIR is limited to several millimeters and NIR illumination can cause abrupt heat generation in body surface. To overcome these issues, we utilize inorganic scintillators that exhibit visible luminescence called scintillation when excited by X-ray. We show that a certain yellow-emitting scintillator can efficiently activate red-shifted opsins upon X-irradiation. With these scintillator/opsin combinations, we successfully activated and inhibited dopamine neurons in the ventral tegmental area of freely moving mice to wirelessly drive related behaviours under X-irradiation. The scintillator crystal was bio-compatible and safely implantable. X-ray irradiations during the behavioural test caused reduction of the number of proliferating cells in the brain and possibly in the other tissues as well but did not affect body weight and home-cage behaviour of the mice. These results demonstrate that X-ray-induced scintillation can be used for wireless optogenetics. (COI:No)

### O14-5

#### Can premedical students critically appraise YouTube "physiological education" videos?

Sean Holroyd (*Weill Cornell Medicine Qatar*)

The use of online videos as learning supplements by medical students has escalated over the past decade[1]. With much of the available material being of varying quality it is of concern that students may not be able to critically appraise online videos. In this study the responses of premedical students (n=44) to an assignment where they evaluated the physiological content of three YouTube videos on the "Mechanics of Breathing" were analyzed. Students had already been exposed to this subject in lectures. The 3 videos met the following criteria; Video 1 – correct and professionally presented, Video 2 – major errors but professionally presented, Video 3 – major errors and unprofessionally presented. Students were asked to rank the videos in order from best to worse as well as to judge them based on the following: were they accurate/factual; was the source of information reputable; could they be used for revising physiological concepts. Interestingly, 52% of the students selected Video 2 as their top-ranking video, 48% selected Video 1, none selected Video 3. Many students commented on the difficulty in choosing between Video 1 and 2 with only 16% of students identifying the major errors in Video 2. Furthermore 64% of students would use Video 2 for revision, with over 50% of these choosing this video alone. Finally, 55% of students chose Video 2 as having come from a reputable source based on the popularity (number of views) of the video and the fact it was associated with a website. The results from this study suggest that a sizeable percentage of premedical students do not have the adequate training to be able to choose appropriate study material from online videos. It is suggested that faculty should either produce their own online material or direct their students to specific online videos or websites.

*1. O'Malley et al. Adv Physiol Educ 43:383-391, 2019*

(COI:No)

# Oral Session 15

## CNS Function

(March 19, 11:00～12:00, Hall 8)

### O15-1

#### Simultaneous in vivo recording of the local field potential and the signal of fluorescent calcium indicator in the hippocampus of Altzheimer's mouse model

Munenori Ono (*Dept Physiol 1, Kanazawa Med Univ*)

Alzheimer disease (AD) patients have severe learning disabilities. In the early stage of AD, several studies have suggested that soluble amyloid beta induced the disorder in the neural activity and the intracellular calcium dynamics, which are very likely to be relevant to the learning disability as well as the progression of cell death in AD. However, the neurophysiological disorder in early stage of AD has not been fully elucidated. In this study, to examine the disorder in the neural activity and calcium dynamics in vivo, we utilized a photometric patch electrode (PME), with which simultaneous recording of fluorescence and electrical signals were available. Using a PME, we recorded the local field potential (LFP), spike activities, and the signals of fluorescent calcium indicator in the hippocampus, where is closely related to learning ability and spatial cognition. The recordings were performed in dorsal hippocampal CA1 region of model mice of AD (3xTg), with which spatial cognitive defects were confirmed in the early stage of AD. During the PME recording, the mouse was placed on a treadmill to monitor the movement, and head-fixed via a metal rod. The spatial learning ability of the individual animal was assessed by Morris Water Maze (MWM) test in advance of the PME recording. The PME recordings showed that the calcium signal increased when the theta oscillation dominated in LFP during locomotion. The transient rise of the calcium signal was also seen sporadically while the animal was stationary. At the rise of the calcium signal, the fast gamma oscillation in the LFP was observed. Compared with the WT animals, AD model animals had less power in the high frequency oscillation at the rise of the calcium signal. Interestingly, the degradation in the high frequency oscillation was correlated with the disorder in the spatial learning test.

(COI:No)

### O15-2

#### Effects of bilateral lesions of amygdala on cardiovascular responses during treadmill running in rats

Kei Tsukioka[1,2], Ko Yamanaka[1], Hidefumi Waki[1] ([1]*Dept Physiol, Grad Sch Health & Sports Sci, Juntendo Univ, Chiba, Japan,* [2]*JSPS Research Fellow*)

It has been reported that the amygdala regulates cardiovascular system in response to emotional events. In our previous study, we observed that maximal exercise performance was greater after bilateral amygdala lesions of rats. Therefore, our hypothesis was that amygdala lesions increased maximal exercise performance by modulating the cardiovascular responses during exercise . To examine this, arterial pressure (AP) in rats were chronically measured by radio-telemetry during treadmill running. Rats were subjected to forced running for 60 min (≦ 20 m / min) per day for 3 days for treadmill habituation. Thereafter, an incremental exercise test was conducted until exhaustion (pre-test). Exercise intensity was initiated at 10 m / min, and the velocity was increased by 2 m / min at every 3 min. In the amygdala lesion group, bilateral lesions in the amygdala were induced by electrical microstimulation. In the sham group, the tip of the electrode was inserted into the amygdala and immediately removed without the electrical microstimulation. After surgery, re-training was conducted similarly as the habituation protocol, and the incremental exercise test was repeated (post-test). We found that the mean AP (MAP) in both groups of animals exhibited a gradual increase during submaximal exercise (low/moderate intensity) followed by a drastic increase during high/maximal-intensity of exercise. However, comparing with the sham group, the MAP response in the amygdala lesion group was significantly lower during submaximal exercise whereas it was greater during high/maximal-intensity of exercise, suggesting that the amygdala may have a role in limiting exercise performance via modulating autonomic cardiovascular control.

(COI:No)

### O15-3

#### Midbrain dopamine neurons drive whisker movements associated with reward processing

Takayuki Yamashita[1], Kohta Mizutani[1,2], Takashi Nakano[3], Yasutaka Mukai[1], Takatoshi Hikida[2], Akihiro Yamanaka[1], Junichiro Yoshimoto[3] ([1]*Dept Neurosci II, RIEM, Nagoya Univ,* [2]*Inst Protein Res, Osaka Univ,* [3]*NAIST*)

Animals including rodents express their internal states as facial movements. However, the cellular and neural circuit mechanisms underlying facial expressions of animals are still enigmatic. Investigating mice performing an auditory Go/No-Go task, we found that notable whisker protractions were induced immediately after Go cue presentation and that reward acquisition evoked a shift of whisker set-point toward a more protracted angle and often triggered whisking. Such reward-related whisker movements were commonly observed among mice we studied, so that behavioral outputs of the mice performing the task could be accurately decoded and predicted only by time plots of their whisker angle. The cue-locked whisker protraction developed over the course of task learning, whereas whisking probability after reward acquisition was not correlated with task proficiency. These properties of reward-related whisker movements were reminiscent of activities of dopamine neurons in the ventral tegmental area (VTA). We therefore tested for optogenetic stimulation of VTA dopamine neurons and found that transient excitation of VTA dopamine neurons induced whisking. Temporal association between an auditory cue and rewarding optogenetic stimulation of VTA dopamine neurons elicited both cue-locked whisker protraction and stimulation-locked whisking, suggesting that licking-associated facial movements are not involved in reward-related whisker movements. Furthermore, virally induced, CRISPR/Cas9-mediated knock-out of Drd1 (or D1R) in the nucleus accumbens attenuated whisker movements induced by activation of VTA dopamine neurons. Our findings thus suggest that midbrain dopamine neurons, via accumbal D1R neurons, trigger whisker movements expressing expectation and acquisition of a reward.

(COI:No)

### O15-4

#### Weak correlated activity of pallidal neurons during task performance in normal and mild parkinsonian monkeys

Woranan Wongmassang[1,2], Taku Hasegawa[1,2], Satomi Chiken[1,2], Atsushi Nambu[1,2] ([1]*Division of System Neurophysiology, National Institute for Physiological Sciences, 38, Nishigonaka, Myodaiji, Okazaki, Aichi, 444-8585, Japan,* [2]*Department of Physiological Sciences, SOKENDAI (The Graduate University for Advanced Studies), 38, NIshigonaka, Myodaiji, Okazaki, Aichi, 444-8585, Japan*)

The globus pallidus is a part of the basal ganglia and divided into the external (GPe) and internal (GPi) segments. Both GPe and GPi neurons change their activity during voluntary movements, suggesting that they play a crucial role in control of voluntary movements. Although not only firing rates of neurons but also firing correlations between neurons have been reported to encode neuronal information, GPe/GPi neurons do not exhibit correlated activity at rest in the healthy state. On the other hand, oscillatory and synchronized activity has been observed in the GPe/GPi in movement disorders, such as Parkinson's disease (PD). To examine the possibility that neuronal correlations in the GPe/GPi contribute to control of movements and pathophysiology of movement disorders, we simultaneously recorded activity of multiple neurons in the GPe/GPi of Japanese monkeys (*Macaca fuscata*) during hand reaching task by using the multi-channel electrodes and analyzed their cross-correlations. We found that only a limited number of GPe/GPi neurons showed correlated activity in relation to task events in healthy monkeys. Next, we generated a mild PD monkey by injecting 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), a dopaminergic neurotoxin. In the mild PD monkey that can perform the motor task, correlated activity was not increased. These results suggest that independent activity of GPe/GPi neurons is essential to normal information processing within the basal ganglia and appropriate control of voluntary movements.

(COI:No)

### O15-5

#### Glutamatergic and GABAergic control of monkey subthalamic activity during motor task

Zlata Polyakova[1,2], Nobuhiko Hatanaka[1,2], Satomi Chiken[1,2], Atsushi Nambu[1,2] ([1]*Div System Neurophysiol, Natl Inst Physiol Sci, Okazaki, Aichi, Japan,* [2]*Dept Physiol Sci, SOKENDAI, Okazaki, Aichi, Japan*)

The subthalamic nucleus (STN) receives cortical inputs through the cortico-STN *hyperdirect* and cortico-striato-external pallido (GPe)-STN *indirect* pathways and drives the internal segment of the globus pallidus, the output nucleus of the basal ganglia. Activity of STN neurons is modulated in relation to voluntary limb movements, and its abnormal activity has been reported in movement disorders, such as Parkinson's disease. However, the control mechanism of STN activity by cortical inputs is still not clear. To address this issue, we recorded neuronal activity of STN neurons in awake monkeys (*Macaca fuscata*) in combination with blockade of neurotransmission by local drug injections. Stimulation of motor cortices induced early and following late excitations in STN neurons. Pharmacological blockade showed that cortically induced early and late excitation in STN neurons are mediated by the *hyperdirect* and *indirect* pathways, respectively, and that cortical inputs to the STN are mainly mediated by N-methyl-D-aspartate receptors. Next, we recorded STN neuronal activity during performance of go/stop reaching task at three different targets. The results showed that the motor region in the STN is involved in both motor execution and cancellation. Task-related STN activity was also controlled through the *hyperdirect* cortico-STN glutamatergic and *indirect* GPe-STN GABAergic inputs. Stop-related activity was mainly transmitted through the *hyperdirect* pathway that caused facilitation in the STN, while the role of the *indirect* pathway was minor. Some neurons with stop-related activity showed their activity in the specific direction, while other neurons showed their activity in all directions.

(COI:No)

# Oral Session 16

## Nutrition・Metabolism・Thermoregulation

(March 19, 11:00～12:00, Hall 9)

### O16-1
#### A novel regulatory mechanism of energy metabolism mediated by Neuronal calcium sensor-1 and its relation to exercise therapies

Tomoe Nakamura-Nishitani.[1], Shu Nakao[2], Hirotsugu Tsuchimochi[1], James Pearson[1], Shigeo Wakabayashi[3] (*[1]Dept Card Physiol, Natl Cereb & Cardiovasc Ctr, Japan, [2]Stem Cell Regen Med Lab, Col Life Sci, Ritsumeikan Univ, Japan, [3]Dept Pharm, Osaka Med Col, Japan.*)

Obesity is a risk factor of life-threatening diseases such as stroke and myocardial infarction, thus it is important to identify novel mechanisms of its regulation and prevention. Neuronal $Ca^{2+}$-sensor-1 (NCS-1) is an EF-hand $Ca^{2+}$- binding protein, playing important roles in excitable tissues via activation of $Ca^{2+}$-signals. We have previously discovered that NCS-1 deficient (KO) mice become obese with age. Analyses using the metabolic cages indicated that both food intake and locomotor activity were not different from those in wild-type (WT) mice, but energy metabolism was significantly decreased in KO mice. At the cellular level, mitochondrial respiration rate and the levels of proteins involved in mitochondrial thermogenesis and biosynthesis, as well as its $Ca^{2+}$-dependent upstream regulators were all decreased in the brown adipocytes in KO mice. These results suggest that decrease in mitochondrial respiration followed by decrease in energy metabolism are major mechanisms of obesity observed in KO mice. Furthermore, we investigated whether exercise therapy would prevent the metabolic disorder induced by NCS-1 deficiency. Eight weeks treadmill training less than lactate threshold level inhibited both increase in body weight and adipocyte hypertrophy in KO mice, while amount of skeletal muscle were increased. Taken together, these results suggest an existence of a novel $Ca^{2+}$- dependent pathway regulating energy metabolism mediated by NCS-1, and aerobic exercise can prevent obesity even that was induced by genetic background difference such as NCS-1 deficiency. (COI:No)

### O16-2
#### Withdraw

### O16-3
#### A combination of high fat diet and nicotine enhances CB1 endocannabinoid receptor in hypothalamic nuclei in mice

Tingting Guo[1, 5], Mami Matsumoto[2], Takashi Yagi[1], Hiroyuki Koyama[1], Daisuke Aotani[1], Kazunobu Sawamoto[2], Yasuhiko Minokoshi[3], Hiroaki Masuzaki[4], Nobuya Inagaki[5], Kazuwa Nakao[6], Tomohiro Tanaka[1] (*[1]Dept Gastro and Metab, Grad Sch Med, Nagoya City Univ, Japan, [2]Devel Regen Neuro, Inst of Brai Sci, Grad Sch Med, Nagoya City Univ, Japan, [3]Divis of Endo and Metab, Nat Ins for Phys Sci, Japan, [4]Divis of Endo, Diab and Metab, Hema, Rheum, Grad Sch Med, Ryukyus Univ, Japan, [5]Dept of Diab, Endo and Nutri, Grad Sch Med, Kyoto Univ, Japan, [6]Med Innov Cent, Grad Sch Med, Kyoto Univ, Japan*)

**Background:** Although combinatorial effect of dietary fat intake and tobacco smoking on body weight has been suggested, underlying mechanisms have not been fully elucidated. Cannabinoid receptor 1 (CB1R) is a GPCR expressed widely in the brain and may play a role both in obesity and tobacco addiction, since its pharmacologic blockade is effective for the both. Here, precise distribution of CB1R within the appetite center and potential changes in a murine model of obese smokers have been examined.
**Methods:** We analyzed mRNA levels of CB1R in micro-dissected hypothalamic nuclei as well as CB1R immunoreactivity in brain slices from male C57BL/6 mice. Mice kept on a high fat diet (HFD) for four weeks were administered with nicotine (12ug/g body weight/day) intraperitoneally, and body weight, food intake and CB1R mRNA levels were examined.
**Results:** CB1R mRNA and protein were detected in hypothalamic nuclei as well as hippocampus. CB1R mRNA in the arcuate (ARC) and lateral nuclei (LH) of the hypothalamus were higher than paraventricular (PVN) and ventral-dorsal medial nuclei (V/DMH). Nicotine suppressed food intake either under standard or HFD by 29 ± 5% and 53 ± 13%, respectively. HFD or nicotine alone slightly lowered CB1R levels in the PVN or LH, respectively. By contrast, treatment of HFD-fed mice with nicotine led to an increase in CB1R levels in ARC, PVN, V/DMH and LH by 45%, 65%, 34% and 50%, respectively.
**Conclusions:** CB1R mRNA was widely distributed in multiple hypothalamic nuclei. The expression of CB1R was augmented only when mice were treated with HFD and nicotine in combination. These data suggest a potential combinatorial effect of the two insult and that an exposure to nicotine in obese subjects may provoke an enhanced central endocannabinoid activity and may lead to the development of cross addiction at the level of the hypothalamus. (COI:No)

### O16-4
#### Effects of Omega-3 fatty acids feeding on high-fat induced impairment of glucose tolerance and behavioral performances

Atsushi Fukushima, Miyako Furuta, Hitomi Fujioka, Toshiya Funabashi (*Dept Physiol, St Marianna Univ Sch Med, Kawasaki, Japan*)

It is well known that a high-fat (HFD) or Western diet increases body weight, impairs glucose tolerance and results in obesity. Since a high-fat diet consumption is suggested to have a profound impact on the brain, learning and memory processes may alter. Omega-3 fatty acids, on the other hand, attenuate the inflammatory response induced by such as HFD. The present study examined whether or not omega-3 fatty acids have beneficial effects on HFD-induced exacerbation. C57BL6 male mice were divided into 3 groups: Fed with an ordinal control diet group, HFD group fed with 45% fat diet, and HFD+omega-3 group fed 45% fat diet but half of fat is from Menhaden oil, instead of Lard. Feeding for 10 weeks had no impact on inhibitory avoidance task and open field test. In HFD+omega-3 group, Y-maze task performed tendency to lowering because intraperitoneal glucose tolerance test was impaired. Next, we examined omega-3 feeding after HFD. This means mice were fed for HFD 5 weeks and switched feeding HFD containing omega-3 for additional 5 weeks. This treatment had no impact on inhibitory avoidance task and open field test. However, omega-3 supplement improved attenuation of alternation in Y-maze task by HFD and significantly recovered impaired glucose tolerance by HFD. We conclude that omega-3 fatty acids is beneficial effects on some HFD-induced impairment. (COI:No)

### O16-5
#### The impact of chronotype on the effect of dietary restriction and serum level of sRAGE among obese patients - preliminary report

Zofia Orzechowska[1], Dominika Kanikowska[1], Katarzyna Korybalska[1], Joanna Grzelak[1], Rafał Rutkowski[1], Agnieszka Zawada[2], Aldona Juchacz[2], Maki Sato[3], Andrzej Bręborowicz[1], Janusz Witowski[1] (*[1]Department of Pathophysiology, Poznan University of Medical Sciences, Poznan, Poland, [2]Department of Internal Diseases, Metabolism and Nutrition, Poznań University of Medical Science, Poznań, Poland, [3]Department of Physiology, Institutional Research, Aichi Medical University School of Medicine, Aichi Medical University, Aichi, Japan*)

A chronotype is an individual, internal biological rhythm, concerning different preferences of the time of day, which depends equally on the properties of the individual biological clock and lifestyle. It is divided into the morning ("lark"), evening ("owl"), or mixed type. It has been reported in previous studies that evening chronotype is closely associated with obesity. This study aimed to assess the chronotype's impact on the effect of low-calorie diet treatment and serum level of Soluble Form of Receptor for Advanced Glycation End Products (sRAGE) in patients with excessive accumulation of adipose tissue in the body.
The study group consisted of 70 obese patients. These individuals underwent a three-week diet treatment. Patients used a diet with a reduction in the daily supply of calories by 25-30% in relation to the total energy demand calculated using the Harris and Benedict formula and the physical activity rate. The patient's weight and serum levels of sRAGE were collected before and after dietary modification. Blood lipids and glucose levels were also assessed. Furthermore, a chronotype evaluation questionnaire was carried out to evaluate the biological rhythm of each patient. The control group consisted of 200 donors from the Regional Blood Donation Center in Poznań (100 men and 100 women), among whom a survey assessing chronotype was also carried out, the anthropometric parameters were measured, and the body mass index (BMI) was determined.
A majority of respondents in both the experimental and control group presented a "lark" chronotype. Preliminary results presented in this study suggest that the highest BMI values were found it the so-called "owl" individuals. At the same time, the low-calorie diet allowed the most effective results to be achieved in this group, which included both weight reduction (mean of 5.3 kg) and average fat loss. For larks it was 4, 6 kg and for intermediate type 4, 7 kg. Furthermore, we found a significant statistical difference in BMI between lark and intermediate chronotype (before 42, 8 vs 38, 6; after 41, 0 vs 37, 2). The initial results presented are based on an incomplete group of patients. The study is currently being continued and will ultimately include a group of 200 individuals. We believe that the final results will allow us to clearly show the correlation between a given chronotype type and the effectiveness of a low-calorie diet. (COI:No)

# Oral Session 17

## Heart・Circulation 2

(March 19, 11:00～12:00, Hall 10)

### O17-1

#### Identification of ANP promotor-driven AcGFP-expressing cardiac progenitor cells in mice

Ryo Fukunaga[1], Mariko Omatsu-Kanbe[1], Kakeru Shimoda[2], Masakazu Agetsuma[3], Junichi Nabekura[3], Motohiro Nishida[2], Hiroshi Matsuura[1] (*[1]Dept Physiol, Shiga Univ Med Sci, Otsu, Japan, [2]Div Cardiocirculatory Signaling, [3]Duv Homeostatic Development*)

The adult mammalian heart comprises heterogeneous cell lineages, including cardiomyocytes, vascular smooth muscle cells, fibroblasts and cardiac stem or progenitor cells. Atypically-shaped cardiomyocytes (ACMs) are identified in the culture of cardiomyocyte-removed fractions obtained from mouse cardiac ventricles as beating cells. Since ACMs show the characteristics of ventricular, atrial and SA-nodal cells based on the expression of cell-specific proteins and electrophysiological properties, those cells are likely to be cardiac progenitor or immature cardiomyocytes. One of the most important characteristics of those cells is the abundant expression of atrial natriuretic peptide (ANP) usually absent in the ventricular myocytes. However, the localization and morphology of these cells in the myocardium has yet to be clarified. In the present study, we prepared ANP promotor-driven AcGFP-expressing mice generated by infection of adeno-associated virus (AAV2-9) encoding pANP-AcGFP to visualize and trace ANP-expressing ACMs. The cells with GFP signal were identified in the interstitial spaces among ventricular myocytes and also GFP-positive beating ACMs were present in the culture. The results indicate that ACMs with ANP-promotor exist in the cardiac ventricles and survive to develop into beating cells in the dispersed culture. (COI:No)

### O17-2

#### Multinucleation of cardiac progenitor cells by cell fusion and nuclear division

Mariko Omatsu-Kanbe, Ryo Fukunaga, Hiroshi Matsuura (*Dept Physiol, Shiga Univ Med Sci, Otsu, Japan*)

The adult mammalian heart comprises several cardiac stem or progenitor cells, though cardiomyocytes do not actually multiply to substitute new cells for damaged ones. Atypical myocardial cells (ACM) derived from mouse cardiac ventricles that develop into beating cells are likely to be cardiac progenitor cells rather than stem cells. Although most of the ACMs possess multiple nuclei, these cells are not observed to be proliferated. In the present study, we prepared ACMs from Fucci2 (fluorescent, ubiquitination-based cell cycle indicator 2) transgenic mice, of which nuclei were fluorescently labelled, to investigate the cell cycling and cocultured these cells with those from wild-type mice. The dynamics of the labelled nuclei were observed using live cell imaging system, and the precise morphology and the protein expression of the nucleus were examined. We found that the cell fusion and nuclear division without cytokinesis occurred in these cells in the culture. The results suggest that the multinucleation process of ACMs, different from that in the myogenesis in skeletal muscle, is close to that observed in the neonatal heart stage. (COI:No)

### O17-3

#### Molecular mechanisms of contraction rhythm homeostasis in warmed cardiomyocytes

Seine Shintani A.[1], Shinichi Ishiwata[2], Norio Fukuda[3] (*[1]Department of Biomedical Sciences, College of Life and Health Sciences, The Chubu University, Aichi 487-8501 Japan, [2]Department of Physics, Faculty of Science and Engineering, Waseda University, Tokyo 169-8555 Japan, [3]Department of Cell Physiology, The Jikei University School of Medicine, Tokyo 105-8461 Japan*)

It is well established that an increase in body temperature, albeit by a slight magnitude, results in dramatic changes in the function of various organs, coupled with altered cellular homeostasis. In the present study, we investigated the effects of infra-red laser irradiation on sarcomere dynamics in living rat neonatal cardiomyocytes, by taking advantage of sarcomere length nanometry. We found that a rapid increase in temperature to 38–42 ℃ induced [$Ca^{2+}$] i-independent high-frequency (~5–10 Hz) sarcomeric auto-oscillations (Hyperthermal Sarcomeric Oscillations; HSOs). In myocytes with intact sarcoplasmic reticular function, HSOs coexisted with [$Ca^{2+}$] i-dependent spontaneous beating in the same sarcomeres, with markedly different frequencies (~7 and ~1 Hz for the former and the latter, respectively). We simulated HSOs with a multi-scale, multi-physics heart simulation model. Our simulation predicts that there exists a "reverse stroke" during the power stroke of myosin, which is vital for HSOs, as well as for rapid myocardial relaxation for subsequent ventricular filling. Based on these findings, we will discuss the physiological significance and molecular mechanisms of HSOs. (COI:No)

### O17-4

#### Pitx2c Overexpression in Atrial Cardiomyocytes Impaired Sinus Atrial Node Function

Shunsuke Baba[1], Toru Akaike[1], Satoko Shinjo[1, 2], Susumu Minamisawa[1] (*[1]Dept Cell Physiol, Grad Sch Med, Jikei Univ, Japan, [2]Dept Cell, Padova Univ, Italy*)

**Introduction:** Sinus atrial node (SAN) dysfunction is associated with fatal diseases. The transcription factor Pitx2c, which is expressed in the left-sided heart may suppress differentiation of SAN cells because Pitx2c knockout mice had aberrant pacemaker cells in the left atria. Hypothesis: Pitx2c overexpression in the right atria may impair SAN formation and function.
**Methods:** We generated the mice harboring the atrial cardiomyocyte-specific overexpression of the Pitx2c by crossing two types of mice; the one has the Cre gene at the endogenous locus of the sarcolipin gene specifically expressed in the atria, and the other was introduced Pitx2c gene between loxP sequences using the CAG promoter. Then, we examined the cardiac phenotypes of Pitx2cflox/cre+ (over expression:OE) and Pitx2cflox/cre- (control:CON) mice.
**Results and Conclusions:** The expression levels of Pitx2c mRNA were more than 100 times higher in the right atrium of OE than CON mice. OE mice were born with normal Mendelian ratios. Echocardiography showed no change in the OE heart (n=7). Telemetry electrocardiography showed the standard deviation of RR interval was significantly larger in OE (15.3 ± 8.40msec) than CON (10.3 ± 5.91msec) mice(n=5). Ectopic atrial beats were found in OE mice. The expression levels of TBX3, SCN5A and Kir3.1 mRNAs were significantly downregulated, and those of podoplanin were significantly upregulated in the right atria of OE (n=7). We assumed these transcriptional alternations involved in SAN formation and channel activities could induce electrical remodeling in the SAN and right atrium of OE mice. In conclusion, atrial cardiomyocyte-specific Pitx2c overexpression decreased heart rate and increased its variability, indicating that the downregulation of Pitx2c in the right atrium is required to maintain SAN function and electrical modeling of the atria. (COI:No)

### O17-5

#### Physiological properties of excitation-contraction relationships in human induced pluripotent stem cell-derived cardiomyocytes sheets

Hiroko Izumi-Nakaseko[1], Koki Chiba[1], Mihoko Hagiwara-Nagasawa[1], Ai Goto[1], Yoshio Nunoi[1], Ryuichi Kambayashi[1], Akio Matsumoto[2], Yasunari Kanda[3], Atsuhiko Naito T.[4], Atsushi Sugiyama[1, 2] (*[1]Dept Pharmacol, Faculty Med, Toho Univ, japan, [2]Dept Aging Pharmacol, Faculty Med, Toho Univ, Japan, [3]Div Pharmacol, NIHS, Japan, [4]Dept Physiol, Div Cell Physiol, Toho Univ Sch Med, Japan*)

Currently available human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been known to show a negative force-frequency relationship as one of their immature properties. In this study, we explored several potential physiological factors which may overcome such limitation of the contraction movement. For this purpose, we prepared one layered, higher cell-density sheets of hiPSC-CMs, and simultaneously recorded the motion vectors and field potentials. First, we analyzed the contraction and relaxation processes under spontaneous activity. Next, we controlled the direction of cell movement by electrical pacing and also tested the effect of supplying the higher oxygen tension on the cell sheet. Under spontaneous activity, an electrical excitation propagated unidirectionally over the cell sheet, but a synchronous movement consisted of multiple contractions which started from various sites. During electrical stimulation, the contraction started around the pacing electrodes and we observed the positive force-frequency relationships in contraction as well as relaxation along with the frequency-dependent shortening of the field potential durations. The use of fractional analysis of motion vectors demonstrated that contraction as well as relaxation processes consisted of fast and slow phases. Increase in oxygen tension from 20 to 95% in mixed gas accelerated the fast phase of relaxation. $\beta$-Stimulation accelerated the timing of fast phase of relaxation, whereas a tyrosine kinase inhibitor dasatinib delayed it. Thus, these observations can indicate that the currently proposed procedure may become a new tool for integrating the drug-induced biological phenomena in vitro extrapolating to clinically observed cardiac efficacy and adverse effects. (COI:No)

# Oral Session 18

## Cell Physiology・Molecular Physiology 2

(March 19, 14:10～15:10, Hall 7)

### O18-1

#### Differential roles of class II PI3-kinase-C2$\alpha$ and -C2$\beta$ in clathrin-mediated fluid phase endocytosis in vascular endothelial cells

Kazuaki Yoshioka[1], Sho Aki[1], Noriko Takuwa[1,2], Yoh Takuwa[1] ([1]*Dept. Physiol., Grad. Sch. Med., Kanazawa Univ., Japan,* [2]*Dept. Health Sci., Ishikawa Pref. Nursing Univ., Japan*)

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases that phosphorylate the 3'-OH group at the inositol ring of phosphoinositides (PIs) on the plasma membrane and intracellular organelle membranes. PI3Ks regulate a diverse array of dynamic membrane events. While class I and class III of PI3K are well-characterized, functional roles of class II PI3K isoforms (PI3K-C2$\alpha$, -C2$\beta$ and -C2$\gamma$) were largely unknown. We previously demonstrated that global C2$\alpha$-knockout (KO) mice were embryonic lethal due to severe defects in angiogenesis. The inducible endothelial cell (EC)-specific C2$\alpha$ deletion resulted in a similar phenotype. Mechanistically, C2$\alpha$ was required for ligand-induced endocytosis of the angiogenic receptors including vascular endothelial growth factor receptor-2 (VEGFR2), sphingosine-1-phosphate receptor-1 (S1P1) and transforming growth factor-$\beta$ receptor (TGF$\beta$R), and thereby receptor-mediated endosomal activation of Rho, Rac, and Smad-2/3. We studied the possible involvement of C2$\alpha$ and C2$\beta$ in clathrin-mediated fluid-phase endocytosis (pinocytosis) in EC. C2$\alpha$ and C2$\beta$ were both required for uptake of FITC-dextran, i.e. pinocytosis. FITC-dextran uptake was partially dependent on both clathrin and dynamin, and both PI3K-C2$\alpha$ and PI3K-C2$\beta$ were required for clathrin-mediated, but not clathrin-non-mediated, FITC-dextran uptake at the step leading up to its delivery to early endosomes. Both C2$\alpha$ and C2$\beta$ were co-localized with clathrin-coated pits and vesicles. However, actin filament staining showed that C2$\beta$ but not C2$\alpha$ was highly co-localized with actin filament-associated clathrin-coated pits and vesicles and required for actin filament formation at the clathrin-coated structures. The C2$\beta$-binding protein, ITSN1, was required for C2$\beta$ co-localization at actin filament-associated clathrin-coated structures and pinosome-associated actin filament formation. These results indicate that C2$\alpha$ and C2$\beta$ are involved in clathrin-mediated endocytosis with cell type-specific functional compensations.

(COI:No)

### O18-2

#### Identification of ROS induced Na+-independent $Mg^{2+}$ efflux in rat ventricular myocytes

Michiko Tashiro[1], Hana Inoue[1], Masato Konishi[2], Utako Yokoyama[1] ([1]*Dept Physiol, Tokyo Med Univ, Tokyo Japan,* [2]*Admission center, Tokyo Med Univ, Tokyo Japan*)

Intracellular $Mg^{2+}$ may function to prevent $Ca^{2+}$-overload caused by ROS (reactive oxygen species) in ventricular myocytes. Although the regulation mechanism of intracellular-free $Mg^{2+}$ concentration ($[Mg^{2+}]_i$) remains unclear, $[Mg^{2+}]_i$ should be regulated by the balance of $Mg^{2+}$ influx and efflux. $Na^+$-gradient dependent Mg extrusion has been widely recognized as a $Mg^{2+}$-efflux system.

To study effects of ROS on $Mg^{2+}$ homeostasis in rat ventricular myocytes, we measured $[Mg^{2+}]_i$ using a fluorescent indicator, mag-fura-2 in $Ca^{2+}$-free Tyrode solution. $[Mg^{2+}]_i$ ($1.05 \pm 0.03$ mM, n=9) was decreased to $0.82 \pm 0.03$ mM (p=0.0005) in 3 min, after 5-min treatment of 500 $\mu$M $H_2O_2$. The lowered $[Mg^{2+}]_i$ was maintained up to ～20 min, and then recovered to the basal level at 50 min after application of $H_2O_2$.

Similar experiments were repeated (i.e., 5-min application of $H_2O_2$) in various extracellular solutions. 1) In the absence of $Mg^{2+}$, $[Mg^{2+}]_i$ was decreased from $1.09 \pm 0.04$ to $0.80 \pm 0.07$ mM in 3 min (n=5, p=0.03), but was not recovered, indicating lack of $Mg^{2+}$ influx. 2) In the absence of $Na^+$ (substitution of NMDG for $Na^+$), $[Mg^{2+}]_i$ was decreased from $0.92 \pm 0.06$ to $0.75 \pm 0.03$mM in 20 min (n=9, p=0.03), and was subsequently recovered. 3) When the cells were first loaded with $Mg^{2+}$ in a low-$Na^+$ and high-$Mg^{2+}$ solution, the raised $[Mg^{2+}]_i$ ($1.67 \pm 0.05$ mM, n=3) was rapidly decreased to $0.54 \pm 0.1$ mM in 3 min (p=0.013).

These results suggest that $Na^+$-independent $Mg^{2+}$ efflux in rat ventricular myocytes, which is activated by ROS, is newly identified. There is a possibility that the ROS-induced $Mg^{2+}$ efflux causes further damage to cells.

(COI:No)

### O18-3

#### CTLA4-Ig (Abatacept) Directly Inhibited Osteoclastogenesis by Interfering With Intracellular Calcium Oscillations via Fc receptor gamma

Hiroyuki Okada[1], Hiroshi Kajiya[2], Yasunori Omata[1], Shunichi Sudo[2], Masashi Shin[2], Fujio Okamoto[2], Jun Hirose[1], Koji Okabe[2], Takeshi Miyamoto[3,4], Sakae Tanaka[1] ([1]*Dept Orthopaedic Surgery, Grad Sch Med, Tokyo Univ, Japan,* [2]*Dept Physiological Science & Molecular Biology, Fukuoka Dental College, Japan,* [3]*Dept Orthopaedic Surgery, Faculty Life Sciences, Kumamoto Univ, Japan,* [4]*Dept Orthopedics, Keio University School of Medicine, Japan*)

CTLA4-Ig (cytotoxic T-lymphocyte antigen 4-Ig; abatacept) is a biologic drug for rheumatoid arthritis. CTLA4 binds to the CD80/86 complex of antigen-presenting cells, and blocks the activation of T cells. Previous reports showed that CTLA4-Ig directly inhibited osteoclast differentiation, the whole inhibitory mechanism of CTLA4-Ig for osteoclast differentiation remained unclear.

Bone marrow macrophages (BMMs) from wild-type mice were cultured with M-CSF and RANKL with or without the recombinant mouse chimera CTLA4-Ig. Intracellular calcium oscillations of BMMs with RANKL were detected by staining with calcium indicator Fura-2 immediately after administration of CTLA4-Ig or after one-day treatment. Calcium oscillations' change with the acute administration were analyzed with wavelet time-series analysis. Finally, LPS-induced osteolysis of calvarial bone was used to examine the negative effect on osteoclastogenesis *in vivo*. Wild-type and Fc receptor gamma (FcR$\gamma$)-deficient C57/BL6 mice were used for these experiments.

CTLA4-Ig inhibited osteoclast differentiation and reduced the expression of the nuclear factor of activated T cells *NFATc1* in BMMs *in vitro*. Calcium oscillations in BMMs were suppressed by CTLA4-Ig both immediately after administration and after one day of treatment. CTLA4-Ig did not affect osteoclastogenesis and did not cause remarkable changes in calcium oscillations in FcR$\gamma$-deficient BMMs. *In vivo*, CTLA4-Ig rescued LPS-induced osteolysis in wild-type mice, and the improvement did not occur in FcR$\gamma$-deficient mice.

In conclusion, CTLA4-Ig inhibited intracellular calcium oscillations via FcR$\gamma$, down-regulated *NFATc1* expression, and directly inhibited osteoclastogenesis *in vitro* and *in vivo*. This is the first report of co-factor (CTLA4 - CD80/86) around co-stimulatory signal (FcR$\gamma$) in osteoclastogenesis.

(COI:No)

### O18-4

#### The Ultrastructural physiology of Bile canaliculus in Porcine Liver Donated after Cardiac Death and Preserved with Machine Perfusion Preservation

Hiroki Bochimoto[1,2], Yo Ishihara[2,3], Daisuke Kondoh[4], Hiromichi Obara[5], Naoto Matsuno[2] ([1]*Div Aerosp Med, Dept Cell Physiol, Jikei Univ Sch Med, Japan,* [2]*Dept transpl technol therapeut develop, Asahikawa Med Univ, Japan,* [3]*Shonan Kamakura Gen Hosp, Japan,* [4]*Lab Vet Anat, Obihiro Univ Agricul Vet Med, Japan,* [5]*Dept Mech Eng, Tokyo Metropol Univ, Japan*)

**Background:** The effects of each type of machine perfusion preservation (MP) of liver grafts donated after cardiac death on the bile canaliculi of hepatocytes remain unclear. Here we analyzed intracellular three-dimensional ultrastructures of the bile canaliculi and endomembrane systems in hepatocytes after warm ischemia followed by successive MP under various temperature conditions.

**Methods:** Porcine liver grafts after warm ischemia for an hour were perfused for four hours with modified University of Wisconsin gluconate solution under hypothermic (HMP, n = 4) or warm MP (WMP, rewarming up to 22℃, n = 5) conditions. Transmission and osmium-maceration scanning electron microscopy were utilized to evaluate the ultrastructure of the bile canaliculi.

**Results:** Scanning electron microscopy revealed that lumen volume of the bile canaliculi decreased after warm ischemia. In the liver grafts preserved by HMP, bile canaliculi tended to recover in terms of lumen volume, although their microvilli were regressed. In contrast, the WMP preserved functional form of microvilli of bile canaliculi.

**Conclusions:** MP potentially restored the bile canaliculus lumen and alleviated the cessation of cellular endocrine processes due to warm ischemia. In addition, WMP prevented the retraction of microvilli of bile canaliculi, suggesting further mitigation of damage of bile canaliculi.

(COI:No)

### O18-5

#### Imaging analysis of spatiotemporally regulated crosstalk between thrombus formation and its lysis

Yuko Suzuki, Hideto Sano, Naoki Honkura, Tetsumei Urano (*Dept Med Physiol, Hamamatsu Univ Sch Med, Japan*)

**Background:** Recent advances in genetic engineering and optical instrumentation have allowed us to analyze the spatiotemporal regulation of thrombus formation and its lysis. Employing human platelet-containing normal plasma (plt-NP), we demonstrated the initiation of tissue factor-triggered clot formation from phosphatidylserine exposing platelets and an uneven fibrin network was formed due to focal generation of thrombin. We also found that the lysis of clot was initiated from activated platelets where both tissue-type plasminogen (tPA) and plasminogen accumulated.

**Aim:** We further analyzed the mechanisms of crosstalk between coagulation and fibrinolysis by focusing on thrombin activatable fibrinolysis inhibitor (TAFI) which can be activated by thrombomodulin (TM)-bound thrombin.

**Methods:** 1) Employing plt-NP or normal platelet-containing TAFI deficient plasma (plt-TAFI-DP), effects of neutralizing antibody of TM (TM-Ab) and activated TAFI inhibitor (TAFIaI) were determined as tPA-induced clot lysis time (CLT) using turbidimetric method. 2) Fibrin network formation and lysis were visualized by confocal microscopy and the localization of fluorescence labeled plasminogen and TAFI were analyzed. 3) Activation of TAFI was evaluated under flow condition employing a microchip-based flow chamber system (Total Thrombus-formation Analysis System).

**Results:** 1) CLT in plt-NP was prolonged in a platelet concentration-dependent manner, which was restored by either TM-Ab or TAFIaI. In plt-TAFI-DP, however, no prolongation of CLT was shown, suggesting that plasma TAFI is essential for platelet-dependent activation of TAFI. 2) Time required for plasminogen accumulation, considered as TAFI activation marker, was shortened by either TM-Ab or TAFIaI. Labeled TAFI localized to both fibrin fiber and activated platelets, in which the former was Lys-binding site dependent- but the latter was independent-manner. 3) TAFIaI significantly prolonged occlusion of flow chamber in the presence of tPA, suggesting that TAFI was efficiently activated under flow condition.

**Conclusion:** Spatiotemporal analysis enabled us to understand the precise crosstalk between coagulation and fibrinolysis mediated by TAFI.

(COI:No)

# Oral Session 19

## Development・Growth・Aging

(March 19, 15:10～16:10, Hall 7)

### O19-1

#### Effects of expression variation in flanking genes on phenotypes of St8sia2-knockout mice

Keisuke Ikegami[1,2], Kazumasa Saigoh[3], Atsuko Fujioka[2], Mamoru Nagano[2], Chihiro Satou[4], Ken Kitajima[4], Susumu Kusunoki[3], Satoru Masubuchi[1], Yasufumi Shigeyoshi[2] (*[1]Dept Physiol, Sch Med, Aichi Med Univ, Japan, [2]Dep Anato, Kindai Univ Fac Med, Japan, [3]Dep Neuro, Kindai Univ Fac Med, Japan, [4]Lab Ani Cell Fun, Biosci Biotech Center, Nagoya Univ. Japan*)

The induction of null mutations by means of homologous recombination is a powerful technique for validating gene function, but the genetic background and closely linked genes flanking the targeted locus often affect phenotypic changes in the null mutant. ST8 alpha-N-acetylneuraminide alpha-2, 8-sialyltransferase 2 (ST8SIA2) synthesizes polysialic acid (PSA), which is essential for brain development. Although previous studies reported that St8sia2-deficient mice that have a mixed 129 and C57BL/6 (B6) genetic background showed mild and variable phenotypes, the reasons for this remain unknown. We hypothesized that this phenotypic difference is caused by diversity in the expression or function of flanking genes of St8sia2. A genomic polymorphism and gene expression analysis in the flanking region revealed reduced expression of insulin-like growth factor 1 receptor (Igf1r) on the B6 background than on that of the 129 strain. This observation, along with the finding that administration of an IGF1R agonist during pregnancy increased litter size, suggests that the decreased expression of Igf1r associated with ST8SIA2 deficiency caused lethality. This study demonstrates the importance of gene expression level in the flanking regions of a targeted null allele having an effect on phenotype. (COI:No)

### O19-2

#### Orthotopic Transplantation of Human iPSC-derived Liver Tissue with Lumenized Blood Vessels is an Effective Cell Therapeutic for Liver Cirrhosis

Tomomi Tadokoro[1], Yoshiki Kuse[2], Megumi Matsuo[3], Yutarou Uchida[1], Kohei Kaida[1], Koudai Kimura[1], Akira Higashibata[4], Toko Hashizume[4], Yasuharu Ueno[2], Satoshi Okamoto[1], Soichiro Murata[1], Hideki Taniguchi[1,2] (*[1]Dept Regen Med, Grad Sch Med, Yokohama City Univ, Japan, [2]Div of Regen Med, Inst of Med Sci, The Univ of Tokyo, Japan, [3]Dept of Gen Srg, Grad Sch of Med, Chiba Univ, Japan, [4]JEM util ctr, Human Spaceflight tech dir, JAXA, Japan*)

Organoid research is making remarkable progress and a partial reconstitution of complex organ system has been achieved by organoids. We previously reported *in vivo* vascularization of human induced pluripotent stem cell (hiPSC)-derived liver organoids and its therapeutic effect on mouse acute liver failure. To treat end-stage liver diseases, we sought to develop the cell culture technology for *in vitro* liver tissue reconstruction including lumenized blood vessels and the novel orthotopic transplantation on liver surface. In this study, 3D imaging of liver bud formation at mouse embryonic stages revealed that vascular network is formed outside of the hepatic cluster by static interactions between hepatic, endothelial and mesenchymal cells. As hepatic progenitors derived from anterior intestinal portal and lateral endoderm are fused to form liver bud, we statically fuse small liver buds derived from hiPSC to mimic embryonic liver bud formation, and successfully generate liver tissue including lumenized blood vessels. We also showed novel orthotopic transplantation of rat embryonic livers can rescue liver cirrhosis concomitant with maturation of donor organ. Finally, we demonstrated that hiPSC-derived liver tissue improved survival rate and the blood biochemical parameters of liver cirrhosis model, and ameliorated liver fibrosis. It is speculated that recovery from liver fibrosis is achieved via MMPs secreted from hiPSC-liver tissue and anti-fibrotic factors derived from macrophages accumulated after transplantation. Taken together, in vitro tissue reconstruction technology could be a promising tool for regenerative medicine when combined with liver surface transplantation. (COI:No)

### O19-3

#### Stress-induced premature senescence caused by inhibition of proteostasis in human fibroblasts

Yasuhiro Takenaka[1,2], Ikuo Inoue[2], Takanari Nakano[3], Masaaki Ikeda[4], Yoshihiko Kakinuma[1] (*[1]Dept Physiol, Nippon Med Sch, Japan, [2]Dept Diabetes and Endocrinol, Saitama Med Univ, Japan, [3]Dept Biochem, Saitama Med Univ, Japan, [4]Dept Physiol, Saitama Med Univ, Japan*)

To investigate how prolonged disturbances of proteostasis is involved in cellular senescence process in proliferating cell, we developed a model in which young normal human fibroblasts MRC-5 were treated with either of a reversible proteasome inhibitor, MG-132, and V-ATPase inhibitor, Bafilomycin A1. After 5-days drug treatment, cells showed senescent phenotypes such as a flattened morphology, permanent cell cycle arrest, expression of senescence-associated $\beta$-galactosidase, and upregulation of p21 and p53. Induction of $\gamma$H2A.X, a marker of DNA damage response, was detected especially during post-treatment period of either drug. Levels of intracellular reactive oxygen species (ROS) such as hydroxyl radicals and hydrogen peroxide also significantly elevated during and after drug treatment, which possibly caused deleterious damage to the nuclear DNA. To clarify the source of ROS, we evaluated mitochondrial function in the course of senescence induction. Mitochondrial signals detected by fluorescence dye and the copy number of mitochondrial DNA gradually increased during and after drug treatment, whereas mitochondrial membrane potential was temporarily downregulated during drug treatment, indicating partial loss of mitochondrial function. Transient upregulation of PGC-1$\alpha$ and sustainable increase in TFAM protein levels were confirmed by immunoblots whereas expression of mitophagy-associated protein Parkin was suppressed during and after drug treatment. Addition of rapamycin to the culture media containing MG-132 or Bafilomycin A1 rescued induction of premature senescence and recovered cellular proliferation potential possibly by suppressing generation of excess ROS. Transport of SOD2 and GPx4, antioxidant enzymes, into the mitochondria was inhibited on only early period of drug treatment (day 1). It is concluded that temporal deterioration of mitochondrial transport could be a trigger of initial generation of ROS and subsequent induction of mitochondrial biogenesis and cellular senescence. (COI:No)

### O19-4

#### Intestinal nutrient absorption and barrier function in SAMP1 senescence accelerated aged mice

Wendy Hempstock, Fumiya Kurihara, Noriko Ishizuka, Hisayoshi Hayashi (*Dept Physiol, Sch Food and Nutr, Univ of Shizuoka, Japan*)

Aging is an inevitable universal biological process, which can be characterized by a general decline in physiological function with the accumulation of diverse adverse changes and increased probability of death. In the intestine, it is widely believed that with aging, the intestinal barrier function decreases, which leads to "leaky gut". Leaky gut is connected to a wide variety of diseases, including inflammatory bowel disease and colon cancer, which affect many elderly people all over the world. In addition, it is thought that nutrient absorption efficiency is decreased in the aged intestine, which may contribute to malnutrition and muscle wasting in the elderly. As a first step toward understanding the details of cellular senescence in the intestine, we conducted experiments using senescence accelerated mouse prone 1 (SAMP1), which is a strain of mice developed by Jackson Laboratories and the University of Kyoto. SAMP1 mice mature normally and then experience accelerated aging. SAMP1 mice have a lifespan around half that of normal mice, which means they reach old age at around 8-9 months of age, with an average lifespan of 9.7 months. To investigate the effect of aging on intestinal nutrient absorption and barrier function we measured glucose and peptide absorption as well as dilution potential using Ussing chambers. Tissue was also collected for quantitative RT-PCR and immunofluorescence analysis. SAMP1 mice were compared to age-matched SAMR1 mice, which are mice of the same background that do not have the accelerated aging phenotype (average lifespan: 16.3 months). Preliminary results suggest that the large intestine becomes more permeable, or leaky. However, surprisingly, the small intestine of SAMP1 mice seems to become tighter compared to SAMR1 controls. Thus this may be a mechanism by which nutrient absorption is decreased in aged mice. (COI:No)

### O19-5

#### Exendin-4 promotes neurite outgrowth, Schwann cell survival/migration, and myelination in vitro

Kazunori Sango, Shizuka Takaku, Naoko Niimi, Hideji Yako (*Diabetic Neuropathy PJ, Tokyo Met Inst Med Sci, Tokyo*)

Besides its insulinotropic actions on pancreatic $\beta$ cells, the localization of glucagon-like peptide-1 receptor (GLP-1R) at the nervous system suggests neuroprotective activities of GLP-1. Previous studies have suggested the efficacy of a GLP-1 receptor agonist exendin-4 (Ex-4) for functional repair after brain and sciatic nerve injury, and amelioration of Parkinson's disease and diabetic neuropathy. However, the underlying mechanisms for these Ex-4 effects remain unclear. In this study, the effects of Ex-4 on neurite outgrowth of 8-week-old female Wistar rat dorsal root ganglion (DRG) neurons, survival and migration and of immortalized adult Fischer rat Schwann cells IFRS1, and myelination in the DRG neuron-IFRS1 co-culture system were investigated. Ex-4 dose-dependently (1 nM < 10 nM < 100 nM) promoted neurite outgrowth of DRG neurons, and these effects were attenuated by co-treatment with PI3 kinase (PI3K) inhibitor LY294002 (5 $\mu$M and 25 $\mu$M). By MTS and scratch wound assays, 100 nM Ex-4 significantly enhanced survival and migration of IFRS1 Schwann cells, respectively. Moreover, immunofluorescence and western blot analyses performed at 21 days of co-culture revealed that 100 nM Ex-4 significantly increased the number of myelin protein 22 (PMP22)-immunoreactive IFRS1 cells surrounding $\beta$III tubulin-immunoreactive neurites and up-regulated the protein expression of PMP22 and myelin protein zero (MPZ). Western blotting performed at 1 day of co-culture resulted in Ex-4-induced phosphorylation of a serine/threonine kinase AKT, suggesting that Ex-4 accelerates the myelination process in the co-culture via activating PI3K/AKT pathway. Because GLP-1R is expressed in both DRG neurons and IFRS1 Schwann cells, and Ex-4 promotes DRG neurite outgrowth and IFRS1 Schwann cell survival and migration, Ex-4 may act on both cells to promote myelination. These findings imply the efficacy of Ex-4 in accelerating axonal regeneration and remyelination following peripheral nerve injury, as well as preventing and restoring diabetic and other peripheral neuropathies. (COI:No)

# Oral Session 20

## Heart・Circulation 3

(March 19, 14:10〜15:10, Hall 9)

### O20-1

#### Donepezil Treatment Prevents the Progression of Chronic Heart Failure and Improves the Prognosis in Spontaneously Hypertensive Rats with Myocardial Infarction

Meihua Li, Can Zheng, Toru Kawada, Kazunori Uemura, Masashi Inagaki, Masaru Sugimachi
(*Dept cardiovascular dynamics, NCVC, Osaka, Japan*)

**Introduction:** We have demonstrated that acetylcholinesterase inhibition by donepezil improves long-term survival of chronic heart failure (CHF) rats with myocardial infarction (MI). This study aimed to investigate whether donepezil is applicable to the treatment of CHF complicated with hypertension.
**Methods:** CHF was induced by permanent extensive MI in 7-week-old male spontaneously hypertensive rats. After one-week recovery, we implanted a blood pressure transmitter for monitoring daily hemodynamics. Survived animals were randomly assigned to untreated (UT, n = 23) or donepezil treated (DT, n = 22) group. In the DT group, rats received average dose of 5 mg/kg/day of donepezil in drinking water (50 mg/L). The dose of donepezil was chosen in order to decrease the heart rate by 20–30 bpm. At the end of 7-week treatment, the impacts of donepezil were evaluated by hemodynamics, neurohumoral states, immunohistochemistry, morphology, and 50-day survival rate.
**Results:** Compared with UT, DT significantly decreased the heart rate (305 ± 12 vs. 335 ± 11bpm, $P<0.05$), but did not change the mean blood pressure. Although there was no significant difference in the MI size between the two groups, DT improved 50-day survival (76% vs. 43%, $P = 0.006$), through suppressing the progression of cardiac hypertrophy (3.83 ± 0.05 vs. 4.09 ± 0.07g/kg, $P<0.05$) and cardiac dysfunction (cardiac index: 101 ± 4 vs. 89 ± 4 ml/min/kg, $P<0.05$; LVEDP: 12 ± 3 vs. 22 ± 2 mmHg, $P<0.05$). Additionally, DT not only decreased plasma levels of norepinephrine, BNP and AVP, but also improved the systemic inflammation.
**Conclusions:** Donepezil treatment prevented the progression of cardiac remodeling, cardiac dysfunction and improved the prognosis of CHF in spontaneously hypertensive rats with MI, suggesting donepezil may be used as a new pharmacotherapy for patients with CHF complicated with hypertension. (COI:No)

### O20-2

#### Occlusal disharmony increased vulnerability to atrial fibrillation via sympathetic activation in mice

Kenji Suita, Yoshiki Ohnuki, Satoshi Okumura (*Dept Physiol, Tsurumi Univ Sch Dent Med, Yokohama, Japan*)

**Background:** Autonomic nervous system plays an important role to improve cardiac function against acute change of hemodynamics. The β-adrenergic receptor (β-AR) signaling is one of the most important regulators to maintain cardiac function by sympathetic activity. However, persistent sympathetic activation is known to induce cardiac remodeling, leading to cardiovascular diseases such as heart failure and arrhythmias. Atrial fibrillation (AF) is the most prevalent arrhythmia and a source of considerable morbidity and mortality. Tooth loss or incorrect positioning causes occlusal disharmony. Furthermore, tooth loss and AF are both risk factors for ischemic stroke and coronary heart disease. Also, it is shown that occlusal disharmony has adverse effects on other systemic organs via stress-induced autonomic imbalance. Here, we hypothesized that the occlusal disharmony may be a risk factor of AF.
**Results:** Occlusal disharmony was induced by means of bite-opening (BO) for a period of 2 weeks. The duration of AF induced by transesophageal atrial burst pacing were significantly elongated in BO mice than control. Atrial myocyte apoptosis and fibrosis were significantly increased by BO as compared to control mice. Oxidative DNA damage in the myocardium was significantly greater in BO-treated mice than control. Consistent with the atrial remodeling, the CaMKII phosphorylation (threonine-286), CaMKII oxidation (methionine-281/282) and RyR2 phosphorylation (serine-2814) were significantly increased in BO-group than control. The indicators of apoptosis such as Bax/Bcl-2 ratio, activated caspase-9 and activated caspase-3 were significantly increased by BO. Akt phosphorylation (serine-473), an anti-apoptotic factor, was significantly decreased by BO. Co-treatment of propranolol, a β-AR antagonist, significantly prevented BO-induced alterations in the atrial myocytes.
**Conclusion:** Occlusal disharmony may be an inducer of increased AF vulnerability through persistent activation of sympathetic nervous system. (COI:No)

### O20-3

#### Sphingosine kinase-2 is required for autophagic lipid degradation in macrophage and inhibits atherosclerosis

Yoh Takuwa[1], Kazuhiro Ishimaru[1], Kazuaki Yoshioka[1], Noriko Takuwa[1,2], Yasuo Okamoto[1]
([1]*Dept Physiol, Kanazawa Univ Sch Med, Japan,* [2]*Dept Health Sci, Ishikawa Pref Nursing Univ, Japan*)

Atherosclerosis, which underlies ischemic coronary heart diseases, is characterized by the infiltration of cholesterol-accumulating macrophages in the intima of the vasculature. Although sphingolipids are implicated in atherosclerosis as both membrane components and lipid mediators, the precise role of sphingolipids in atherosclerosis remains elusive. We show that genetic disruption of sphingosine kinase-2 (SphK2) but not SphK1 aggravates the formation of atherosclerotic lesions in mice with ApoE deficiency. Bone marrow chimaera experiments show the involvement of SphK2 expressed in myeloid cells. In macrophages, deficiency of SphK2, a major SphK isoform in this cell type, increases cellular levels of sphingosine and ceramides. SphK2-deficient macrophages have increases in lipid droplet-containing autophagosomes and autolysosomes and defective lysosomal degradation of lipid droplets via autophagy with impairment of luminal acidification and proteolytic degradation in the lysosomes. Transgenic overexpression of SphK1 in SphK2-deficient mice prevented aggravation of atherosclerosis and abnormalities of autophagosomes and lysosomes in macrophages, which suggested that two SphKs have at least partially overlapping actions. These results collectively demonstrate that SphK2 is necessary for autophagosome- and lysosome-mediated catabolism of intracellular lipid droplets to prevent the development of atherosclerosis; therefore, SphK2 may be a novel therapeutic target of atherosclerosis. (COI:No)

### O20-4

#### Multi-layer Cardiac Pacemaker Mechanisms demonstrated in Human Induced Pluripotent Stem Cells by Developing Mathematical Cell Models

Hirohiko Kohjitani[1], Shigeya Koda[2], Yukiko Himeno[2], Takeru Makiyama[1], Yuta Yamamoto[1], Yimin Wuriyanghai[1], Daisuke Yoshinaga[3], Asami Kashiwa[1], Akira Amano[2], Akinori Noma[2], Takeshi Kimura[1]
([1]*Dept Cardiovasc Med, Grad Sch Med, Kyoto Univ, Japan,* [2]*Dept Physiol, Facl Life Sci, Sch Life Info, Ritsumeikan Univ, Japan,* [3]*Dept Ped Med, Grad Sch Med, Kyoto Univ, Japan*)

Undifferentiated myocardial cells derived from Human induced pluripotent stem cell (hiPSC-CM) mostly shows spontaneous activity. The difference in the configuration and frequency of spontaneous action potential (AP) may be due to variable expression levels of the ion channels, and thereby the hiPSC-CM may embody mechanisms of various slow diastolic depolarization (SDD). To clarify mechanisms of SDD, we first created whole 3 types of hiPSC-CM mathematical models. Then, specific role of each ion channel in generating SDD was examined by changing the relative amplitude of delayed rectifier K+ current (IKr), inward-rectifier K+ current (IK1), L-type Ca2+ current (ICaL + Ist), hyperpolarization-activated current (Iha), and IbNSC to determine the range of combinations that allow generation of spontaneous action potential. The lead potential analysis well quantified the contribution of these currents, and we could identify two basic mechanisms and two additional mechanisms. The primary mechanism 1 is the removal of inactivation of IKr on repolarization (y1 gate) and subsequent deactivation (y2 + y3 gates) during 100 to 200 ms of DD. The primary mechanism 2 is caused by positive feedback process among inward currents i.e. ICaL and Ist, T-type Ca2+ current (ICaT), inward Na+ current (INa), and current of Na+/Ca2+ exchanger (INCX). These primary mechanisms induce the typical sinus node low membrane potential oscillation. High membrane potential oscillation is induced by the secondary two channel mechanisms; activation and subsequent deactivation of Iha, and unblocking and re-blocking of IK1 by intracellular factors. These mechanisms work between the two primary mechanisms, but gradually relax by themselves and evoke relatively lower rhythm of SDD. Finally, IbNSC is mainly responsible for determining the negative limit of the maximum diastolic potential, while IKr, IKur, IKto induces repolarization from the plateau potential. If these repolarizing reserves are limited, the oscillation is stopped at the plateau potential near -20 mV. (COI:No)

### O20-5

#### Regulation of Blood Pressure and Cardiac Output during Chronic Alpha1-Adrenergic Stimulation

Jean-Pierre Montani[1], Bruce N. Van Vliet[2]
([1]*Faculty of Science and Medicine, University of Fribourg, Switzerland,* [2]*Division of Basic sciences, Memorial University of Newfoundland, St. John's, Canada*)

**Background:** The alpha1-adrenergic receptor plays a key role in cardiovascular regulation. The main objective of this study was to evaluate the long-term control of blood pressure and cardiac output during chronic vasoconstriction induced by alpha1-adrenergic stimulation.
**Methods and results:** We infused phenylephrine (PE) intravenously (1 microg/kg/min) for 10 days in 7 dogs housed in metabolic cages and maintained on a fixed sodium intake. Mean arterial pressure (MAP) and cardiac output (CO) were monitored continuously 20 hours/day. On the first day, PE caused a rapid increase in MAP from a control value of 91 ± 4 mmHg to 113 ± 8 mmHg, and decreased both CO and heart rate from 2.32 ± 0.10 L/min and 69 ± 3 beats/min to 1.77 ± 0.06 L/min and 51 ± 2 beats/min, respectively. The initial increase in MAP was not sustained as MAP stabilized then at a value of 99 ± 4 mmHg (days 8-10) whereas CO and heart rate remained decreased at 1.74 ± 0.09 L/min and 54 ± 2 beats/min, respectively (days 8-10). Total peripheral resistance increased initially by 62% and remained elevated by ~50% throughout the infusion. However, due to an increase in hematocrit from 37.9 ± 1.9 to 52.2 ± 3.6, arterial oxygen delivery, estimated by the product of CO and hematocrit, was maintained.
**Summary and conclusions:** Chronic vasoconstriction led to a mild hypertension with a sustained decrease in heart rate and cardiac output. However, despite the low cardiac output, oxygen delivery to the tissues was maintained secondary to an increase in hematocrit. Our data suggest that arterial oxygen delivery rather than cardiac output itself is a strongly regulated variable. (COI:No)

# Oral Session 21

## Heart・Circulation 4

(March 19, 15:10～16:10, Hall 9)

### O21-1
#### Contribution of afferent stimulation to hemodynamic and myocardial acetylcholine release during vagal nerve stimulation

Toru Kawada[1], Takashi Sonobe[2], Takuya Nishikawa[1], Meihua Li[1], Tsuyoshi Akiyama[2], James Pearson[2], Masaru Sugimachi[1] (*[1]Dept Cardiovasc Dynamics, Natl Cereb Cardiovasc Ctr, [2]Dept Cardiac Physiol, Natl Cereb Cardiovasc Ctr*)

**Purpose:** Although vagal nerve stimulation (VNS) is considered to be a new treatment strategy for heart failure, clinical trials of VNS showed diverse efficacy results. This study aimed to elucidate the contribution of afferent stimulation to hemodynamic and myocardial interstitial acetylcholine (ACh) release during VNS.
**Methods:** We measured arterial pressure (AP), heart rate (HR), and ACh responses to 10-min right VNS in anesthetized Wistar-Kyoto rats. The experiment was performed in the rats with intact vagi (INT, n=7) and the rats whose right vagus was sectioned at the afferent portion of the stimulation site (VAX, n=6). VNS was tested with a stimulation voltage of 3 V or 10 V and a stimulation frequency of 5 or 20 Hz. The pulse duration was fixed at 0.1 ms.
**Results:** The high-voltage VNS (10 V, 5 Hz or 20 Hz) produced significant initial reductions in AP and HR in less than 10 s in the INT group. These initial AP and HR responses were not observed in the VAX group. The high-frequency VNS (20 Hz, 3 V or 10 V) significantly increased myocardial interstitial ACh levels in both the INT and VAX groups.
**Conclusion:** The initial hemodynamic responses, observed during high-voltage VNS in the INT group, were attributable to afferent stimulation. Unmyelinated afferent C fibers may be responsible for these initial hemodynamic responses because high-voltage VNS was required for the responses. Myocardial interstitial ACh release, observed during high-frequency VNS in both the INT and VAX groups, was mainly attributable to efferent stimulation. Lightly myelinated efferent B fibers may be responsible for the myocardial ACh release. (COI:No)

### O21-2
#### A potential role for GRK2 in the development of right ventricular dysfunction in pulmonary arterial hypertension

Mark T. Waddingham [1], Hirotsugu Tsuchimochi[2], Ryotaro Asano[1], Mikiyasu Shirai[1], James T. Pearson [2], Takeshi Ogo[1] (*[1]Pulmonary Hypertension, NCVC, Osaka, Japan, [2]Cardiac Physiology, NCVC, Osaka, Japan*)

**Purpose:** Pulmonary arterial hypertension (PAH) is associated with right ventricular (RV) dysfunction. The mechanisms leading to RV dysfunction remain unclear, although overactivation of the sympathetic nervous system (SNS) appears to be a central event. G protein-coupled receptor kinase 2 (GRK2) overexpression is known to enhance SNS overactivation in heart failure. GRK2 has also been shown to negatively modulate nitric oxide bioavailability, which can promote diastolic dysfunction and myofilament dysfunction. However, the connection between GRK2, the myofilaments and diastolic dysfunction has not been examined in RV dysfunction in PAH. Therefore, our aim was to evaluate changes in GRK2 expression over the time-course of RV dysfunction development and relate these to changes in global RV function and myofilament function in a rat model of PAH.
**Methods:** PAH was induced in rats using the Sugen/10% chronic hypoxia (SuHX) method, and rats were subsequently housed in normoxic conditions for 3 or 6 weeks. We simultaneously examined global RV function and myofilament function in anesthetized rats using cardiac catheterization and muscle x-ray diffraction, respectively.
**Results:** SuHx rats exhibited significantly increased RV end systolic pressure ($P<0.05$), indicative of PAH. An increase in RV end diastolic pressure and RV tau relaxation constant was also observed, suggesting early diastolic dysfunction in PAH rats. Muscle x-ray diffraction experiments revealed a progressive decline in RV diastolic myosin mass in PAH rats. We found a progressive decline in relative eNOS phosphorylation and a significant increase in iNOS ($P<0.01$) and GRK2 ($P<0.01$) expression in SuHx rats 6 weeks after induction. Importantly we found a correlation between GRK2 expression and iNOS ($P<0.0001$) expression and eNOS phosphorylation ($P<0.05$).
**Conclusion:** These data suggest that GRK2 may drive the development of RV dysfunction, potentially by modulating myofilament function in PAH. (COI:No)

### O21-3
#### The effects of fragrance inhalation of Lavender essential oil on blood pressure and muscle sympathetic nerve activity

Eriko Kawai[1], Daiki Imai[1, 2], Kosuke Saho[1], Emiko Morita[1], Yuta Suzuki[1, 2], Hisayo Yokoyama[1, 2], Kazunobu Okazaki[1, 2] (*[1]Dept of Environ Physiol for Exerc, Osaka City Univ Grad Sch of Med, Osaka, Japan, [2]Res Ctr for Urban Health & Sports, Osaka City Univ, Osaka, Japan*)

**Introduction:** Previous animal studies reported that fragrance inhalation of Lavender essential oil decreased sympathetic nerve activity as well as blood pressure (BP). We recently found in humans that fragrance inhalation of Grapefruit essential oil increased diastolic BP which was associated with an increase in muscle sympathetic nerve activity (MSNA). The purpose of this study was to investigate the effect of the fragrance inhalation of Lavender essential oil on BP and MSNA.
**Methods:** Young healthy subjects were participated in this study (men; n=7, woman; n=1). During subjects in a sitting position, they inhaled brank air via face mask for 10 min as baseline (BL), then inhaled air included fragrance components of Lavender essential oil collected in a Douglas bag for 10 min. Beat-by-beat BP and heart rate (HR), and MSNA (peroneal nerve, microneurography) were continuously recorded during experiment.
**Results:** Diastolic BP (BL, 56.4 ± 5.9 mmHg; inhalation, 55.0 ± 5.6 mmHg) and MSNA burst frequency (BL, 18.0 ± 6.2 bursts/min; inhalation, 15.8 ± 6.4 bursts/min) decreased significantly during fragrance inhalation of Lavender essential oil compared to BL. On the other hand, systolic BP and HR remained unchanged with fragrance inhalation.
**Conclusion:** The fragrance inhalation of Lavender essential oil has the effects to decrease diastolic BP and MSNA. (COI:No)

### O21-4
#### Pressure-volume analysis of rat's micturition cycles in vivo

Tzer-Bin Lin (*Dept Physiol School Med, Taipei Med Univ, Taiwan*)

Though comprehensive works have established the pressure-volume (PV) analysis for cardiac functions, the potential application of this technique in the urinary bladder, which functions resemble to the heart as it is continuously filled with fluid and periodically contracts to propel fluid against resistance, has not been established.
In forty urethane-anesthetized (1.2 g/kg) female Sprague-Dawley rats, cystometry was conducted to record voiding contractions induced by a constant saline infusion (0.04 ml/min); and the PV relationship was established by plotting intra-vesical pressure (IVP) against intra-vesical volume (IVV).
In a micturition cycle, PV data point moved counterclockwise and the PV trajectory shaped an enclosed loop in the plane. PV loops coincided well and the loop-derived urodynamic parameters kept stable under a constant saline infusion (0.04 ml/min).
Enhancing preload by elevating infusion rates (to 0.08 and 0.12 ml/min) increased the area enclosed by the PV loop (Apv) and shifted loops to the right and slightly upward. Augmenting afterload by increasing outlet resistance (to 1/4 and 1/2 urethra clamping) increased Apv and shifted loops to the right and upward. Without affecting Apv, muscarine (0.01 and 0.1 mM)-induced inotropic states enhanced peak and voiding pressures but reduced end-filling and filling volumes that was as opposed to the atropine (0.01 and 0.1 mM)-induced anti-inotropic state.
Collectively, PV analysis not only reliably assayed baseline bladder function but also validly assessed modified bladder functions both in response to altered external environment and bladder contractility itself; thereby could provide a novel dimension in cystometry exploring bladder functions/diseases. (COI:No)

### O21-5
#### Prediction of the hemodynamic impact of positive end-expiratory pressure using the framework of circulatory equilibrium in dog

Takuya Nishikawa, Kazunori Uemura, Toru Kawada, Yohsuke Hayama, Masaru Sugimachi (*Dept Cardiovascular Dynamics, NCVC, Japan*)

**Background:** Positive end-expiratory pressure (PEEP) is the mechanical ventilation that applies pressure above atmospheric pressure at the end of expiration. PEEP prevents alveolar collapse, improves pulmonary congestion, and improves respiratory status in patients with congestive heart failure (HF). However, PEEP suppresses venous return through an increase in intrapleural pressure, and reduces cardiac output (CO). The lack of understanding of quantitative relation between PEEP and hemodynamics makes it difficult to set optimal PEEP. Therefore, we aimed to quantitatively predict the impact of PEEP on hemodynamics using the framework of circulatory equilibrium.
**Theoretical consideration:** The framework of circulatory equilibrium defines circulatory equilibrium as the intersection of cardiac output curve (COC) and venous return surface (VRS). PEEP increases the intrapleural pressure and shifts COC rightward and VRS upward. The intersection of shifted COC and VRS represents hemodynamics under PEEP.
**Method:** Two mongrel dogs were pre-implanted with a flow probe to measure CO. We measured CO, central venous pressure (CVP) and left ventricular end-diastolic pressure (LVEDP) under closed-chest and then increased PEEP stepwise. Using our proposed framework, we predicted the hemodynamic impact of PEEP and compared them with those measured. To reproduce the various pathological conditions, we induced volume overload by the plasma expanders and HF by embolizing coronary artery with the glass microspheres. We repeated the hemodynamic prediction protocol under each condition of volume load and HF.
**Result:** The estimated CO ($r^2$=0.95, root mean squared error [RMSE] 1.4 ml/min/kg), CVP ($r^2$=0.86, RMSE 0.33 mmHg) and LVEDP ($r^2$=0.88, RMSE 0.17 mmHg) matched well with those measured regardless of the conditions of volume overload or HF.
**Conclusion:** The framework of circulatory equilibrium can quantitatively predict the hemodynamic modulation by PEEP. It may contribute to the optimal setting of PEEP in patients with HF. (COI:No)

# Poster Presentations

## Day 1

（March 17, 14:20～15:20）

| | |
|---|---|
| **1P-001～1P-012** | Ion Channel・Receptor（1） |
| **1P-013～1P-024** | Heart・Circulation（1） |
| **1P-025～1P-036** | Neuron・Synapse（1） |
| **1P-037～1P-046** | Sensory Function（1） |
| **1P-047～1P-053** | Behavior Science・Biorhythm（1） |
| **1P-054～1P-056** | Neurochemistry（1） |
| **1P-057～1P-061** | Autonomic Nervous（1） |
| **1P-062～1P-067** | Muscle Physiology（1） |
| **1P-068～1P-072** | Oral Physiology（1） |
| **1P-073～1P-077** | Endocrinology（1） |
| **1P-078～1P-079** | Kidney・Urination（1） |
| **1P-080～1P-085** | Motor Function（1） |
| **1P-086～1P-088** | Development・Growth・Aging（1） |
| **1P-089～1P-095** | Cell Physiology・Molecular Physiology（1） |
| **1P-096～1P-101** | Environmental Physiology（1） |
| **1P-102～1P-104** | Drug Actions（1） |
| **1P-105～1P-111** | CNS Function（1） |
| **1P-112～1P-117** | Nutrition・Metabolism・Thermoregulation（1） |
| **1P-118～1P-123** | Pathophysiology（1） |
| **1P-124～1P-125** | Physical Fitness・Sports Medicine（1） |
| **1P-126～1P-127** | Respiration（1） |
| **1P-128～1P-129** | Study Methodology（1） |
| **1P-130～1P-132** | Others（1） |

## 1P-001

### Anti-integrin antibodies enhance axon extension and electric guidance

Masayuki Yamashita (*Centr Basic Med Res, Int Univ Health & Welfare, Japan*)

Growing axons are directed not only by chemical signals but also by electric fields in a process known as galvanotropism. The axons of embryonic brain, spinal cord, and retina extend along the extracellular voltage gradient towards the cathode. In the embryonic central nervous system, endogenous positive DC potentials are generated by neuroepithelial cells' sodium transport, of which disruption results in erroneous path-finding of newborn neurons' axons (Yamashita, ***BBRC***, 2013). This means that the electric field is essential for orienting axons during embryonic development. However, there is no experimental evidence for the cell surface molecule that is responsible for galvanotropism. The axons of embryonic neurons express integrin, although the role for integrin in axon development was unknown. Here I show that integrin regulates axon extension and is involved in the electric axon guidance. Retinal strips of chick embryos were cultured in the electric field of the same strength as that *in vivo* (15 mV/mm). To maintain the environment for retinal ganglion cell (RGC) axon extension, the retinal strip was embedded in Matrigel®, since Matrigel® and the inner limiting membrane, onto which RGC axons extend expressing integrin $\alpha 6\beta 1$, contain the extracellular matrix ligands, laminin and collagen. RGC axons extended from the retinal strip towards the cathode. A monoclonal anti-chicken integrin $\beta 1$ antibody TASC significantly enhanced the cathodal growth. The enhancement of the cathodal growth depended on the concentration of TASC. Another monoclonal anti-chicken integrin $\beta 1$ antibody W1B10 also enhanced the cathodal growth in a different dose-dependent manner: it was more effective than TASC at low concentrations ($\leqq 50\,\mu g$/mL). The negative control isotype antibody mouse IgG1 showed no enhancement of the cathodal growth even at a high concentration ($200\,\mu g$/mL). TASC and W1B10 also increased axon extension without electric fields. This indicated that integrin regulates axon extension itself. (COI:No)

## 1P-002

### TRPV1 is a physical and regulated component of the natriuretic peptide signaling system

Tatsuki Kurokawa[1,2], Jaime Horton S.[3], Takuya Shiraishi[2], Naghum Alfulaij[3], Andrea Small-Howard L.[4], Helen Turner C.[5], Yasuo Mori[2], Alexander Stokes J.[3]
([1]*Dept Physiol, Grad Sch Med, Oita Univ, Japan,* [2]*Grad Sch Eng, Kyoto Univ,* [3]*Univ Hawaii,* [4]*GB Sci Inc,* [5]*Chaminade Univ*)

Activation of the atrial natriuretic signaling pathway is intrinsic to the pathological responses associated with a range of cardiovascular diseases that stress the heart, especially those involved in sustained cardiac pressure overload which induces hypertrophy and the pathological remodeling that frequently leads to heart failure. We identify transient receptor potential cation channel, subfamily V, member 1, as a regulated molecular component, and therapeutic target of this signaling system. TRPV1 is expressed in cardiac myocytes, but we understand relatively little of the potential regulatory coupling of TRPV1 to pathways that control heart physiology, and the longitudinal impact of TRPV1 inhibition in heart health under conditions of applied pathology has some attendant controversies. Data show that TRPV1 is a physical component of the natriuretic peptide A, cGMP, PKG signaling complex, interacting with the Natriuretic Peptide Receptor 1 (NPR1), and upon binding its ligand, Natriuretic Peptide A (NPPA, ANP). TRPV1 activation is subsequently suppressed through production of cGMP and PKG mediated phosphorylation of the TRPV1 channel. Further, inhibition of TRPV1, with orally delivered drugs, suppresses chamber and myocyte hypertrophy, and can longitudinally improve in vivo heart function in mice exposed to chronic pressure overload induced by transverse aortic constriction, reversing pre-established hypertrophy induced by pressure load while restoring chamber function. TRPV1 is a physical and regulated component of the natriuretic peptide signaling system, and TRPV1 inhibition may provide a new treatment strategy for treating, and reversing the loss of function associated with cardiac hypertrophy and heart failure. (COI:No)

## 1P-003

### Long-lasting calcium increase is induced by dopamine via D1 receptor in hypothalamic orexin neurons

Yasutaka Mukai[1,2,3,4], Hidenori Aizawa[5], Kenji Tanaka F.[6], Takeharu Nagai[7], Akihiro Yamanaka[1,2,3]
([1]*Neuroscience II, RIEM, Nagoya Univ, Japan,* [2]*Neural regulation, Grad Sch Med, Nagoya Univ, Japan,* [3]*CREST, JST, Japan,* [4]*Research Fellowship, JSPS, Japan,* [5]*Dept Neurobiol, Grad Sch Biomed and Health Sci, Hiroshima Univ, Japan,* [6]*Dept of Neuropsychiatry, Sch of Med, Keio Univ, Japan,* [7]*Dept Biomol Sci and Eng, ISIR, Osaka Univ, Japan*)

Sleep/wakefulness switches in seconds to hours-time scale in mammals from mice to humans. Orexin neurons which produce a neuropeptide orexin in the lateral hypothalamic area (LHA) are important for maintenance of wakefulness. Preceding studies reported several substances regulate activity of the orexin neurons. However, most of the studies based on electrophysiological recordings measured by in milliseconds to minutes-time scale. Therefore, a mechanism underlying activity change in range of seconds to hours-time scale which comparable with sleep/wakefulness state change is still elusive. We previously found that dopamine (DA) induced long-lasting increase of intracellular calcium concentration in orexin neurons in acute brain slices for more than 1 hour. Furthermore, we found the DA-induced increase in intracellular calcium concentration was diminished by dopamine $D_1$-like receptor antagonist, SCH-23390.

In this study, we introduced in vivo genome-editing to examine receptors implicated in the mechanism of long-lasting calcium increase. We used an adeno associated-virus (AAV) encoding both Cas9 (SaCas9) and a short guide RNA (sgRNA) designed to recognize dopamine $D_1$ receptor (D1R) encoding gene (*Drd1*). To knockout D1R, we unilaterally injected the AAV into the LHA of transgenic mice expressing a calcium indicator yellow Cameleon-Nano50 exclusively in orexin neurons. Four weeks after AAV injection, we made acute brain slices and separated them into hemispheres, D1R-knockedout side and D1R-intact side. Then we applied DA for 2 minutes through perfusion and monitored calcium activity for more than 2 hours. As a result, D1R-knockedout side showed diminished calcium increase induced by DA compared with D1R-intact slice. This result suggested that long-lasting increase induced by DA is mediated through D1R. We are now trying to find out its physiological roles in vivo. (COI:No)

## 1P-004

### Analysis of the structural dynamics of Two-pore $Na^+$ channel 3

Ki-ichi Hirazawa[1,2], Takushi Shimomura[1,2], Yoshihiro Kubo[1,2] ([1]*Div Biophys and Neurobiol, Natl Inst Physiol Sci,* [2]*Dept Physiol Sci, SOKENDAI*)

Two-pore $Na^+$ channel 3 (TPC3) is a voltage-gated $Na^+$ channel. Its single polypeptide has 2 repeats of canonical motif of voltage-gated cation channels, composed of 6 transmembrane helices (6TM). Each of the 6TMs has one voltage sensor domain (VSD) and one pore domain (PD). Previously we revealed that the 2nd VSD of TPC3 is responsible for voltage sensing, whereas the 1st 6TM contains a phosphoinositide (PI) binding site, which potentiates voltage sensitivity of TPC3 gating. We also revealed PI dependent potentiation needs a functional linkage between the 1st 6TM and 2nd 6TM, through one electrostatic interaction between the 1st PD and 2nd PD. These findings suggest that PI binding to the 1st 6TM can modulate voltage dependent structural change of the 2nd VSD via functional coupling between the 1st 6TM and 2nd 6TM. To reveal how this PI binding affects voltage dependent structural changes of the 2nd VSD, we performed voltage clamp fluorometry analysis (VCF) of TPC3 heterologously expressed in *Xenopus* oocytes. In VCF, along with TPC3 current, we simultaneously recorded local structural changes of TPC3 based on the fluorescent intensity from fluorophore attached to the specific site in TPC3. We successfully detected voltage dependent structural changes of the 2nd VSD which could cause pore gating. Furthermore, when we manipulated PI concentration within oocyte by the co-expression of voltage sensitive phosphatase, structural change of the 2nd VSD was affected by PI concentration change. The effect of PI binding on the 2nd VSD movement could be further supported by the analysis of one TPC3 mutant which has a deficiency of its PI binding. Therefore, PI binding may regulate the voltage sensitivity of TPC3 gating through potentiation of the movement of the 2nd VSD. (COI:No)

## 1P-005

### Analyzing PI(4, 5)P2 sensitivity of $GABA_A$ receptor by voltage sensing phosphatase

Shunichi Sugimoto, Takafumi Kawai, Yasushi Okamura (*Dept Phyiol, Grad Sch Med, Osaka Univ, Japan*)

Ion channels play major roles in physiological processes in diverse biological systems. Recent studies indicate that many types of ion channels are directly controlled by phosphatidylinositol-4, 5-bisphosphate (PIP2), which is important for diverse cellular processes. However, PIP2-sensitivity of type $\gamma$-aminobutyric acid receptor ($GABA_AR$), major inhibitory pentameric ligand-gated ion channels in the central nervous system, remains unknown. Interestingly, recent studies in structural biology found that each $GABA_AR$ binds to PIP2 and that there are potential PIP2-binding sites in the $\alpha 1$ subunit, raising a possibility that function of $GABA_AR$ is regulated by PIP2 (Laverty D, et al. Cryo-EM structure of the human $\alpha 1\beta 3\gamma 2$ $GABA_A$ receptor in a lipid bilayer. Nature. 2019). Furthermore, in that paper, there were significant differences in the potential PIP2-binding sites among different $\alpha$ subunits. Therefore, we hypothesized that $GABA_AR$ is sensitive to PIP2 and that there is a difference in PIP2-sensitivity among different $\alpha$ subunits. To examined it, we used voltage sensing phosphatase (VSP), which shows voltage-dependent phosphatase activity against PIP2. We studied *Xenopus* oocytes expressing Ciona intestinalis VSP (Ci-VSP) and $GABA_AR$, and evaluated the PIP2-sensitivity of $GABA_AR$ by depolarization-induced depletion of PIP2. High PIP2-sensitivity of $GABA_AR$ was observed in the *Xenopus* oocytes expressing some types of $\alpha$ subunits together with $\beta$ and $\gamma$ subunits. We also discuss the relationship of the present results with the PIP2-binding sites revealed by structural biology. (COI:No)

## 1P-006

### Alteration in the coupling between voltage sensor movement and phosphatase activity in voltage-sensing phosphatase with mutation in voltage sensor domain

Natsuki Mizutani, Yasushi Okamura (*Dept Integrative Physiol, Grad Sch Med, Osaka Univ, Suita, Japan*)

Voltage-sensing phosphatase (VSP) shows phosphatase activity toward phosphatidylinositol 4, 5-bisphosphate (PI(4, 5)$P_2$) upon membrane depolarization. Voltage sensor deficient mutants lack phosphatase activity and mutations in a short linker between voltage sensor domain (VSD) and the PTEN-like phosphatase domain reduce phosphatase activity, suggesting that activation of VSD "couples" with phosphatase activity via the linker. However, it is unclear how VSD regulates phosphatase activity. We have recently found that amino acid mutation at F234 (two amino acids downstream of the 4th arginine (R4) of S4) in *Ciona intestinalis* VSP (Ci-VSP) causes a remarkable decrease in the phosphatase activity whereas the gating current is normal. To understand what structural detail of VSD is optimal for inducting enzyme activity in Ci-VSP, we analyzed both VSD motions and enzyme activities of F234 mutants and other VSD mutants. Mutants of Ci-VSP were expressed in *Xenopus* oocytes and analyzed by the two-electrode voltage clamp. We studied the voltage-driven motion of VSD or cytoplasmic catalytic region with thiol-reactive fluorescent dye or with unnatural fluorescent amino acid, Anap, respectively. Surprisingly, in multiple mutants including F234 mutant, phosphatase activity remarkably decreased whereas the gating current remained intact. We interpret these findings based on the hypotheses: (1) VSD cannot be fully activated, leading only partial phosphatase activity, (2) the structure of the fully-activated VSD differs from that of the normal full activation state, failing to induce the full phosphatase activity, (3) local membrane structure or environment facing the cytoplasmic side of the VSD may be altered by the mutations, possibly preventing the interaction between enzyme region and the plasma membrane which may be critical for phosphatase activity (the second and the third idea are not exclusive to each other). Detailed analysis will provide insights into molecular mechanisms by which VSD motion leads to phosphatase activity in VSP. (COI:No)

## 1P-007

### Activation of the THIK-2 channel by Gi/o and Gq coupled receptors

Michihro Tateyama[1,2], Yoshihiro Kubo[1,2] ([1]*Div Biophys & Neurosci, NIPS, Japan,* [2]*SOKENDAI, Dept Physiological Sci*)

We previously reported that the THIK-1 channel, a member of the two-pore-domain potassium channel family, is activated both by Gi/o- and Gq-coupled receptors. As the primary structure is highly conserved in the THIK channel family, we expected that Gi/o- and/or Gq-coupled receptors might regulate the THIK-2 channel. Here we examined the effects of receptors on the activity of the THIK-2 channel expressed in CHO cells by patch clamp recording. As the intracellular N-terminal cytoplasmic residues of the THIK-2 channel is known to inhibit the expression on the plasma membrane, most of the N-terminal residues were deleted (ΔN-THIK-2) to increase the expression level. Although the current density of the ΔN-THIK-2 channel was small (nearly 4 pA/pF), the density was increased about 3 or 10 times by stimulation of the co-expressed Gi/o-coupled metabotropic glutamate receptor (mGlu2) or Gq-coupled adrenergic $\alpha$1A-receptor ($\alpha$1A-AR), respectively. The effect of mGlu2 was inhibited by incubation of cells with pertussis toxin (300 ng/mL) for more than 16 hours, indicating that the effect of mGlu2 is mediated by Gi/o. The effect of $\alpha$1A-AR was inhibited by the treatment of cells with a PLC inhibitor, U73312 (2 $\mu$M, 10 min), showing that the effect is mediated by PLC. These results clearly showed that the THIK-2 channel can be activated both by Gi/o- and Gq-coupled receptors. (COI:No)

## 1P-008

### Physiological roles of Prrt3, an orphan metabotropic receptor: Comprehensive behavioral test battery analysis using homozygous full gene knock-out mice derived from flox mice

Tomomi Yamamoto[1], Satoko Hattori[2], Li Zhou[3], Rie Natsume[3], Kohtarou Konno[4], I-Shan Che[1], Masahiko Watanabe[3,4], Kenji Sakimura[3], Tsuyoshi Miyakawa[2], Yoshihiro Kubo[1] ([1]*Div Biophys & Neurobiol, Natl Inst Physiol Sci,* [2]*Div Sys Med Sci, Inst Comprehensive Med Sci, Fujita Med Univ,* [3]*Dept Cellular Neurobiol, Brain Res Inst, Niigata Univ,* [4]*Dept Anatomy, Faculty of Med, Hokkaido Univ*)

Prrt3 is an orphan metabotropic receptor with a long N-terminus extracellular region, and it belongs to family C which mGlu and GABAB receptors also belong to. We previously showed by immunohistochemical study that Prrt3 protein is chiefly expressed in the thalamus, cerebellar cortex, hippocampus and substantia nigra in the brain and that it is preferentially expressed in the presynaptic terminal. We previously raised gene knock out (KO) mice using ES cells obtained from KOMP Repository in which all exons are targeted, but homozygous (homo) KO mice showed high mortality. By the DNA microarray analysis, the influence of dramatic change of expression of flanking genes was suspected. Thus, we constructed Prrt3 gene flox mice and raised, by crossing with ActB-Cre mice, full KO mice whose exon 2 and 3 of Prrt3 genome is lost. A complete loss of Prrt3 protein in the homo KO mice brain was confirmed by immunohistochemical staining. We performed comprehensive behavioral test battery analysis using homo flox and flox derived homo KO mice. By the Barnes probe test to analyze the spatial memory, we observed a significant decrease in the retention of spatial memory after 4 weeks interval from the completion of training. The scores of homo KO mice of Latency to the 1st arrival at the target hole, Error to 1st and Distance to 1st were all significantly higher than those of homo flox mice. However, the time spent around the target hole did not show significant difference. The data could be interpreted that that the homo KO mice remember the target location but do not remember well the way to reach there. These results provide us with clues toward the understanding of physiological roles of Prrt3. (COI:No)

## 1P-009

### Two aromatic residues in the extracellular loop structure of the FMRFamide-gated Na+ channel is critical for the channel activation

Yasuo Furukawa[1], Iori Tagashira[2] ([1]*Lab Neurobiol, Grad Sch Int Sci Life, Hiroshima Univ, Japan,* [2]*Lab Neurobiol, Grad Sch Int Arts & Sci, Hiroshima Univ, Japana*)

FMRFamide-gated $Na^+$ channel (FaNaC) is a homo-trimeric peptide-gated sodium channel, which is activated by a molluscan cardioactive peptide, FMRFamide. FaNaC is a member of DEG/ENaC family, and the extracellular domain of the DEG/ENaC channels is divided into five subdomains (Finger, Knuckle, Thumb, Palm, and beta-ball domains).

We previously reported that six aromatic residues in the Finger domain which are conserved among molluscan FaNaCs are involved in the FMRFamide sensitivity of *Aplysia* FaNaC, AkFaNaC, (Furukawa and Tagashira, FAOPS2019). In the same study, we also carried out the docking simulation, and found that the mutation of those aromatic residues affects the docking of FMRFamide. These results suggest that the conserved aromatic residues in the Finger domain may construct the FMRFamide binding site.

In the present study, we further examined the function of two other conserved aromatic residues (F188, Y189) in the loop structure within the Finger domain. We found that the FMRFamide sensitivity of AkFaNaC is substantially decreased if either F188 or Y189 is changed to valine, and that the double mutant (F188V-Y189V) is practically insensitive to FMRFamide. The FMRFamide docking simulations, however, failed to show obvious differences in the results between the wild-type channel and the mutant channels. These results may be explained if F188 and Y189 are involved in the activation steps following the initial ligand binding step. (COI:No)

## 1P-010

### Temperature preference analysis by using thermal gradient assay

Tomoyo Ujisawa[1], Sandra Derouiche[1,2], Makoto Tominaga[1,2] ([1]*Thermal Biology Group, ExCELLS, Natl Inst of Natural Sci, Japan,* [2]*Div of Cell Signaling, Natl Inst for Physiol Sci, Natl Inst of Natural Sci, Japan*)

Temperature perception is a critical ability for the survival of all animals in order to determine viable thermal environments and to avoid damage caused by extreme temperatures. The thermosensitive transient receptor potential (thermoTRP) ion channels are the molecular thermal sensors that allow the monitoring of environmental temperature. Each of these ion channels has a specific range of temperature sensitivity. However, the precise involvement of the thermoTRPs in temperature selection and temperature avoidance behaviors remains unclear. Several temperature behavioral assays are used to quantify thermal preference such as a two-plate choice assay or a linear gradient assay. But, these assays lack thermal resolution and many biases are present. A circular thermal gradient assay using Thermal Gradient Ring (TGR) was introduced recently. It allows to determine more accurately the thermal selection behaviors of mice by providing high thermal resolution and by eliminating corner artifacts. We hope to find features between temperature thresholds at a molecular level and temperature preference at an individual level, and assess more precise contribution of each thermoTRP to thermal preference by using this new circular gradient assay and several TRP-deficient mice. (COI:No)

## 1P-011

### Molecular mechanism of the GON domain in maintaining calcium balance

Sawako Yoshina[1], Shohei Mitani[1,2] ([1]*Dept Physiol, TWMU,* [2]*TIIMS*)

ADAMTS9 is a metalloprotease that cleaves components of the extracellular matrix and is also implicated in intracellular protein transport. ADAMTS9/GON-1 has a unique C-terminal domain called the GON domain. The function of intracellular protein transport is dependent on the GON domain but independent of protease activity. However, molecular mechanisms of ADAMTS9 in cells remain unknown.

To investigate the intracellular role of ADAMTS9/GON-1, we searched for genes whose depletion suppressed the *gon-1* phenotype. We identified several suppressor genes. To determine whether the GON domain interacts with the suppressor genes, we performed immunoprecipitation experiments in HEK293 cells transfected with the Flag-tagged GON domain and Myc-tagged candidate proteins. We found that the GON domain interacts with several suppressor gene products. The suppressor genes included a molecule involved in ubiquitination of inositol 1, 4, 5-trisphosphate receptor (IP3R). IP3R is an IP3-gated ion channel that releases $Ca^{2+}$ from the ER. Next, we investigated whether ADAMTS9/GON-1 is involved in intracellular calcium homeostasis. We found that the GON domain depletion increased $Ca^{2+}$ leak from the ER lumen to the cytosol. Furthermore, ubiquitination of IP3R was increased by ADAMTS9 depletion. Now, we are investigating how the GON domain is involved in calcium homeostasis. (COI:No)

## 1P-012

### Involvement of thermosensitive TRP channels in temperature-dependent microglia movement

Sandra Derouiche[1,2], Rei Nishimoto[3], Kei Eto[4], Junichi Nabekura[4], Makoto Tominaga[1,2] ([1]*Division of Cell Signaling (NIPS), Okazaki Japan,* [2]*Thermal Biology Group (ExCELLS), Okazaki, Japan,* [3]*Department of Anesthesiology and Resuscitology, Okayama University Hospital, Okayama, Japan,* [4]*Division of Homeostatic development (NIPS), Okazaki, Japan*)

Microglia maintain the homeostasis of the central nervous system and migrate via chemotaxis in their activated state. Interestingly, hypothermia was shown to reduce the microglial migration induced by ischemia, suggesting a modulation of microglia movement by temperature. Although several ion channels and transporters are known to support the microglia movement, the molecular mechanisms behind the temperature-dependent movement of microglia have not yet been elucidated. Some members of the transient receptor potential (TRP) channels superfamily with thermosensitivity, constitute strong candidates for the mediation of this phenomenon. Here, we investigate the regulation of mouse microglia movement by temperature and the involvement of thermosensitive TRP channels. All together our in vitro and in vivo results suggest a role of TRPM2, TRPM4 and TRPV4 channels in the temperature-mediated microglia movement, both in physiological and pathological conditions. (COI:No)

## 1P-013

### GATA transcription factors participate in recanalization of the lymphatic vessels

Tomomi Watanabe-Asaka[1], Moyuru Hayashi[1], Akane Suzuki[2], Satoshi Uemura[3], Jun Takai[3], Takashi Moriguchi[3], Yoshiko Kawai[1] (*[1]Dept Physiol, Sch Med, Tohoku Med Pharm Univ, Japan, [2]Sch Med, Tohoku Med Pharm Univ, Japan, [3]Div MedBiochem, Sch Med, Tohoku Med Pharm Univ, Japan*)

Lymphedema occasionally emerges after extensive lymph node dissection as well as sentinel lymph node biopsy. Meanwhile, mechanism underlying the recanalization of lymphatic vessels upon lymph node resection has been largely elusive. GATA family of transcription factors regulate multitude of developmental processes through binding to GATA motif in a series of target genes. Recently, it has been suggested that GATA2 and GATA3 are important for development of lymphatic vessel and lymph node in embryonic stages. Given this, we hypothesized that GATA2 and GATA3 play a role for recanalization and growth of lymphatic vessel in adult animal. To address this issue, we examined lymphatic formation and recanalization upon lymph node resection using adult Gata2 heterozygous deficient ($Gata2^{+/-}$) mice and endothelial cell-specific Gata3 conditional knock-out (ECKO) mice. We found that popliteal lymph node formation was impaired in the Gata3 ECKO mice, whereas the $Gata2^{+/-}$ mice showed normally formed popliteal lymph nodes. We thereafter examined recanalization of lymphatic vessels 3 weeks after the resection of popliteal lymph node in the $Gata2^{+/-}$ mice. Recanalization was evaluated by visualization of lymph vessels using Evans Blue dye injection technique. Of note, the recanalization rate was significantly diminished in the $Gata2^{+/-}$ mice in comparison with the control group. Our results thus clearly demonstrate that GATA2 plays a crucial role for the lymphatic recanalization upon lymph node excision. Prompt regeneration of lymphatic vessels during wound repair promotes smooth lymphatic circulation and thereby confers substantial clinical benefits. We believe that further analyses of roles played by GATA2 in the lymphatic regeneration will open new therapeutic avenues. (COI:No)

## 1P-014

### The increased prefrontal oxygenation prior to and at the onset of overground walking using multichannel wireless near-infrared spectroscopy

Ryota Asahara[1], Kei Ishii[1], Nan Liang[2], Kanji Matsukawa[3] (*[1]AHFRC, AIST, Tsukuba, Japan, [2]Dept Human Health Sci, Grad Sch Med, Kyoto Univ, Kyoto, Japan, [3]Dept Integ Physiol, Grad Sch Biomed and Health Sci, Hiroshima Univ, Hiroshima, Japan*)

Our laboratory has reported using wireless 2-channel near-infrared spectroscopy (NIRS) that oxygenated-hemoglobin concentration (Oxy-Hb, as index of regional cerebral blood flow) in the prefrontal cortex (PFC) increased prior to and at the onset of natural overground walking with arbitrary start. The increase in prefrontal Oxy-Hb prior to and at the onset of walking was absent when walking was forced to start by cue, suggesting that the initial increase in prefrontal Oxy-Hb may appear in association with central command. However, the focal location of the initial increase in Oxy-Hb over the PFC during overground walking remained unsolved. The aim of the present study was to identify by using multichannel NIRS the specific prefrontal area activating prior to and at the onset of natural overground walking. The Oxy-Hb responses in multiple prefrontal areas were measured during overground walking in 15 subjects. Furthermore, we examined to what extent the initial increase in prefrontal Oxy-Hb during overground walking was dependent on walking speed. Overground walking with arbitrary start evoked the initial increase in Oxy-Hb of the dorsolateral PFC (DLPFC) and ventrolateral PFC (VLPFC) but not the frontopolar area. Furthermore, the initial increases in Oxy-Hb of the DLPFC and VLPFC were independent of walking speed. Taken together, it is suggested that the DLPFC and VLPFC are activated prior to and at the onset of natural overground walking and the prefrontal activities may play a role in driving neuronal activity in the caudal brain responsible for the generation of central command. (COI:No)

## 1P-015

### Analysis of hypoxic in utero environments that maintain cardiomyocyte proliferation and its implications for regeneration in adults

Ken Hashimoto[1], Aya Kodama[1], Momoko Ohira[1], Misaki Kimoto[1], Akira Hanashima[1], Yoshihiro Ujihara[2], Satoshi Mohri[1] (*[1]First Dept. Physiol., Kawasaki Med. Sch., Japan, [2]Dept. Electr. Mech. Eng., Nagoya Inst. Tech., Japan*)

Mammalian cardiomyocytes lose their proliferative capacity shortly after birth, which is a major obstacle for therapeutic heart regeneration in adults. We and others have recently shown the importance of hypoxic in utero environments for active fetal cardiomyocyte proliferation. In this study, we found two novel cardiomyocyte cell cycle promoters in mice, Fam64a and Novex3. These two molecules were abundantly expressed in hypoxic fetal cardiomyocyte nuclei, but this expression was drastically repressed by oxygen exposure, and in postnatal cardiomyocytes following the onset of breathing and the resulting elevation of oxygen tension. Sufficient expression of Fam64a and its degradation in M phase by ubiquitin ligase APC/C, was both shown to be required for the completion of cardiomyocyte mitosis and cytokinesis. Transgenic mice overexpressing Fam64a in postnatal cardiomyocytes exhibited enhancement of cell cycle activity in neonatal and adult hearts. Novex3 is the short splice variant of the giant sarcomeric protein connectin (titin). We unexpectedly found that it was localized in the cardiomyocyte nuclei specifically during the fetal stage, in addition to sarcomeric expression. Mechanical analysis by atomic force microscopy and microneedle-based tensile tests demonstrated that Novex3 contributes to the elasticity/compliance of the nucleus at interphase and facilitates cardiomyocyte proliferation, by promoting phosphorylation-induced disassembly of multimer structures of nuclear lamins. Interestingly, Novex3 knockdown inhibited Fam64a expression, and vice versa. Therefore, these three molecules (Fam64a, APC/C, and Novex3) seem to work cooperatively to maximize cardiomyocyte proliferation during hypoxic fetal stage. We now set out to achieve novel regeneration strategy by introducing these molecules into adult hearts, which would create cardiomyocytes with high proliferative capacity and compliant nuclei. (COI:No)

## 1P-016

### The central role of Ulk1-dependent autophagy in mediating mitophagy in the heart during ischemia and reperfusion

Toshiro Saito[1], Junichi Sadoshima[2] (*[1]Dept [1]st Surgery, Grad Sch Med, Yamaguchi Univ, Japan, [2]Dept CBMM, New Jersey Med Sch, Rutgers Univ, USA*)

Removal of damaged mitochondria by mitophagy is essential for cardiac homeostasis. However, activation of mitophagy and the underlying mechanism have not been clearly demonstrated in the heart during ischemia and reperfusion. Mito-Keima is a pH-sensitive marker that indicates mitophagy by a high 560nm/440nm signal ratio. We crossed transgenic mice expressing Mito-Keima with mouse models of autophagy loss-of-function, namely mice with cardiac-specific knockout of atg7 (atg7cKO) or ulk1 (ulk1cKO). Tg-Mito-Keima (Keima-WT), Tg-Mito-Keima-atg7cKO (Keima-atg7cKO), and Tg-Mito-Keima-ulk1cKO (Keima-ulk1cKO) mice were subjected to 48 hours of fasting. In Keima-WT hearts, the area with high-ratio dots was $1.1 \pm 0.1$ % of the total heart area at baseline and starvation increased it to $2.3 \pm 0.1$ % ($p<0.05$), indicating stimulation of mitophagy. The area with high-ratio dots during starvation was significantly smaller in Keima-ulk1cKO ($0.9 \pm 0.2$ %, $p<0.05$) but not in Keima-atg7cKO ($2.0 \pm 0.2$ %), suggesting that mitophagy occurs in an Ulk1-dependent but Atg7-independent fashion. During ischemia, mitophagy was transiently activated, peaking at 30 minutes after coronary artery ligation in Keima-WT ($3.1 \pm 0.2$ %) and Keima-atg7cKO ($2.7 \pm 0.2$ %), but it was significantly blunted in Keima-ulk1cKO ($1.1 \pm 0.2$ %, $p<0.05$). ulk1cKO mice displayed significantly larger infarcts ($34.9 \pm 4.3$ %, $p<0.05$) than WT mice ($19.7 \pm 2.8$ %), suggesting that Ulk1 protects the heart against ischemia. During reperfusion following 30 minutes of ischemia, mitophagy was transiently augmented in Keima-WT ($3.5 \pm 0.2$ %) and Keima-atg7cKO ($3.3 \pm 0.2$ %), but not in Keima-ulk1cKO ($1.1 \pm 0.2$ %). These results suggest that Ulk1-dependent autophagy is essential for the induction of mitophagy in the heart during ischemia and reperfusion. (COI:No)

## 1P-017

### Effect of ATP released from the maxi-anion channel on left ventricular contractile function in Langendorff-perfused mouse heart model

Hiroshi Matsuura[1], Akiko Kojima[2], Yutaka Fukushima[1,2], Yu Xie[1], Xinya Mi[1], Sabirov Ravshan Z[3], Yasunobu Okada[4,5] (*[1]Dept Physiol, Shiga Univ Med Sci, [2]Department of Anesthesiology, Shiga University of Medical Science, Otsu, Japan, [3]Institute of Biophysics and Biochemistry, National University of Uzbekistan, Tashkent, Uzbekistan, [4]Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan, [5]National Institute for Physiological Sciences (NIPS), Okazaki 444-8787, Japan*)

We have previously shown i) that the organic anion transporter SLCO2A1 constitutes an essential core component of the ATP-conductive large-conductance anion (Maxi-Cl) channel, and ii) that this Maxi-Cl channel contributes largely to the release of ATP into coronary effluent observed during and following the oxygen-glucose deprivation in the perfusate. The present study was undertaken to examine the effect of ATP released via Maxi-Cl channels on left ventricular contractile function, using a Langendorff-perfused mouse heart model. Isolated heart was perfused at 37℃ with oxygenated normal Tyrode solution at a constant hydrostatic pressure of 80 mmHg. Left ventricular function was assessed by measuring left ventricular pressure using a fluid-filled balloon (made of plastic film) connected to a pressure transducer. After the initial 30-min stabilization period, heart was then perfused with oxygen-glucose-deprived (OGD) Tyrode solution for 6 min, which was followed by 10-min perfusion with oxygenated normal Tyrode solution in the absence and presence of the ATPase apyrase and the A1 adenosine receptor antagonist 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX). In the absence of apyrase and DPCPX, left ventricular developed pressure (LVDP) was decreased from baseline value of 68.55.2 mmHg to 49.81.7 mmHg at the end of 6-min perfusion with OGD Tyrode solution, which was followed by a transient increase to 84.03.2 mmHg during subsequent perfusion with oxygenated normal Tyrode solution. On the other hand, in the presence of apyrase and DPCPX, LVDP was decreased during 6-min perfusion with OGD Tyrode solution to the same degree but without exhibiting a transient increase in LVDP during subsequent perfusion with oxygenated normal Tyrode solution. These results strongly suggest that ATP released via Maxi-Cl channels contributes to the development of transient positive inotopy during reperfusion after the short-period hypoxia/ischemia in the heart. (COI:No)

## 1P-018

### Analysis on Combinations of Ion Channel Permeabilities of Guinea-pig Ventricular Myocyte Model that Generate Similar Action Potential Waveforms

Maho Yamamoto[1], Kazuki Okumura[1], Tomohisa Kawakami[1], Rina Sato[2], Yukiko Himeno[2], Akira Amano[2] (*[1]Dept Bioinfo, Grad Sch Life Sci, Ritsumeikan Univ, Shiga, Japan, [2]Dept Bioinfo, Col Life sci, Ritsumeikan Univ, Shiga, Japan*)

Drug effect estimation system was developed to reduce cost for drug discovery by estimating the drug effects to specific ventricular myocyte ion channels in silico, which is necessary for evaluating drug safety. The system estimates the effects of drugs on ion channels by using action potential waveforms (APWs) of ventricular myocytes. APWs are used as input data which are measured from guinea-pig ventricular myocytes before and after drug administration with suction electrode method. This system estimates the effect of drug on ion channels by searching for the similar APWs that were generated by various combinations of ion channel permeabilities. The similarities are evaluated excluding phase 0 and 1, since they are difficult to measure accurately, and also excluding the end of repolarization phase where the waveform often becomes unstable. The problem of the system is that the estimation accuracy is low because similar APWs are generated from different combinations of channel permeabilities. We analyzed the characteristics of these combinations by searching for the similar APWs to several typical waveforms. As a result, there were combinations of potassium channel permeabilities to compensate for changes in sodium channel and/or calcium channel permeabilities. (COI:No)

## 1P-019

### Changes in Left Ventricular Elastance During Ejection Phase --- Model Study ---

Shiro Kato, Kota Kishida, Akari Sanechika, Akira Amano (*Dept Bioinf, Col Lifesci, Ritsumeikan Univ, Shiga, Japan*)

Left Ventricular End-Systolic Pressure and Volume Relation (ESPVR) becomes linear in large mammals such as human or canine. Emax which is slope of ESPVR, is well known as an index of cardiac contraction because of its independence of loading conditions of Left Ventricle (LV). Time-Varying Elastance Model (TVEM) was proposed as a mathematical model of LV by using the linear characteristics of ESPVR. In TVEM, the LV elastance increases during ejection phase, thus each isochronous Pressure and Volume Relation (PVR) lies on a line. On the other hand, detailed cardiac contraction model has been proposed, and by using such model, LV model is also proposed. Interestingly, the characteristics of isochronous PVRs become different for these models. Although the linear characteristics of the isochronous PVRs were the same, the slope of isochronous PVRs for the detailed cardiac contraction model slightly decreased during ejection phase, and the unloaded volume largely decreased. From the mathematical analysis, the PVR characteristics of contraction model comes from the combination of well-known force length relation and force velocity relation. (COI:No)

## 1P-020

### The serotonergic system mediates cardiovascular responses evoked by stimulating the lateral habenula of rats

Tri Doan Huu[1,2,6], Yuma Sato[1,3], Masayuki Matsumoto[4,5], Tadachika Koganezawa[1,5] ([1]*Dept Physiol, Fac Med, Univ Tsukuba, Japan,* [2]*Dr Prog Biomed Sci, Grad Sch Comp Human Sci, Univ Tsukuba, Japan,* [3]*Mstr Prog Med Sci, Grad Sch Comp Human Sci, Univ Tsukuba, Japan,* [4]*Dept Cogn Bahav Neurosci, Fac Med, Univ Tsukuba, Tsukuba, Japan,* [5]*Transborder Med Res Ctr, Univ Tsukuba, Japan,* [6]*Ctr Adv Trn Clin Sim, Univ Med Pharm Ho Chi Minh City, Vietnam*)

Neurons in the lateral habenula (LHb) are activated by stressful events such as pain, physical constraint, open field exposure and social defeat. Although these events induce cardiovascular responses, whether and how the lateral habenula regulates the cardiovascular autonomic nervous system remains unclear. To investigate this issue, here we electrically stimulated the LHb and examined the effect on cardiovascular responses in anesthetized rats. We found that the electrical stimulation of the LHb increased mean arterial pressure (MAP) and decreased heart rate (HR), while stimulating the outside of the LHb at 0.25 mm distance affected neither of them. Notably, denervating the bilateral vagus nerves, which include the cardiac parasympathetic nerves, completely suppressed the effect of the LHb stimulation on HR but did not change that on MAP. On the other hand, administration of propranolol (5-10 mg/kg, *i.v.*), a nonselective $\beta$ adrenergic receptor antagonist, partly attenuated the effect of the LHb stimulation on MAP but did not affect that on HR. Furthermore, systemic administration of methysergide (1 mg/kg, *i.v.*), a nonselective serotonergic receptors antagonist, attenuated the responses of MAP and HR to the LHb stimulation.

These results indicated that the activation of the LHb neurons induces cardiovascular responses through both the sympathetic and the parasympathetic nervous systems. The serotonergic system also mediates the cardiovascular responses. Our findings suggest that the LHb and the serotonergic system cooperate to induce cardiovascular responses when animals encounter stressful events. (COI:No)

## 1P-021

### Mitochondrial contribution to automaticity of murine sinoatrial nodal cells

Yukari Takeda, Satoshi Matsuoka (*Dept of Integrative and Systems Physiol, Univ of Fukui*)

The automaticity of sinoatrial nodal cells (SNCs) attributes to the activities of ion channels at the plasma membrane. There is however growing evidence that spontaneous local calcium release (LCR) from the sarcoplasmic reticulum (SR) regulates the pacemaker activity. Previously, we have reported that mitochondrial $Na^+$-$Ca^{2+}$ exchanger, NCXm, is involved in the generation of LCR and automaticity in murine SNCs. Although it is anticipated that mitochondrial $Ca^{2+}$ efflux through NCXm modulates $Ca^{2+}$ handling of nearby SR, the spatial association between LCR and mitochondria has never been assessed. In the current study, we investigated the local orientation of LCR in relation to location of mitochondria in murine SNCs. For LCR detection, $Ca^{2+}$ dynamics were measured by 2D imaging of Calbryte-520™-loaded murine SNCs. Mitochondria areas were simultaneously identified by co-labeling of SNCs with tetramethylrhodamine ethyl ester (TMRE). High-speed and high-resolution imaging of the SNCs detected LCRs between rhythmic firings of $Ca^{2+}$ transient in all SNCs. The probability of LCR occurrence was found to be higher at mitochondria area (69.1 ± 13.8%). Moreover, the distance between LCR and adjacent mitochondria area was significantly shorter in LCRs evoked in the early phase of $Ca^{2+}$ transient cycle (average 0.68 $\mu$m, median 0.00 $\mu$m), when compared to that evoked in the late phase (average 1.52 $\mu$m, median 0.16 $\mu$m). Taken together, LCRs, especially those in the early phase of $Ca^{2+}$ transient cycle were generated in close proximity to mitochondria in murine SNCs. (COI:No)

## 1P-022

### Preservation of active cardiac force after release from repetitive overstretch

Shin Takeo, Yoichiro Kusakari, Naritomo Nishioka, Susumu Minamisawa (*Dept Cell Physiol, Med, Jikei Univ, Japan*)

**Background:** The overstretch of cardiac muscle is known to drastically decrease active force development. Cardiac muscle can produce greater active force in response to stretching within an optimal range of sarcomere length (1.8 $\mu$m≦SL≦2.2 $\mu$m) according to Starling's law of the heart. Therefore, repetitive overstretch would further worsen cardiac contractility. How cardiac contractility recovers when its length is recovered to the optimal range after repetitive overstretch has not yet been examined, however.

**Purpose:** We exposed cardiac papillary muscle to repeated overstretch and release to investigate the changes in active tension before and after overstretch.

**Methods:** We used male Sprague-Dawley rats (BW≧350 g) and dissected the right ventricular papillary muscles. A papillary muscle was stretched to Lmax, at which point the active tension reached the maximal level. We then stepwisely stretched it within 2 s up to 120% of Lmax, and stimulated the muscle (1 Hz, 36℃) with tension measurement. Experiments were performed with rats at 14 weeks and 2 years of age.

**Result:** The active tension 4 min after overstretch was decreased to 32.8% of Lmax, but recovered to 92.1% of Lmax 20 min after overstretch release. Although the active tension gradually decreased as the papillary muscle was repeatedly overstretched and released, it remained around 75% of Lmax after the fourth overstretch release (experimental time 2 h). In contrast, the active tension of the papillary muscle whose length was kept at Lmax decreased to 10% of Lmax at 2 h. This phenomenon was much more obvious in younger rats than in older rats.

**Conclusion:** The contractile force of cardiac muscle that undergoes several overstretches intermittently was much stronger than that which continuously kept its length within normal limits. This result indicates that overstretch may be effective in protecting the contractility of cardiac muscle. (COI:No)

## 1P-023

### Decrease in the forearm muscle oxygenation assists recovery of arterial blood pressure during supraventricular tachycardia but not during ventricular tachycardia

Kei Ishii[1], Hidehiko Komine[1], Takeshi Machino[2], Fumi Yamagami[2], Yasuhiro Hatori [1], Jongseong Gwak [1], Tsubasa Izaki[1], Akihiko Nogami [2], Kazutaka Aonuma [2], Hideo Tsurushima [3], Satoshi Kitazaki [1], Motoyuki Akamatsu [1] ([1]*AHFRC, AIST, Tsukuba, Japan,* [2]*Cardiovascular Division, University of Tsukuba, Tsukuba, Japan,* [3]*Department of Nuerosurgery, University of Tsukuba, Tsukuba, Japan*)

Rapid hypotension caused by cardiac tachyarrhythmias is counteracted by reflexly-evoked peripheral vasoconstriction. However, it was still unclear whether peripheral vasoconstriction does assist recovery of arterial blood pressure (AP) during various types of actual tachyarrhythmia. The degree of AP recovery would differ between paroxysmal supraventricular tachycardia (PSVT) and ventricular tachycardia (VT) because of lesser cardiac output and/or ability to evoke vasoconstriction in VT cases. We hypothesized that AP recovers via peripheral vasoconstriction during PSVT but it remains decreased during VT. To estimate peripheral blood flow responses, forearm tissue oxygen index (TOI) was measured by near-infrared spectroscopy (NIRS). The NIRS and hemodynamic data were collected during electrophysiological study. Mean AP (MAP) decreased ($P<0.05$) rapidly in both PSVT and VT, while the arrhythmia rates were similar ($P > 0.05$). Thereafter MAP returned to the pre-arrhythmia level at 40 s from the onset of PSVT. The decreased MAP was maintained during VT. PSVT caused a decrease in the forearm TOI, which did correlate with MAP recovery ($P<0.05$). During VT, there was no such relationship between the TOI response and MAP recovery ($P > 0.05$). These relationships were also evident when the data with drugs (e.g., isoproterenol) infused during EPS was excluded. These results provide a positive suggestion that the decrease in the forearm muscle oxygenation probably via vasoconstriction does recover MAP during PSVT but fails to recover MAP during VT. (COI:Properly Declared)

## 1P-024

### Comparison of passive mechanical properties of rat, chicken, frog and turtle ventricles

Megumi Ito[1], Yoshihiro Ujihara[2,3], Akira Hanashima[3], Takeshi Honda[3], Aya Kodama[3], Shukei Sugita[2], Masanori Nakamura[2], Ken Hashimoto[3], Satoshi Mohri[3] ([1]*Creative Eng Program, Nagoya Inst Tech, Japan,* [2]*Dept Electric Mech Eng, Nagoya Inst Tech, Japan,* [3]*First Dept of Physiol, Kawasaki Med Sch, Japan*)

Vertebrate hearts have undergone marked morphological changes to adapt to different environments and lifestyles during evolution. Mammals and birds have the hearts consisting of two atria and two ventricles, while amphibians and most reptiles have the hearts consisting of two atria and one ventricle. However, functional changes in the ventricles during the course of evolution from one to two ventricles have remained unclear. Here, we compared passive mechanical properties of the ventricle of frogs and turtles with left ventricle (LV) of rats and chickens. To analyze the passive mechanical properties of the ventricles, we obtained the relationship between the ventricular pressure and the inflow volume when the cardioplegic solution was introduced into diastolic-arrested ventricle or the LV at a constant speed. To compare different-sized hearts, the inflow volume was normalized by the ventricular weight. The results showed that LVs of rats and chickens were significantly stiffer than ventricles of turtles and frogs. Isolated cardiomyocytes in rats were significantly wider than in chickens, turtles and frogs, suggesting that rat cardiomyocytes were stiffer than others in the longitudinal direction at a single cell level. Because the extensibility of cardiomyocytes is regulated by the elastic protein connectin (also called titin) that is striated muscle-specific and the largest protein that connects the Z-line to the M-line in half-sarcomeres, we compared the primary structure of connectin. We found that the elastic regions, which function to confer extensibility to connectin, were greatly shorter in rat and chicken hearts than in the frog and turtle hearts, indicating that connectin in rat and chicken hearts was stiffer than that in turtle and frog hearts. These results suggested that ventricular extensibility was restricted at the molecular level during the course of evolution from one to two ventricles. (COI:No)

## 1P-025

### Alteration of astrocytes and extracellular matrix molecules in the cortex of pentylenetetrazol-kindled mice

Hiroshi Ueno[1], Yu Takahashi[2], Shunsuke Suemitsu[2], Motoi Okamoto[3], Takeshi Ishihara[2] (*[1]Dept Med Tech, Kawasaki Univ Med Welf, Japan, [2]Dept Psych, Kawasaki Med Sch, [3]Dept Med Tech, Okayama Univ*)

Although epilepsy is one of the most common chronic neurological disorders with a prevalence of approximately 1.0%, the underlying pathophysiological processes remain to be elucidated. Understanding the molecular and cellular mechanisms involved in the development of epilepsy is important for the development of appropriate therapeutic strategy. Kindling is widely accepted as a functional model of temporal lobe epilepsy. Pentylenetetrazol (PTZ) is commonly used in kindling and to study seizure mechanisms, neurobiology of epilepsy, learning and memory disorders caused by seizures, and to evaluate the effects of new treatments. In this study, we investigated the effects of status epilepticus on astrocytes, microglia, and extracellular matrix (ECM) molecules in the somatosensory cortex and piriform cortex of mice. The purpose of this study was to reveal changes in the astrocytes and ECM in the cortical region of experimental epilepsy models. Activation of astrocytes was observed in many cortices except the retrosplenial granular cortex after PTZ-induced kindling acquisition in mice. Activated astrocytes in the cortex were found in layers 1-3 but not in layers 4-6. In the somatosensory and piriform cortices, no change was observed in the number of parvalbumin (PV)-positive neurons and PV-positive neurons covered with perineuronal nets. However, the amount of ECM in the extracellular space was increased. The expression of VGLUT1- and GAD67-positive synapses was also increased. Thus, in the PTZ-kindling epilepsy mice model, an increase in the number of ECM molecules and activation of astrocytes were observed in the somatosensory cortex and piriform cortex. These results indicate that PTZ-induced seizures affect not only the hippocampus but also other cortical areas. Our study findings may provide an opportunity to develop new therapeutic approaches to prevent seizures or their consequences. (COI:No)

## 1P-026

### Optical analysis of functional development of the glossopharyngeal nerve pathway in the mouse fetus brainstem

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

The glossopharyngeal nerve (N.IX) transfers motor and sensory information related to visceral and somatic functions, such as salivary secretion, gustation and the control of blood pressure. N.IX-related neural circuits are indispensable for these essential functions. Compared with the strenuous analysis of morphogenesis, we are only just starting to elucidate the functiogenesis of these neural circuits during ontogenesis. In the present study, we applied voltage-sensitive dye recording to the embryonic mouse brainstem, and examined the functional development of the N.IX-related neural circuits. First, we optically identified the motor nucleus (the inferior salivatory nucleus (ISN)) and the first-order sensory nucleus (the nucleus of the tractus solitarius (NTS)). We also succeeded in recording optical responses in the second/higher-order sensory nuclei via the NTS, including the parabrachial nucleus. Second, we pursued neuronal excitability and the onset of synaptic function in the N.IX-related nuclei. The neurons in the ISN were excitable at least at E11, and functional synaptic transmission in the NTS was first expressed at E12. In the second/higher-order sensory nuclei, synaptic function emerged at around E12-13. Third, by mapping optical responses to N.IX and vagus nerve (N.X) stimulation, we showed that the distribution patterns of neural activity in the NTS were different between the N.IX and the N.X from the early stage of ontogenesis. We discuss N.IX-related neural circuit formation in the brainstem, in comparison with our previous results obtained from chick and rat embryos. (COI:No)

## 1P-027

### Prenatal exposure to nicotine disrupts synaptic network formation by inhibiting spontaneous correlated wave activity

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

Correlated spontaneous activity propagating over a wide region of the central nervous system is expressed during a specific period of embryonic development. In a previous study using the optical imaging technique with a voltage-sensitive dye, we demonstrated that this wave-like activity, which we referred to as the depolarization wave, plays a fundamental role in the early process of synaptic network formation. We found that *in ovo* application of bicuculline/strychnine or *d*-tubocurarine, which blocked neurotransmitters mediating the wave, significantly reduced functional synaptic expression in the brainstem sensory nucleus. This result, especially for *d*-tubocurarine, an antagonist of nicotinic acetylcholine receptors, raised the possibility that prenatal nicotine exposure associated with maternal smoking affects the development of neural circuit formation by interfering with the correlated wave. In the present study, we tested this hypothesis by examining the effects of nicotine on the correlated wave and assessing the chronic action of nicotine on functional synaptic expression. The application of nicotine transiently increased electrical bursts and embryonic movements associated with the wave, but subsequently inhibited these activities. Furthermore, chronic exposure to nicotine *in ovo* markedly reduced functional synaptic expression in the brainstem sensory nucleus, the parabrachial nucleus. This study suggested that prenatal nicotine exposure disrupts the initial formation of neural circuitry by inhibiting the correlated spontaneous activity. (COI:No)

## 1P-028

### Functional organization of response-selective inputs on dendrites of mouse primary visual cortex neurons

Satoru Kondo[1], Kohei Kikuta[2], Kenichi Ohki[1,2] (*[1]IRCN, UTIAS, Univ.Tokyo, Japan, [2]Dept Phyiol, Grad Sch Med, Univ Tokyo, Japan*)

Understanding how neurons integrate thousands of synaptic inputs is critical to discern cortical information processing. Substantial evidences suggest the importance of spatial arrangement of synaptic inputs onto dendrites for neuronal computation. However, the principle of spatial arrangement and integration mechanisms of inputs remain largely unsolved.
Recent advances of functional imaging technique enable recordings of activities of individual spines using various calcium sensors. Due to the low time resolution of calcium signal transient, back propagating action potential (bAP) invades spines and makes the accurate spine analysis difficult. To solve this problem, we developed a new method to overcome by using inhibitory optogenetics. We sparsely co-expressed GCaMP6s and inhibitory optogenetic protein with soma-localized signal in mouse primary visual cortex (V1) by adeno associated virus (AAV) and recorded visually evoked spine signals from layer 2/3 excitatory neurons. With photo-inhibition by targeted one-photon laser, we successfully suppressed the somatic depolarization and individual spine signal was detected. Firstly, we recorded the visually-evoked signals form the spines without inhibition followed by with inhibition and compared them. We found that the bAP-subtracted method does not always estimate the accurate visual response of spines especially when the spine shows the simultaneous response with the soma. Next, to investigate the input-output relationship, we recorded ~1,000 spine responses from individual orientation or direction selective neurons. We found that ~30% of recorded spines were visually responsive and among them ~90% were selective for either orientation or direction. These selectively responded spine were distributed all over the dendrites but showed some clustering of orientation or direction selective inputs that matched with the somatic selectivity. We also investigated the spatial frequency selectivity of these inputs and their functional organization on the dendrites. (COI:No)

## 1P-029

### GABA release from cerebellar Purkinje cells is insensitive to endocannabinoid-mediated synaptic modulation

Moritoshi Hirono[1,2], Yuchio Yanagawa[3] (*[1]Dept Physiol, Sch Med, Wakayama Med Univ, Japan, [2]Grad Sch Brain Sci, Doshisha Univ, Japan, [3]Gunma Univ Grad Sch Med, Japan*)

Endocannabinoids (eCBs) act as ubiquitous modulators of synaptic transmission via the activation of cannabinoid receptors (CBRs) in the CNS. Cerebellar Purkinje cells (PCs) project to the deep cerebellar nuclei (DCN), where they make synaptic contacts with DCN neurons. PCs also extend axon collaterals that terminate in the vicinity of the PC layer and make synaptic contacts with Lugaro cells and globular cells. However, the effects of eCBs on GABAergic transmission from PC axon terminals have yet to be investigated thoroughly. In this study, we examined the modulatory actions of eCBs on the GABAergic transmission at PC-globular cell synapses and PC-DCN neuron synapses using whole-cell patch-clamp recordings from mouse cerebellar slices. We showed that the CBR type 1 agonist WIN55212 did not affect either spontaneous or miniature inhibitory postsynaptic currents (IPSCs) recorded from globular cells under control conditions and in a state of enhanced synaptic activity. By contrast, another $G_{i/o}$ protein-coupled receptor agonist, baclofen, significantly reduced the frequency of miniature IPSCs in globular cells. Moreover, we showed that WIN55212 had no effects on IPSCs in large DCN neurons, the primary targets of PCs. A type 2 CBR agonist, HU308, also had no significant effects on IPSCs in either globular cells or large DCN neurons. Globular cells and large DCN neurons did not elicit depolarization-induced suppression of inhibition (DSI). Taken together, our results suggest that GABA release from PC axon terminals is insensitive to exogenous CBR agonists and devoid of regulation by DSI, indicating that PCs do not express functional CBRs at their axon terminals. This is in sharp contrast to the fact that PCs receive abundant excitatory and inhibitory inputs that are under eCB-mediated presynaptic inhibitory modulation. The actions of eCBs are selective to distinct synapses and possibly contribute to both information processes and rigorous signal transmission in the cerebellum. (COI:No)

## 1P-030

### Live cell imaging of endogenous drebrin using camelidae single-domain antibody

Hiroyuki Yamazaki[1], Kenji Hanamura[1], Banri Segawa[1], Narutoshi Tsukahara[2], Akikazu Murakami[2], Tomoaki Shirao[1] (*[1]Dept. Neurobiol. Behav. Gunma Univ. Grad. Sch. Med., Maebashi, Japan, [2]Dept. of Parasitol. Grad. Sch. Med., Univ. Ryukyus, Okinawa, Japan*)

Drebrin functions as an F-actin stabilizing factor in dendritic spines, where it regulates spine morphogenesis and is involved in synaptic plasticity. GFP-fused drebrin (GFP-drebrin) has been used for many studies, particularly for real-time imaging. However, there are some difficulties in use of GFP-drebrin. For example, when it is expressed at higher level in neurons, the spines and filopodia abnormally change their shapes by F-actin-remodeling activity of drebrin. In this study, we made a novel tool for drebrin-imaging using the camelidae antibody that consists of a single monomeric variable antibody domain. We isolated some "variable domains of heavy chains of camel heavy-chain antibody" (VHH) by affinity selection to drebrin using VHH domain-phage library. All selected VHH clones recognized exogenous and endogenous drebrin in western blotting. A drebrin-VHH clone (3E3) showed a similar immunostaining pattern in cultured neurons to an authentic drebrin monoclonal-antibody (clone M2F6). Next, we fused the VHH to IgG-Fc with GFP to visualize endogenous drebrin in living cells. When GFP-3E3 expression vector was transfected to cultured neurons, GFP signals were observed mainly in dendritic spines. The signals translocated to dendrites from dendritic spines by depolarizing stimuli with high potassium, which is consistent with our previous studies. Furthermore, we delivered GFP-3E3 into developing mouse neocortex via in utero electroporation and observed them in living brains of adult mice. In vivo imaging showed that GFP-3E3 normally localized in dendritic spines, suggesting that the expressed GFP-3E3 were not toxic. These indicate that GFP-3E3 is useful tool for observation of endogenous drebrin *in vitro* and *in vivo*. (COI:No)

## 1P-031

### Protocadherin 10 delays developmental climbing fiber synapse elimination in a subset of aldolase C-positive Purkinje cells in the cerebellum

Takaki Watanabe[1,2], Honoka Suzuki[1], Kazuto Sakoori[1], Shutaro Inoue[1], Tsubasa Akamatsu[1], Manabu Abe[3], Kenji Sakimura[3], Naofumi Uesaka[1,2], Masanobu Kano[1,2] (*[1]Dept of Neurophysiol, Grad Sch of Med, Univ of Tokyo, Japan, [2]WPI-IRCN, UTIAS, Univ. of Tokyo, Japan, [3]Dept of Cell Neurobiol, Brain Res Inst, Niigata Univ, Japan*)

Accumulating evidence suggests that the cerebellum is involved in autism spectrum disorder (ASD). Here, we focused on protocadherin 10 (Pcdh10), an ASD-associated gene that is expressed in subsets of cerebellar Purkinje cells (PCs), and investigated whether Pcdh10 is involved in developmental elimination of early-formed redundant climbing fiber (CF) to PC synapses, a representative model of synapse pruning in the developing brain. We generated Pcdh10-DIO-tdTomato mice and crossed them with GluD2-Cre mice to obtain conditional Pcdh10 knockout (Pcdh10-cKO) mice in which PCs that have endogenous Pcdh10 will express tdTomato instead of Pcdh10. We found that the expression of tdTomato in Pcdh10-cKO was restricted to a subset of aldolase C (Aldoc)-positive PCs, which was consistent with a previous report about Pcdh10 expression in PCs. We performed whole-cell recordings from PCs in cerebellar slices at various postnatal days and examined CF synapse elimination. We found that in the cerebellar vermis of wild-type mice, CF synapse elimination was delayed in Aldoc-positive/Pcdh10-positive PCs when compared with Aldoc-negative PCs or Aldoc-positive/Pcdh10-negative PCs. This delay of CF synapse elimination in a subset of Aldoc-positive PCs was absent in Pcdh10-cKO. Similar delay of CF synapse elimination was also found in Aldoc-positive PCs in the right cerebellar hemisphere including Crus I, Crus II, and Lobule simplex of wild-type mice, whereas the delay was not seen in Pcdh10-cKO. Conversely, ectopic overexpression of Pcdh10 in PCs by in utero electroporation at E12 increased the degree of multiple CF innervation at young adult stage. Pcdh10-cKO mice had a mild motor deficit and exhibited repetitive behavior in marble burying tests. These results indicate that Pcdh10 maintains CF synapses and counteracts their elimination in a subset of PCs during postnatal development, and suggest that deletion of Pcdh10 might be related to abnormal repetitive behavior relevant to a core symptom of ASD. (COI:No)

## 1P-032

### Function of metabotropic glutamate receptor 1 in the neonatal hippocampal marginal zone

Megumi Taketo (*Dept Cellular Functional Biol, Facult Med, Kansai Med Univ*)

Metabotropic glutamate receptors (mGluRs) are distributed in the central nervous system and play important roles as synaptic plasticity and memory formation in various neuronal processes. mGluRs are classified into three groups I to III, and group I mGluRs consist of two subtypes, mGluR1 and mGluR5. Group I receptors couple to $G_q$ protein and increase intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) through the release of $Ca^{2+}$ from intracellular stores. These receptors also regulate the channel activity of plasma membranes. Hippocampal marginal zone contains early-developed neurons including Cajal-Retzius cells (CR cells), which regulate neuronal migration through secretion of glycoprotein, reelin. CR cells also project their dendrites to other hippocampal neurons and regulate network activity. Though the function of the secreted reelin has been studied considerably, less is known about the regulation of CR cell excitability. In the present experiments, functional expression of mGluR1 was detected by fluorescence $Ca^{2+}$ imaging, in acute slices of neonatal rat hippocampus. In CR cells, $[Ca^{2+}]_i$ elevation was induced by application of group 1 mGluR-specific agonist in the presence of mGluR5 specific antagonist, MPEP. Whereas, the $[Ca^{2+}]_i$ elevation was prevented by mGluR1-specific antagonist, CPCCOEt. Characteristics of the $Ca^{2+}$ mobilization after activation of mGluR1 in neurons of marginal zone were further investigated. Elimination of extracellular $Ca^{2+}$ could not prevent the mGluR1-induced $[Ca^{2+}]_i$ increase in CR cells. Effects of $Ca^{2+}$-permeable channel blockers on mGluR1-mediated $Ca^{2+}$ mobilization were also determined. Possibility of a cross-talk between mGluR1 and other receptors expressed in CR cells is under investigation. (COI:No)

## 1P-033

### Direct measurements of transmitter release kinetics at lemniscal fiber terminals in the somatosensory thalamus

Mitsuharu Midorikawa, Mariko Miyata (*Dev Neurophys, Dept Physiol, Sch Med, Tokyo Women's Med Uni, Tokyo, Japan*)

Fast synaptic transmission is important for information processing at central nervous systems, but how it acquired through the development is largely unknown especially on presynaptic side. Here, we focused on the presynaptic terminals of afferent fiber at mouse sensory thalamus, lemniscal fiber, where we managed to perform direct patch-clamp recordings from the presynaptic terminals throughout synaptogenesis to synapse strengthening periods.
Somatosensory information from the maxillary region of rodent is conveyed to the ipsilateral trigeminal nuclei via the infraorbital nerve and then to relay neurons in the contralateral ventral posteromedial thalamic nucleus (VPM) via medial lemniscal fibers. VPM relay neurons are innervated by multiple afferent fibers before the maturation, but synapses are eliminated upon the developmental maturation and eventually dominated by a single strong lemniscal fiber.
The kinetics of exocytosis was examined by membrane capacitance measurements, as well as by preterminal-postsynaptic neuron paired recordings. With development, the total releasable pool of the terminal becomes larger during immature periods first, and the enlargement of the fast component of transmitter release occurs thereafter. The fast component is mediated by vesicles tightly coupled to the calcium channels. Together with remarkable shortening of action potential durations, the developmental changes could fine-tune the speed, reliability, and plasticity of transmitter release in mature synapse, which underlies precise afferent sensory signal transmission. (COI:No)

## 1P-034

### Spatio-temporal analysis of synaptic integration in hippocampal neurons by membrane potential imaging

Masato Morita, Tomoo Hirano, Shin-ya Kawaguchi (*Dept Biophys, Grad Sch Sci, Kyoto Univ, Japan*)

Neurons form many synapses and receive lots of inputs there. Synaptic inputs are integrated spatially and temporally in the somatodendritic compartment, generating output firing at the axon initial segment. In hippocampal pyramidal neurons, it is well known that synaptic potentials summate supralinearly, however, its spatio-temporal property remains unclear. To study this issue, using a genetically encoded voltage indicator and rapid multi-spots local glutamate uncaging, we have quantitatively examined the supralinear summation of EPSPs in cultured hippocampal neurons. Hippocampal neurons were transfected with an improved version of ASAP1, which showed larger fluorescence changes upon membrane potential changes (~50 %/100 mV). EPSPs upon glutamate uncaging at dendritic spines were recorded by voltage imaging and/or the whole-cell patch clamp method. We find that simultaneous EPSPs in response to glutamate uncaging by 405 nm laser illumination at 2 distinct sites exhibit higher depolarization than the linear sum of each EPSP. The closer the distance between 2 sites stimuli is, the higher the supralinear summation becomes. Notably, the supralinear augmentation of EPSPs is evident even when the individual EPSP is tiny as ~1 mV. In this poster, we are also going to show the molecular mechanisms underlying the supralinear dendritic computation in hippocampal neurons. (COI:No)

## 1P-035 (AP-2)

### Synaptic plasticity at cortico-striatal pathway in functional recovery after cortical damage

Susumu Jitsuki[1], Sayaka Kogami[1], Aoi Jitsuki-Takahashi[2], Tomomi Yamanoue[1], Takuya Takahashi[1] (*[1]Dept Physiol, Grad Sch Med, Yokohama City Univ, Japan, [2]Dept Biochem, Sch Med, Tokyo Women's Med Univ, Japan*)

Brain damage such as stroke is a devastating neurological condition that may severely compromise patient quality of life. Restoration of motor impairment after brain damage is considered to be the result of compensative neural plasticity in intact brain regions, mediated by the reorganization of cortical motor maps. Experience-dependent synaptic AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid) receptor (AMPAR) delivery underlies behaviors that require neural plasticity such as learning. We have previously found that rehabilitation-dependent synaptic delivery of AMPAR in the peri-injured area facilitate functional recovery after cortical damage. For execution of appropriate motor function, it requires involvement of motor-related brain regions such as striatum, thalamus or brain stem. However, it remains unclear that the role of other brain region in recovery after brain damage. Here, using cortical injury rat model, we found that AMPAR-mediated miniature EPSC (mEPSC) of the layer 5 pyramidal neurons in the periinjured cortex were positively correlate with recovery rate of forelimb reaching motor performance after rehabilitative training. On the other hand, mEPSC of the medium spiny neurons in the periinjured striatum were negatively correlate with recovery rate of motor performance. Furthermore, mIPSC of the periinjured cortex showed no correlation with motor performance. These results suggest that changes of excitatory input in the peri-injured region could contribute functional recovery after cortical damage. (COI:No)

## 1P-036

### Neuronal activities underlying an experience-dependent synaptic remodeling in the developing sensory thalamus

Hisako Nakayama, Mariko Miyata (*Dept Neurophysiol, Grad Sch Med, Tokyo Women's Medical Univ, Tokyo, Japan*)

Synaptic remodeling during the postnatal development is a process to establish functional neuronal circuits suitable for individual growth environment and sensory experience. The synaptic remodeling usually consists of strengthening of specific synaptic inputs and elimination of others. In the developing sensory thalamus, each VPm neuron receives synaptic inputs from more than three afferents (MLs, medial lemniscal fibers) which convey sensory information from whisker and perioral regions in the brain stem, PrV2 and PrV3, respectively. Among the competing synaptic inputs, those arise from one PrV2-ML are selectively strengthened and maintained but others are eliminated by postnatal day 20 (P20). Those processes have been shown to depend on sensory experience using whiskers during P12-P14, however, mechanisms underlying the sensory experience dependent processes are almost unknown. In the present study, we examined how neuronal activities of postsynaptic VPm neurons have impacts on the selective strengthening and elimination. Neuronal activities were reduced by injecting AAV vectors expressing Kir2.1 to thalamic VPm region at P3. We confirmed that excitability of the Kir2.1-expressing neurons was reduced at P10. The amplitude of ML-EPSCs elicited in the Kir2.1-expressing VPm neurons was significantly smaller than that in Kir2.1-unexpressing VPm neurons at P21-P25. As for elimination, the numbers of discrete steps in the EPSCs in response to gradually strengthened stimulus in most Kir2.1-expressing VPm neurons were one as is the case in Kir2.1-unexpressing VPm neurons. Our results suggest that selective strengthening and elimination during developmental synapse remodeling are differentially regulated by neuronal activities of postsynaptic VPm neurons. (COI:No)

## 1P-037

### Immunohistological and behavioral analysis of peripheral nerve injury in methylmercury-exposed rats

Yo Shinoda[1], Yuta Yamada[1], Mari Kikuta[2,3], Momoko Sakamoto[1], Yayoi Tsuneoka[1], Tsutomu Takahashi[1], Eiko Yoshida[4], Toshiyuki Kaji[4], Yasuyuki Fujiwara[1] (*[1]Dept Env and Health, Sch Pharm, Tokyo Univ Pharm and Life Sci, Japan, [2]Tokyo Gakugei Univ Int Sec Sch, Japan, [3]Keio Univ, JST/GSC, Japan, [4]Dept Env and Health, Dept Pharm, Tokyo Univ Sci, Japan*)

Methylmercury (MeHg) is known as the causal substrate of Minamata disease, which induces central and peripheral nerve injury. MeHg induced neural degeneration have been investigated mainly by using brain because of the principal pathological phenotype is caused by the impairment of central nervous system (CNS). In contrast, MeHg induced neural injury in peripheral nervous system (PNS) is not well investigated. To clarify the aspects of neural injury in PNS induced by MeHg, 9 weeks Wistar male rats were exposed by MeHgCl solution orally for 5 days and not exposed for subsequent 2 days (Day 7). This cycle was continued to the next week again (Day 14). In Day 7 and 14, rats were anesthetized, fixed and their dorsal root ganglion (DRG), motor and sensory fibers were excised and cryosectioned. Co-staining of axonal marker NF and myelin marker MBP showed axonal degeneration in sensory, but not in motor fibers in Day 14. The peripheral sensory neurons in DRG were also degenerated especially middle to large sized neurons in Day 14. In fact, DRG has many different subtypes of neurons that sense pain, pressure, itch, etc., so that neurons were classified by different markers such as NF, PLXNC1, TrkA and TH, however, these classified staining did not show any differences of neural degeneration between each subtypes. We also analyzed another cell type using several cell-type specific markers such as microglia (Iba1), macrophage (CD68), astrocyte (GFAP), fibroblast (Vimentin), epithelial cell (CD31) and Schwann cell (SOX10). Microglia, macrophage and Schwann cell were significantly increased in Day 14 DRG and sensory fibers. These results suggest that MeHg affects peripheral neural degeneration in DRG and axonal degeneration in sensory fiber but not in motor fiber with concerting several cells. (COI:No)

## 1P-038

### Transduction of sodium taste in the taste buds

Akiyuki Taruno[1,2] (*[1]Dept Mol Cell Physiol, Kyoto Prefec Univ Med, Japan, [2]PRESTO, JST*)

Sodium as an ion ($Na^+$) is an essential mineral and the major cation in the extracellular fluid, and sodium taste regulates $Na^+$ intake. Amiloride-sensitive epithelial sodium channel (ENaC) in taste cells is the $Na^+$ sensor mediating behavioral attraction to sodium salts. However, how taste cells process oral $Na^+$ downstream of ENaC remains to be defined. I performed simultaneous patch-clamp and $Ca^{2+}$ imaging experiments in ENaC $\alpha$-expressing (ENaC $\alpha^+$) taste cells acutely dissociated from the fungiform taste buds of mice expressing GCaMP3 under the control of ENaC $\alpha$ promoter. Electrically excitable ENaC $\alpha$+ taste cells that generated spikes by 2 mM $Ba^{2+}$ responded to ENaC-mediated $Na^+$ influx by a suprathreshold depolarization and action potential firing. In the whole-cell voltage-clamp recordings, the same cells exhibited an amiloride-sensitive ENaC current, a voltage-gated $Na^+$ (Nav) current, and a voltage-gated non-selective outward current. The outward current was abolished by 10 μM carbenoxolone, a blocker of CALHM1/3 channel, and knockout of Calhm3, demonstrating that the current was mediated by CALHM1/3. As CALHM1/3 is the action potential-dependent neurotransmitter-release channel originally found in sweet-, bitter-, umami-sensing type II taste cells, it is suggested that taste cells transduce ENaC-mediated $Na^+$ influx directly into a burst of action potential and thereby CALHM1/3 channel-dependent neurotransmission. Meanwhile, I also found taste cells that express functional ENaCs but neither Nav nor CALHM1/3 channels. Thus, these studies identified functional ENaC expression in two functionally distinct taste cell populations. Further studies are required to determine how each cell population contributes to sodium taste perception. (COI:No)

## 1P-039

### The cells and neurotransmission underlying sodium taste in the taste buds

Miho Nakanishi[1], Kengo Nomura[1], Akiyuki Taruno[1,2] (*[1]Dept Mol Cell Physiol, Kyoto Prefec Univ Med, Japan, [2]PRESTO, JST*)

The excessive intake of salt (NaCl) can cause hypertension, and salt intake is regulated by the taste of sodium. Thus, understanding the mechanism of sodium taste is important. It is known that sodium-sensing in the tongue is mediated by amiloride-sensitive epithelial sodium channel (ENaC). Our recent electrophysiological studies showed that ENaC function exists in two distinct taste cell populations. One harbors Nav and CALHM1/3 channels, and the other harbors neither Nav nor CALHM1/3. CALHM1/3 is the action potential-dependent neurotransmitter release channel. However, it remains unclear how each cell population contributes to sodium taste perception.

We first performed immunohistochemical examinations to detect ENaC $\alpha$- and Calhm3-expressing cells. Consistent with the electrophysiological findings, ENaC $\alpha$-expressing cells were divided into two distinct populations based on Calhm3-expression. We subsequently carried out a behavioral taste test, called brief-access lick test, using mice lacking ENaC $\alpha$ in Calhm1-expressing cells (ENaC-cKO) and Calhm3-knockout mice. In the test, mice were depleted of sodium and then presented with various concentrations of NaCl in randomized order, and licks of each solution were counted in 5-sec window. The control mice showed behavioral attraction to NaCl; they preferred higher concentration of NaCl. In contrast, the attractive responses to NaCl were strongly attenuated in ENaC-cKO mice, and abolished in Calhm3-knockout mice. Furthermore, the amiloride-sensitive component of the gustatory nerve responses to oral NaCl stimuli was absent in both ENaC-cKO and Calhm3-knockout mice.

In summary, ENaC function in CALHM1/3-expressing cells is indispensable for the gustatory nerve response and behavioral attraction to NaCl, and CALHM1/3 function is also necessary for taste responses to NaCl. We concluded that the taste cells expressing both ENaC and CALHM1/3 are responsible for sodium taste perception, and the sodium taste cells employ the CALHM1/3 channel for the neurotransmitter release to the gustatory nerves. (COI:No)

## 1P-040

### Immunohistostaining and $Ca^{2+}$-imaging of tracheal solitary chemosensory cells in mice

Yoshitaka Ohtubo, Aiko Miyazaki (*Dept Human Intelligence Sys, Grad Sch Life Scie Sys Eng, Kyutech, Japan*)

The respiratory tract contains solitary chemosensory cells (SCCs) and chemosensory cell clusters for chemical detection. These cells express taste receptors, Tas1Rs and Tas2Rs, and are thought to use intracellular signaling molecules, such as gustducin and IP3 receptor type 3 ($IP_3R3$), the molecules used in the signal transduction of taste to convert chemical into biological information. In this study, we investigated the co-expression of gustducin and $IP_3R3$ and performed $Ca^{2+}$-imaging of SCCs in mouse trachea. Gustducin-immunoreactive SCCs were observed throughout the trachea. In double immunohistostaining, 75% (632/842 cells) of $IP_3R3$-expressing cells were also immunoreactive to gustducin, whereas 34% (632/1878 cells) of gustducin-expressing cells were $IP_3R3$ immunoreactive. Before measuring the intracellular $Ca^{2+}$ changes with fura-2, fura-2-stained cells were identified with immunohistochemistry, because these cells were scattered on the tracheae surface. Almost all (98%) fura-2-stained cells were gustducin-immunoreactive. In a few SCCs, a spontaneous intracellular $Ca^{2+}$ increase occurred in physiological saline. Further, some, but not all SCCs responded to 10 mM denatonium benzoate. Bacteria in the tracheae produce chemicals, including bitter substances used in quorum sensing. SCCs in tracheae increase the intracellular $Ca^{2+}$ in response to bitter substances, which may trigger physiological reactions, such as an epithelial inflammatory response. (COI:No)

## 1P-041

### Correlations of oral capsaicin thresholds and genotypes of capsaicin receptor gene in our medical students

Yoshihiro Murata[1], Kiwamu Shibano[1], Masahiro Yamaguchi[1], Fumino Okutani[2] (*[1]Dept Physiol, Kochi Med Sch, Japan, [2]Dept Occup Health, Kochi Med Sch, Japan*)

We reported in the previous meetings that detection thresholds to capsaicin at the anterior part of the tongue showed a bimodal distribution. To explore the mechanisms that underlie the individual differences, we examined the correlations of oral capsaicin thresholds with single nucleotide polymorphisms (SNPs) of capsaicin receptor gene *TRPV1*. Oral capsaicin thresholds were measured on the anterior part of the tongue by the "filter-paper disc" method. *TRPV1* SNPs (rs222747, rs224534, and rs8065080) were detected by TaqMan SNP Genotyping Assays using real-time PCR (StepOnePlus, Thermo Fisher Scientific). Among 227 subjects with a median age of 21-year-old from our medical students, we found a haplotype significant for those with the higher-threshold of oral capsaicin (haplotype frequency, 0.029 for the "lower" group vs. 0.141 for the "higher" group; permutation test, P = 0.012; SNPAlyze, dynacom). Individual differences in oral capsaicin thresholds could be caused by genetic factors. This study was approved by the Ethic Committee of Kochi Medical School 23-102 and partly supported by KAKENHI (19K02296) to Y.M. (COI:No)

## 1P-042

### Pharmacological blockade of spinally-sensitized dorsal horn neurons in a reserpine-induced fibromyalgia model

Toru Taguchi[1,2], Daisuke Uta[3], Katsuyuki Tsuboshima[4], Hisao Nishijo[4], Kazue Mizumura[5] (*[1]Dept. Phys. Ther., Niigata Univ. Health Welfare, Niigata, [2]Inst. Hum. Mov. Med. Sci, Niigata Univ. Health Welfare, Niigata, [3]Dept. Appl. Pharmacol., Grad. Sch. Med. Pharm. Sci., Univ. Toyama, Toyama, [4]Syst. Emot. Sci., Grad. Sch. Med. Pharm. Sci., Univ. Toyama, Toyama, [5]Dept. Phys. Sch. Dent. Nihon Univ., Tokyo*)

Chronic widespread pain such as fibromyalgia (FM) is a major medical problem. Behavioral pharmacological studies showed that some drugs were effective for reducing pain, yet the spinal anti-nociceptive mechanisms are poorly understood. The present study was conducted to examine whether therapeutic drugs for FM (amitriptyline, duloxetine, and pregabalin) could affect neuronal responses of the superficial dorsal horn (SDH) neurons using a rat model of FM. Subcutaneous injections of reserpine (1 mg/kg), a depleter of biogenic amines in the nervous system, were administered to make the FM model (Nagakura et al. Pain, 2009). Under urethane anesthesia, extracellular recordings in vivo were made from the SDH neurons at the lumbar segments L4-L5. The SDH neurons showed the higher spontaneous discharges in the FM group compared with the control group. Responsiveness to quantitative mechanical stimuli, applied to the receptive field of the SDH neurons, induced stimulus intensity-dependent increases in the discharge rate, and the response magnitude was significantly greater in the FM group compared with the control. Bath application of amitriptyline (0.1～1 mM) and duloxetine (0.1～1 mM) on the surface of the spinal cord remarkably suppressed the increased spontaneous and mechanical discharges in a dose-dependent manner, while that of pregabalin (1 mM) did not. These results suggest that the SDH neurons are sensitized in an FM model, and that the neurons are the site of analgesic actions for amitriptyline and duloxetine, but not pregabalin, for the treatment of FM. (COI:No)

## 1P-043

### The gate mechanism of sensation-respiration in the parabrachial nucleus

Akiko Arata, Hirotaka Ooka, Chiaki Uchida, Sotatsu Tonomura (*Dept Physiol, Hyogo Coll Med, Nishinomiya, Japan*)

The multi-sensational signals were projected to the lateral parabrachial nucleus (LPB) in the pons. LPB has also known as the system of inspiratory-expiratory (I-E) phase switching that contributes to the control of respiratory rate. Thus, the tight interaction between respiration and pain signals as nociception-respiratory coordination was expected in LPB. In this study, we investigated the pain-respiratory coordination system using the pons-medulla-spinal cord preparation intact forelimb isolated from postnatal 0-4 days-rats. The spontaneous inspiratory activity was recorded from cervical fourth (C4) ventral nerve root, and we examined the responses of C4 activity when 2% capsaicin was injected into the forelimb. The C4 inspiratory rate increased significantly in the preparation with pons, but not the removal of pons ($P<0.01$). Moreover, we examined the properties of LPB neurons in this preparation. First, the responded area of LPB from C8 dorsal root stimulation was detected by optical imaging using voltage-sensitive dye; the LPB neurons (n=45) were recorded from the responded area using whole-cell patch-clamp. I-E neurons, which were synchronized with the I-E phase of C4 ventral root activity, located in the extra LPB. The spontaneous or non-spontaneous firing neurons, which was not synchronized with the C4 activity called non-respiratory neurons. All I-E neurons and eight non-respiratory neurons, which existed in the extra lateral PB. Each neuron tested the current-voltage (I-V) relationship in current-clamp mode. According to the responses of hyperpolarizing current pulses, post-inhibitory rebound (PIR) was observed in 13 non-respiratory neurons. These results suggested that 1) the pons contribute to the increase of respiratory rate by noxious stimulation; 2) I-E neurons could directly receive noxious information, so I-E neurons were thought to be the core mechanism of pain-respiratory coordination; 3) the non-respiratory LPB neurons which expressed PIR might be contributed to the onset-switching mechanism of the pain-respiratory coordination network. (COI:No)

## 1P-044

### An implantable cranial window using a collagen membrane for chronic voltage-sensitive dye imaging

Nobuo Kunori[1], Ichiro Takashima[2] ([1]*Neurorehabilitation research group, AIST, Tsukuba, Japan,* [2]*Human Informatic Research Institute, AIST*)

Incorporating optical methods into implantable neural sensing devices is a challenging approach for brain-machine interfacing. Specifically, voltage-sensitive dye (VSD) imaging is a powerful tool enabling visualization of the network activity of thousands of neurons at high spatiotemporal resolution. However, VSD imaging usually requires removal of the dura mater for dye staining and thereafter the exposed cortex needs to be protected using an optically transparent artificial dura. This is a major disadvantage that limits repeated VSD imaging over the long term. To address this issue, we propose to use an atelocollagen membrane as the dura substitute. The membrane used in the present study was optically transparent and enough to pass excitation/emission light for VSD imaging. We made a small cranial chamber device, which is a tubular structure equipped with a collagen membrane at one end of the tube, and implanted the device over the sensorimotor cortex of rats. When performing the VSD imaging 1 week following implantation surgery, we successfully observed the forelimb-evoked neuronal activities in the sensorimotor cortex through the collagen membrane. We also tried pharmacological modulation of neuronal responses through the implanted-cranial window. The gabazine, GABAA receptor antagonist, placed into the implanted chamber clearly increased forelimb-evoked neuronal responses. These results indicate that the atelocollagen membrane was chemically transparent, allowing VSD staining and pharmacological interventions across the membrane material. Because of its ideal chemical and optical manipulation capability, this collagen membrane may be widely applicable in various implantable neural sensors to monitor the changes of neuronal activity due to variety of reasons such as motor learning and stroke. (COI:No)

## 1P-045

### Neuronal mechanism of contrast sensitivity modulation via noradrenergic $\beta$ receptor

Keisuke Tsunoda (*Grad Sch Frontier Biosci., Osaka Univ, Osaka, Japan*)

Noradrenaline (NA) secreted throughout the brain by noradrenergic neurons in the locus coeruleus regulates various brain functions, including vision. Previous electrophysiological studies on the primary visual cortex (V1) revealed that iontophoretically-administered NA modulates the visual responses and the modulatory effect differs from neuron to neuron, being facilitative or suppressive. The NA-induced neuronal response modulation in V1 suggests to modulate the visual information processing, affecting perceptual visual detectability. However, it has not been unclear whether and how the noradrenergic response modulation of V1 neurons affects perceptual visual performance.

In order to investigate this point, we performed multipoint extracellular recordings from the V1 of the rats performing the visual stimulus detection task, and first examined the relationship between task performance and neural activity, and then, tested the effect of a noradrenergic Beta-receptor antagonist. The behavior task was Go/No-go visual detection task. Grating stimulus with various contrasts was presented on the front monitor of a rat under head-restrained condition, and the perceptual contrast threshold as a lower contrast limit enabling to detect the stimulus was estimated. The rat was rewarded by making a response (pulling a lever) to the stimulus presentation. During the rat performed the task, propranolol (Beta-receptor antagonist, PRP) was topically administered on the cortical surface of V1.

About 10% of V1 neurons changed their firing rates corresponding on the task performance, in which the magnitude of the visual responses was higher in the correct trial than the miss trial. PRP administration significantly lowered the perceptual contrast threshold, suggesting an improvement of the perceptual detectability for low contrast stimulus. Also, the neuronal signal-to-noise ratio was improved at low contrast conditions but not at high conditions. Therefore, NA might reduce animal's visual ability to detect stimuli at low contrast conditions via Beta-receptor in V1. (COI:No)

## 1P-046

### Steroidal substances derived from the abdominal gland of the male newt, Cynops pyrrhogaster, may act as female-attracting pheromones

Fumiyo Toyoda[1], Shogo Haraguchi[2], Kazutoshi Yamamoto[3], Masahiro Matsunaga[4], Minoru Takase[5], Tomoaki Nakada[6], Kazuyoshi Tsuitsui[3], Sakae Kikuyama[3] ([1]*Dept Neurophysiol, Nara Medical Univ, Japan,* [2]*Department of Biochemistry, Showa Univ Sch. Med, Tokyo, Japan,* [3]*Dept Biol, Fac Educ Integr Sci, Waseda Univ, Japan,* [4]*Dept Health and Psychosocial Med, Aichi Med Univ Sch Med, Nagakute, Aichi, Japan,* [5]*Amphibian Research Center, Hiroshima University, Hiroshima, Japan,* [6]*Dept Comp Behav Med, Nippon Vet Life Sci Univ, Japan*)

Previously, we identified a decapeptide (sodefrin) in the abdominal gland (AG) of the male newt as a major female-attracting pheromone. Meanwhile, we found that several steroidal substances are synthesized in the gland and that among them, pregnenolone and androstenedione are released from the gland. We confirmed that both steroids exert a female-attracting activity. The minimum effective amount were approximately 0.4ng in both cases when they were subjected to a preference test that was developed by ourselves. When both steroids were applied in combination, they showed an additive effect. The minimum effective amount of these steroids was estimated to be the amount contained in 10-2 piece of the AG of the sexually developed male newts. The minimum effective amount of sodefrin had previously been determined to be 10ng, which is contained in 10-3 piece of the gland. Accordingly, we concluded that sodefrin is the major pheromone exerting a female-attracting activity in the field during the breeding season. When the combination of 10 ng sodefrin with 0.04 ng of both steroids, all of which are equivalent to 10-3 piece of AG contents, was compared with sodefrin alone or both of the two steroids in terms of attracting the female newts, sodefrin in combination with the steroids exhibited the most potent activity. In order to determine the responsiveness of the receptor cells in the vomeronasal epithelium of the lateral nasal sinus region to these steroids, electro-olfactograms (EOGs) were recorded. In the sexually developed females, the vomeronasal epithelium showed a considerable response to the steroids. When subthreshold amount of the steroids was applied together with sodefrin, the EOG response was markedly enhanced as compared with the response to sodefrin alone. BSA-conjugated androstenedione also induced EOG response in the epithelium, indicating that the steroid acts through the membrane receptors of the vomeronasal cells. (COI:No)

## 1P-047

### Circadian clock disruption in mice with adenine-induced tubulointerstitial nephropathy

Yu Tahara (*Lab Physiol Pharm, Sch Adv Sci Eng, Waseda Univ, Japan*)

Chronic Kidney Disease (CKD) is increasing in incidence and has become a worldwide health problem. Sleep disorders are prevalent in CKD patients raising the possibility that these patients have a disorganized circadian timing system. Here, we examined the effect of adenine (0.2%, 2 weeks)-induced tubulointerstitial nephropathy on the circadian system in mice. Compared to controls, adenine-treated mice showed serum biochemical measures of renal dysfunction as well as increased expression of renal markers for inflammation and fibrosis. The CKD mice exhibited fragmented sleep behavior and locomotor activity, with lower amounts of activity compared to controls. On a molecular level, the CKD mice exhibited low amplitude rhythms in their central circadian clock (suprachiasmatic nucleus) as measured in vitro PER2::LUCIFERASE driven bioluminescence. Whole animal imaging revealed that the treated mice also exhibited dampened oscillations in the intact kidney, liver, and submandibular gland. Consistently, dampened circadian oscillations were observed in several circadian clock genes and clock-controlled genes in the kidney of the CKD mice. Finally, mice with a genetically disrupted circadian clock (Clock mutants) were treated with adenine and compared to WT controls. The treatment evoked worse kidney damage as higher gelatinases (MMP-2 and 9) and adenine metabolite deposition in the kidney. Adenine also caused non-dipping hypertension and lower heart rate. Taken together, the data indicate that central and peripheral circadian clocks are disrupted in the adenine-treated mice, and suggest that the disruption of the circadian clock accelerates CKD progression. (COI:No)

## 1P-048

### Glucocorticoid exposure during the circadian inactive phase induces aberrant expression of hypothalamic orexigenic and anorexigenic neuropeptides and alters feeding behaviour in rats

Mitsuhiro Yoshimura[1], Benjamin Flynn[1], Yvonne Kershaw[1], Zidong Zhao[1], Becky Conway-Campbell[1], Yoichi Ueta[2], Stafford Lightman[1] ([1]*THS, BMS, Univ of Bristol, UK,* [2]*Dept of Physiol, Sch of Med, UOEH*)

Adrenal glucocorticoid secretion is characterised by both circadian and ultradian rhythmicity. Here, we have assessed how dysregulated corticosterone (CORT) rhythm affects feeding behaviour in rats. Adult male SD rats were adrenalectomised, implanted with a jugular cannula and an intraperitoneal telemetry probe. Rats were kept under 12/12 light/dark cycle and provided with food and saline *ad libitum*. They were divided into 2 groups: FORWARD and REVERSE. We used a programmable pulsatile infusion system to deliver CORT-HBC, which is a water-soluble form of CORT. In the FORWARD group, the CORT-HBC infusion pattern was programmed to deliver both circadian and ultradian components of an endogenous CORT profile determined by automated blood sampling of adrenal-intact animals. In the REVERSE group, the same dose and pattern of CORT-HBC was infused, except phase-shifted by 12h. Food and saline intake were measured every 12h and body weight was measured daily for 5 days. Brains, subcutaneous and epididymal fat, and blood samples were collected at zeitgeber time (ZT) 1 and ZT13. In the FORWARD group, the percentage of food intake was significantly greater in the dark phase compared to the light phase. In the REVERSE group, food intake profile was significantly dysregulated. No change in body mass, subcutaneous or epididymal fat mass was observed between groups. Locomotor activity profiles were similar; however, core body temperature was altered in REVERSE compared to FORWARD. *In situ* hybridization histochemistry revealed that hypothalamic gene expression of the anorexigenic neuropeptides were significantly upregulated at ZT13, whereas, that of orexigenic neuropeptides were markedly upregulated at ZT1 in REVERSE compared to FORWARD. Clock genes expression in the suprachiasmatic nucleus was unaffected by infusion pattern. These results suggest that desynchronization of light/dark cues and circadian CORT rhythms can induce inappropriate feeding behaviour by directly altering hypothalamic neuropeptide expression. (COI:No)

## 1P-049

### Prefrontal Parvalbumin Interneurons are Essential for Social Behavior Development

Kazuhiko Yamamuro[1], Hirofumi Morishita[2], Toshifumi Kishimoto[1] (*[1]Dept Psychiatry, Nara Medical Univ, Japan, [2]Dept Psychiatry, Icahan School of Medical Sinai Univ, USA*)

Social isolation (incl. loneliness experience) during developmental critical windows could be highly detrimental to proper functioning of mature prefrontal cortex (PFC) and establishment of appropriate adult behaviors. However, the specific circuits that undergo social experience-dependent maturation to regulate social behavior development are poorly understood. Here we show that juvenile social isolation in mice leads to reduced intrinsic excitability and input drives of adult parvalbumin-positive interneurons (PVIs) in medial PFC (mPFC), suggesting juvenile social experience is required for their proper activation in adulthood. In vivo imaging of mPFC-PVI activity by fiber photometry demonstrated that adult mPFC-PVIs are preferentially activated by social signals. Recapitulating decreased activity of PVIs through acute chemogenetic suppression revealed that normal social behavior requires physiological mPFC-PVI activity. Conversely, chemogenetic restoration of mPFC-PVIs activity in the adult animal rescued juvenile isolation-induced social deficits. Therefore, PVI development in the juvenile m PFC is critically linked to long-term impacts on social behavior. (COI:No)

## 1P-050

### The suprachiasmatic nucleus regulates wakefulness via CRF neurons in the hypothalamus

Daisuke Ono, Hung Chi Jung, Srikanta Chowdhury, Wang Guanyi, Akihiro Yamanaka (*RIEM, Nagoya Univ, Japan*)

Living organisms exhibit endogenous circadian rhythms and adapt to the 24 hour daily cycles on Earth. Circadian rhythms are known to organize temporal timing of physiology and behavior such as body temperature, metabolism, and sleep/wakefulness. In mammals, the suprachiasmatic nucleus of the hypothalamus (SCN) functions as master circadian pacemaker including thousands of neurons that express self-sustained and synchronizing circadian rhythms in firing activity and gene expression. Nevertheless, little is known about neuronal projections from the SCN that regulate sleep/wakefulness. Here we show that corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus mediate circadian rhythms in the SCN. Optogenetic activation of CRF neurons promotes wakefulness. Furthermore, in vivo Ca2+ recording using fiberphotometory revealed that CRF neurons in the PVN were active during wakefulness, but not NREM and REM sleep. We also found that neuronal activity of CRF neurons in the PVN were regulated by the SCN. We will discuss mechanisms by which the SCN regulated sleep and wakefulness in mice. (COI:No)

## 1P-051

### A corticohypothalamic pathway for psychological stress-induced social avoidance

Yuta Shima, Naoya Kataoka, Kazuhiro Nakamura (*Dept Integrative Physiol, Nagoya Univ Grad Sch Med, Japan*)

Psychological stress causes various physiological responses by stimulating sympathetic mechanisms (e.g., increases in body temperature, heart rate and blood pressure) and behavioral mechanisms in mammals. Recently, we discovered a group of glutamatergic excitatory neurons in the dorsal peduncular cortex and dorsal tenia tecta (DP/DTT), located at the ventral limit of the medial prefrontal cortex, and found that they mediate master stress signaling to the dorsomedial hypothalamus (DMH) to drive various sympathetic stress responses. However, it is unknown whether this DP/DTT→DMH pathway contributes to behavioral changes induced by stress or emotion. Here, we examined the effect of the DP/DTT→DMH pathway on social avoidance induced by social defeat stress, an animal model of psychosocial stress. A male Wistar rat underwent social defeat stress from a dominant, male Long-Evans rat, and was subsequently placed in an open field. Then, the same Long-Evans rat, which was caged, was placed in the field. The stressed Wistar rats mostly stayed at the corners of the field, being away from the Long-Evans rat and avoiding interaction. This stress-induced social avoidance contrasted with active social interaction exhibited by naïve Wistar rats, which had not undergone social defeat stress. To selectively inhibit the DP/DTT→DMH pathway in the stressed Wistar rats, the animals beforehand received injections with adeno-associated virus (AAV) to transduce DP/DTT→DMH projection neurons with iChloC, a photo-activated chloride channel shown to suppress neuronal activity. Photoinhibition of the DP/DTT→DMH pathway completely reversed the social defeat stress-induced social avoidance: the Wistar rats often entered the interaction zone surrounding the cage of the Long-Evans rat and actively interacted. These results demonstrate that the stress signaling mediated by the DP/DTT→DMH pathway is essential to drive stress-induced social avoidance as well as sympathetic stress responses. (COI:No)

## 1P-052

### Odor induced anti-pruritic effect on pruritogen-induced scratching behavior in mice

Tatsuroh Kaneko, Tomoyuki Kuwaki, Hideki Kashiwadani (*Dept Physiol, Med, Kagoshima Univ, Japan*)

Pruritus is one of the uncomfortable feelings on the skin and sometimes impairs our quality of life. Antihistamines are the initial drug of choice for the treatment of pruritus, but there are several types of pruritus on which antihistamines do not efficiently work. Thus, the development of treatment for antihistamines-resistance pruritus is one of the pressing issues.

Previously we have shown that odor of linalool, one of the monoterpene alcohols in lavender extracts, induced analgesic effects triggered by olfactory input. The linalool analgesia significantly attenuate chemical pains induced by formalin or capsaicin, raising the possibility that the linalool odor may also attenuate antihistamine-resistance itch evoked by chemical pruritogen. To address the possibility, we observed the scratching behaviors evoked by intradermal administration of pruritogens in mice under linalool odor exposure.

Male C57/BL6 mice (9 weeks-old) were used in the experiments. As peripheral pruritogens, serotonin (50ug/50uL/site) and chroloquine (200ug/50uL/site) were intradermaly injected into the nape of the neck of mice. Immediately after the injection, mice were placed in observation chamber in which was filled with linalool odor or odorless air (as control). Then scratching behavior was video-recorded for 30 minutes and analyzed.

In serotonin-induced scratching mice model, linalool odor exposure significantly reduced scratching behavior for first 6 minutes after serotonin injection. Linalool odor also significantly reduced scratching behavior in chroloquine-induced itchy mice for 20 minutes after chroloquine injection.

In conclusion, linalool odor exposure decreased scratching behavior in peripherally induced itchy mice models. These data suggest the potential benefit of linalool odor on the control of pruritus in clinical situation. (COI:No)

## 1P-053

### Physiological function of VRK 2 in zebrafish model

Ryohei Umeda[1], Kazumasa Hada [2], Kenshiro Shikano[1], Ryoko Higa[1], Hirotaro Urushibata [2], Hiroshi Shiraishi [2], Toshikatsu Hanada [2], Reiko Hanada [1] (*[1]Dept Neurophysiol, Fac Med, Oita Univ, Japan, [2]Cell Biol, Grad Sch Med, Oita Univ, Japan*)

Vaccinia related kinase 2 (VRK2), a serine / threonine kinase belonging to the Vaccinia-related kinase family, which plays an important role in cell survival and stress response to hypoxia. Recently, VRK2 gene mutations in humans have been reported to be one of the causes of schizophrenia. In previous studies, schizophrenia and its related diseases were shown by Genome-wide association study (GWAS) analysis, and it was found VRK2 as one of candidate genes of their causes. The functional analysis of VRK2 has been done by in vitro studies using cells, however in vivo analysis has not been performed yet. Thus, it is not clear the physiological function of VRK2 in vivo. Therefore, we have established a VRK2 gene-deficient zebrafish (VRK2 KO) using the CRISPR / Cas9 system, and analyzed a series of behavior experiments. At first, their growth conditions such as body length and body weight were analyzed in VRK2 KO and control zebrafish (WT). Since VRK2 gene mutations is reported to be a cause of mental illness, we next analyzed morphological changes of the brain using HE staining. In addition, we performed a series of behavioral analysis of aggression, sociality, and anxiety related to schizophrenia using Mirror test, Social interaction test, and Novel tank diving test, respectively. Here, we will present these data. (COI:No)

## 1P-054

### The ontogeny of glycine transporter 1 (GlyT1) during development in the spinal cord

Chigusa Shimizu[1], Daisuke Omata[1], Ryuji Tomoyose[1], Kie Okano[1], Shiori Kobayashi[1], Akihito Okabe[2], Chitoshi Takayama[1] (*[1]Dept.Mol.Anatomy, Grad.Sch.Med, University of the Ryukyus, [2]Seinan Jo Gakuinn University*)

In the spinal cord, glycine and Gamma-amino butyric acid (GABA) are inhibitory neurotransmitters. Released glycine and GABA is removed from the synaptic cleft by glycine transporter1 (GlyT1) and GABA transporter 3 (GAT-3). We have previously investigated the developmental changes in GABA-removal system (Kim et al. 2014). However, the development of glycine-removal system in astrocytes remains unclear. The present study aimed to reveal the ontogeny of the glycine-removal system in the astrocytes by examining the immunohistochemical localization of GlyT1 in the embryonic and postnatal mouse cervical spinal cord.

On embryonic day 12 (E12), GlyT1 was expressed in the mantle layer. GlyT1 was localized in the process of radial glia. On E14, GlyT1 was localized in the ventral horn. This expression pattern was different from GAT-3. Weak GAT-3 immunolabeling was localized to several radial fibers extending from the central canal to the pial surface on E12. On E14, GAT-3 was detected in the radial processes. After E18, GlyT1 was colocalized with GAT-3 in astrocytes. In our previous study, GlyT2, which uptakes glycine in presynaptic terminal, was first detected in the ventral horn on E16 after GlyT1 was expressed (Sunagawa et al. 2017).

These results suggested that glycine and GABA were uptaken in different region of the radial glia in E12-14, but after that, both neurotransmitters were removed by the same astrocytes. Before the formation of glycinergic terminal, glycine-removal system in astrocytes might be ready. (COI:No)

## 1P-055

### Modulatory effects of repeated psychophysical stress on nociceptive neural activities in the rostral ventromedial medulla (RVM) evoked by noxious stimulation to the craniofacial tissue in the rats

Mana Hasegawa[1,3], Masayuki Kurose[2], Noritaka Fujii[1,3], Kensuke Yamamura[2], Keiichiro Okamoto[2] (*[1]Div Dental Clinic Edu, Niigata Univ Grad Sch Med Dent Sci, [2]Div Oral Physiol, Niigata Univ Grad Sch Med Dent Sci, [3]General Dent Clinic Edu Unit, Niigata Univ Med Dent Hosp*)

Dysfunction of the descending pain controls, indicated by neural changes in the rostral ventromedial medulla (RVM), are known to increase craniofacial nociception under repeated psychophysical stress conditions. Previous reports showed that repeated psychophysical stress conditionings impaired serotonergic (5HT) mechanisms in the brain. However, it remains unclear if neural changes in the RVM, which had facilitatory effects on craniofacial nociception, could be due to dysfunction of 5HT mechanisms under stress conditions. SD male rats were assigned to repeated Forced Swim Stress (FST) and sham conditionings for 3 days (10 min/d). Fluoxetine (selective serotonin reuptake inhibitor) or vehicle (saline) was administered daily just after each FST. Single unit activities were isolated in the RVM, and units were classified into three types of units (ON-, OFF- and NEUTRAL-cell) based on the responsiveness to noxious heating stimulation (52 ℃) to the facial skin over the masseter muscle under general anesthesia. Heat-evoked neural discharges for those units are quantified in each group after repeated FST. Further, nociceptive EMG activity evoked by heating stimulation to the facial skin was recorded in the suprahyoid muscle, simultaneously. Repeated FST increased neural activities of ON- cells due to increases in prolonged after-discharges. Further, FST also had modulatory effects on OFF-cell activity indicated by prolonged the pause duration in spontaneous activities. FST-induced changes of response properties in ON- and OFF cells were prevented by the systematic administration of fluoxetine. FST had no effects on neural activities in NEUTRAL-cell. Nociceptive EMG activities were significantly increased after repeated FST, which was reduced by daily administration of fluoxetine. Application of fluoxetine alone did not affect the neural and EMG activities. These results indicated that increases in craniofacial nociception in the RVM after repeated FST could be due to neural changes in ON- and OFF cell activities through the changes in 5HT mechanism. (COI:No)

## 1P-056

### The effect of an acupuncture "press tack needle" treatment on orexin secretion under acute stress

Aki Fujiwara[1], Mana Tsukada[1], Takuji Izuno[1], Hideshi Ikemoto[1], Yasunori Takayama[1], Shi-yu Guo[1], Yan-qing Liu[1,2], Tadashi Hisamitsu[1], Masataka Sunagawa[1] (*[1]Dept Physiol, Sch Med, Showa Univ, Japan, [2]Dept of Combined Traditional Chinese and Western Med, Yangzhou Univ Sch Med, China*)

Orexins are neuropeptides secreted mainly from the hypothalamus and are involved in the control of stress responses. We previously reported that press tack needle (PTN) treatment at the GV20 acupuncture point (Baihui) suppresses orexin A secretion, which is increased by chronic mental stress. However, this decrease in orexin A secretion is inhibited by electroacupuncture treatment at the SP6 (Sanyinjiao) and ST36 (Zusanli) acupoints in rats with acute pain. In the present study, we investigated whether or not the effects on orexin secretion differ depending on the types of stress experienced using a rat acute stress model.

Rats were randomly divided into three groups as follows: 1. no stress, 2. acute stress, and 3. PTN-treated acute stress. In the PTN+Stress group, the PTN (length: 1.2 mm) was fixed at the acupuncture point that corresponds to human GV20. As acute stress, a restraint stress load was applied for 90 minutes, after which the amount of defecation induced by restraint stress and the plasma orexin A concentration were measured. Compared with the Stress group, the amount of defecation was significantly suppressed in the PTN + Stress group. The plasma orexin A concentration was significantly decreased in the Stress group compared with the Control group, but in the PTN + Stress group, this decrease was significantly suppressed.

In the chronic stress model, the increased orexin A secretion was suppressed by PTN. In contrast, in an acute stress model, PTN inhibited the decrease in the orexin A secretion. These findings suggest that PTN treatment is also effective under acute stress and that the effect has a moderating effect on the secretion of orexin A. (COI:No)

## 1P-057

### Microinjections of an ionotropic excitatory amino acid receptors activator L-cysteine identified a parasympathetic carotid vasodilator response zone that spans the rat brainstem?

Yumi Takemoto (*Neurophysiol, Grad Sch Biomed, Hiroshima Univ, Japan*)

Our previous study demonstrated that microinjection of an ionotropic excitatory amino acid receptors activator L-cysteine into the rostral ventrolateral medulla pressor area (RVLM) produce equi-pressure common carotid (CA) vasodilation in the superior cervical sympathetic denervated rat (SCD), different from pressor and CA vasodilator responses to L-cysteine in the ventral pons of the salivatory nucleus level. It may suggest any parasympathetic functional relation to the pre-sympathetic area in the rat RVLM. The present study was performed to find the exact place where L-cysteine stimulation produces carotid vasodilation in the brainstem around the RVLM. In anesthetized intact rats or SCDs, a window was ventrally opened to access the brainstem, then common carotid flow (CAF), arterial blood pressure (AP) and carotid flow resistance (AP/CAF) were monitored. L-Cysteine microinjections produced carotid vasodilation with equi-pressure or pressor responses at the dorsal and rostral sites from the RVLM, forming a plate-like response zone in individual rats. The plate atlas varied from rat to rat. Vasodilator sites in intact rats and SCDs extended 1.4 mm anterior to and 2.5 mm dorsal to the RVLM, corresponding to the parvicellular reticular and salivatory nuclei which were identified with dye injections. Vasodilator region was rostral and just adjacent to localized carotid vasoconstrictor region within the RVLM pressor area in intact rats, but the region included the whole RVLM pressor area in SCDs which had no carotid vasoconstrictor region. Thus, L-cysteine microinjections into the brainstem parasympathetic related zone produced common carotid vasodilation in rats. It may have functionally identified an autonomic region capable of regulating common carotid flow across the rat brainstem where pre-sympathetic neurons and parasympathetic related fibers may interact. Further studies are required. (COI:No)

## 1P-058

### Fos expression in rat MLR neurons projecting to the RVLM following the voluntary treadmill exercise

Yui Yamane, Tatsuo Watanabe, Satoshi Koba (*Div Integr Physiol, Tottori Univ, Japan*)

Neurons in the mesencephalic locomotor region, MLR, send axonal projections to the rostral ventrolateral medulla, RVLM (MLR-RVLM neurons). Here we tested the hypothesis that the MLR-RVLM neurons are excited in association with voluntary locomotion. Male rats that had received bilateral microinjections into the RVLM with a retrograde adeno-associated virus vector that encoded GFP were accustomed to the voluntary treadmill exercise for seven-to-nine days. On the experimental day, they were treadmill exercised at 16 m/min for 40 min (n = 7) or placed on the treadmill for a comparative control period (n=7). The paraformaldehyde-fixed brains were then processed for double-immunofluorescence microscopy. The MLR of exercised rats contained more expression of Fos protein in both GFP- and ChAT- immunoreactive (IR) neuronal cells than that of control rats (19 ± 4 vs. 8 ± 2% in GFP-IR cells and 22 ± 5 vs. 8 ± 2% in ChAT-IR cells, respectively, $P<0.05$). Interestingly, GFP-IR, RVLM-projecting cells in the MLR did not correspond with ChAT-IR cells, which were abundantly distributed in the pedunculopontine nucleus within the MLR. These results suggest that a portion of RVLM-projecting MLR neurons, which are unlikely cholinergic, is excited during locomotion. (COI:No)

## 1P-059

### The lateral parabrachial nucleus and Kölliker-Fuse nucleus involve in the reflex responses of heart rate to noxious mechanical stimulation of the hindpaw in anesthetized rats

Hana Nozawa[1,2], Rie Shimoju[1,3], Takamichi Taniguchi[1,2], Hideshi Shibata[4], Mieko Kurosawa[1,3] (*[1]Grad. Sch. Health & Sci., Int. Univ. Health & Welfare, Otawara 324-8501, Japan, [2]Dept. Occupational Ther., Intl. Univ. Health & Welfare, Otawara 324-8501, Japan, [3]Center Med. Sci., Intl. Univ. Health & Welfare, Otawara 324-8501, Japan, 4Lab. Vet. Anat., Ins. Agric., Tokyo Univ. Agric & Tech., Fuchu, Tokyo 183-8509, Japan*)

Noxious mechanical stimulation (pinching) of the hindpaw reflexly increases arterial pressure and heart rate via the supraspinal structure in anesthetized rats. We have recently shown that the lateral parabrachial nucleus (LPBN) and the Kölliker-Fuse nucleus (KF) were involved in the reflex responses of arterial blood pressure to pinching of a contralateral hindpaw. The present study was aimed to clarify the involvement of these nuclei in the reflex responses of heart rate. Heart rate was measured by a pulse rate tachometer. Muscimol, a widely used neuronal inhibitor, was nanoinjected into the unilateral LPBN or KF. Pinching was applied with a surgical clamp at a force of 3-5 kg to the hindpaw for 20 s. The administration of muscimol into the LPBN or KF nuclei had no influence on basal values of the heart rate. On the other hand, the heart rate values at 20 s after pinching of the hindpaw were significantly attenuated after the administration of muscimol when pinching was applied to the hindpaw contralateral to the site of muscimol injection. The heart rate values in response to pinching of the ipsilateral hindpaw were slightly attenuated; however, the attenuation was not statistically significant. The effects of muscimol injected into the KF on the reflex responses were similar to those injected into the LPBN. The present results demonstrate that both the LPBN and KF are involved in the reflex responses of heart rate elicited by pinching of the contralateral hindpaw. (COI:No)

## 1P-060

### GABAergic neurons in the rostral medullary raphe nucleus regulate the cardiac parasympathetic system

Taro Minoura, Kazuhiro Nakamura (*Dep Integrative Physiol, Nagoya Univ Grad Sch Med, Japan*)

When mammals are exposed to environmental stressors, glutamatergic sympathetic premotor neurons in the rostral raphe pallidus nucleus (rRPa) of the medulla oblongata are activated to drive sympathetic responses, such as tachycardia and thermogenesis. We have found that GABAergic neurons in the rRPa are also activated at the same time. However, the functions of the GABAergic neurons are unknown. In this study, we sought for their functions. To optogenetically stimulate GABAergic rRPa neurons, these neurons were transduced with ChIEF-mCherry, a channelrhodopsin variant by injecting a Cre-dependent adeno-associated virus vector into the rRPa of Gad2-ires-Cre mice. It was confirmed that most of neurons expressing ChIEF-mCherry were GABAergic. Furthermore, their axon terminals were found to be closely associated with cholinergic neurons in the nucleus ambiguus, which were potentially cardiac parasympathetic preganglionic neurons. This observation suggests that GABAergic rRPa neurons innervate cardiac parasympathetic preganglionic neurons to inhibit them. To test this hypothesis, we examined the effect of photostimulation of GABAergic rRPa neurons on heart rate under anesthesia. Photostimulation of either cell bodies in the rRPa or their axon terminals in the nucleus ambiguus significantly increased heart rate. Prior intravenous injection of atropine, a muscarinic receptor antagonist, suppressed the increase in heart rate caused by photostimulation of cell bodies of GABAergic rRPa neurons. These results suggest that activation of GABAergic rRPa neurons elicits tachycardic response by inhibiting parasympathetic outflow to the heart. The rRPa is likely to be a brain site that controls the balance between sympathetic and parasympathetic efferent tones by regulating the glutamatergic and GABAergic neurons therein. (COI:No)

## 1P-061

### Role of orexin neurons during social defeat stress and descending projections from the hypothalamus in the rat

Ena Yamamoto[1], Takatoshi Horiuchi[1], Misaki Ichikawa[2], Mio Matsuyama[1], Nao Suzuki[2], Joji Horiuchi[1,2] (*[1]Dept Biomedical Engineering, Toyo Univ, Japan, [2]Depat Biomedical Engineering, Toyo Univ, Japan*)

It is known that the hypothalamus plays an important role in the cardiovascular response evoked by psychological stress. Orexin (ORX) neurons are localized within the dorsomedial hypothalamic area (DMH) and the perifornical area (PeF). Therefore, it is suggested that the ORX neurons are involved in the stress induced-cardiovascular response. However, a neural network of the stress response is still unknown. In the present study, we investigated the role of the ORX neurons on the cardiovascular response evoked by social defeat stress (SDS) and its descending projection from the hypothalamus in conscious rats. A telemetry probe was implanted into an experimental animal to measure blood pressure (BP) and heart rate (HR), and the rat was exposed to a single SDS (SSDS) or repeated SDS (14 days). In addition, some rats were microinjected anterograde neural tracer, BDA and then exposed to the SSDS. After both SDS challenges, the brains were removed and stained c-Fos, ORX and BDA. BP and HR were significantly increased during both SDSs. After the repeated SDS challenge, however, baselines of BP and HR did not change. The number of c-Fos immunoreactive neurons in the DMH and the PeF increased after both SDSs. In contrast, the number of ORX neurons in these areas did not change in both SDSs. The percentage of c-Fos-expressed ORX neurons in the DMH profoundly increased (~40%) in the repeated SDS group compared to that of the SSDS (~20%). The nerve terminals from the DMH were observed in the midbrain ventrolateral (vl) periaqueductal grey (PAG) and the medullary raphe. These results suggest that the ORX neurons in the hypothalamus play a crucial role in the cardiovascular response evoked by the SDS, and that the stress response is mediated via neurons in the vlPAG and the medullary raphe. (COI:No)

## 1P-062

### Role of P2Y signals in generating spontaneous contractions in the guinea pig seminal vesicles

Mitsue Takeya[1], Hikaru Hashitani[2], Ryuhei Higashi[3], Kei-ichiro Nakamura[4], Makoto Takano[1] (*[1]Dept Physiol, Kurume Univ Sch Med., Kurume, Japan, [2]Dept Cell Physiol, Grad Sch Med Sci, Nagoya City Univ, Nagoya, Japan, [3]Advanced Imaging Research Center, Kurume Univ Sch Med, Kurume, Japan, [4]Dept Anat, Kurume Univ Sch Med, Kurume, Japan*)

In seminal vesicles (SVs) of the guinea pig, the mucosa is essential for generating spontaneous phasic contractions and corresponding electrical and $Ca^{2+}$ activity in SV smooth muscles. Recently, we have reported that subepithelial synchronous interstitial cells (SSICs), distributed in the lamina propria of SV mucosa, generate spontaneous electrical slow waves to trigger synchronous $Ca^{2+}$ transients in SV smooth muscles. Dye-coupling between SSICs and SV smooth muscles were also demonstrated, suggesting that SSICs function as pacemaker cells to drive the spontaneous contractions by sending depolarising signals via gap junctions. Here we further explored whether purines released from mucosal cells are involved in the SV spontaneous contractions using fluorescent $Ca^{2+}$ imaging and immunohistochemistry. In mucosa-intact preparations, SV smooth muscles developed spontaneous $Ca^{2+}$ flashes and associated contractions at 3.9 ± 0.6 $min^{-1}$. The frequency of the SV spontaneous contractions were reduced by partial removal of epithelium but restored by 10 $\mu$M ADP (1.8 ± 1.1 $min^{-1}$ in control perfusate, vs 3.1 ± 1.5 $min^{-1}$ in ADP, $p<0.05$). In contrast, mucosa-denuded preparations, SV smooth muscles were quiescent and failed to contract in response to ADP, suggesting that P2Y signals within the SV mucosa play a role to accelerate the spontaneous contractions. In the SV mucosa, SSICs were distributed just beneath the layers of columnar and basal epithelial cells. SSICs and both types of epithelial cells were immunopositive for $P2Y_1$. 10 $\mu$M ADP or 0.1 $\mu$M MRS2365, a $P2Y_1$ agonist, evoked $Ca^{2+}$ transients in the basal cells and SSICs. However, 1-10 $\mu$M MRS2500, a $P2Y_1$ antagonist, and additional application of suramine, a non-selective P2 antagonist, did not suppress the generation of either $Ca^{2+}$ transients in SSICs or SV spontaneous contractions. These results indicated that purines play a role to facilitate mucosa-dependent SV contractions. (COI:No)

## 1P-063

### Mathematical model to understand the exercise-induced skeletal muscle fatigue during intense exercise

Yuttamol Muangkram (*Dept Bioinformatics, Life Sciences, Ritsumeikan Univ, Japan*)

During intense exercise activity, it leads to a drop in maximal voluntary contraction or the inability of contractile and metabolic processes of contracting muscle to sustain the desired work output defined as muscle fatigue. It is widely accepted that a close relationship between an increase in lactate concentration and a decrease in pH, and the accumulation of muscle inorganic phosphate have been regarded as the major causes of skeletal muscle fatigue. Intense exercise greatly activates ATPase activity and promotes ATP production, thus leading to an alteration of the metabolic by-products. However, the biological mechanisms underlying the development of muscle fatigue remain not fully understood. In this study, we aimed to construct a basic mathematical model which can reproduce the biological processes of metabolic fatigue in a variety of exercise intensity which is consistent with known experimental data. The novel model captures the key biological reactions including; 1) the role of creatine kinase reaction in maintaining ATP homeostasis, 2) the role of bicarbonate buffer system in regulating blood pH, and 3) the characteristic of metabolic alteration at transition state. Moreover, we revised our previous hybrid contraction model by introducing the inhibitory effect of metabolic by-products based on the structural and experimental data. The accumulation of metabolic by-products reduces the number of attached cross-bridge enhancing the reduction in maximal contraction. In conclusion, the current model which consists of reliable biological fluxes and metabolic concentration in good agreement with experimental results can provide a better understanding of metabolic fatigue during intense exercise. For further study, the combination of the current model with other mechanism-related fatigue, e.g. the shortage of substrates, may help elucidate the nature of metabolic exercise-induced metabolic fatigue. (COI:No)

## 1P-064

### Effects of omecamtiv mecarbil on the contractile properties of skinned porcine left atrial and ventricular muscles

Tomohiro Nakanishi[1], Takako Terui[1,2], Fuyu Kobirumaki[1], Norio Fukuda[1] (*[1]Dept Cell Physiol, Jikei Univ, Japan, [2]Dept Anesth, Jikei Univ, Japan*)

**Background:** Omecamtiv mecarbil (OM) is a compound that has been developed to treat systolic heart failure via targeting cardiac myosin to increase myocardial contractility. The effects, however, have not been systematically investigated in muscles with different expression profiles of myosin heavy chain (MHC).
**Methods and Results:** Active force was measured with skinned porcine left atrial (LA; ~100% $\alpha$-MHC) and ventricular (LV; ~100% $\beta$-MHC) fibers. Sarcomere length was set at 2.1 $\mu$m. OM left-shifted the mid-point of the force-pCa curve ($\Delta pCa_{50}$) by 0.07 and 0.25 pCa units in LA at 0.5 and 1.0 $\mu$M, respectively. The magnitude of the shift of $\Delta pCa_{50}$ significantly more pronounced in LV, in that the values were 0.17 and 0.32 pCa units at 0.5 and 1.0 $\mu$M, respectively. In both LA and LV, the rate of rise of active force ($t_{1/2}$) became slower in the presence of OM, with the magnitude greater in LV; i.e., ~5% and ~10% at 0.5 and 1.0 $\mu$M in LA, as compared to ~20% and ~30% at 0.5 and 1.0 $\mu$M in LV. Finally, we investigated the effects of inorganic phosphate (Pi) on maximal force at pCa 4.5. In LA, Pi decreased maximal force in an inverse-sigmoidal manner with and without OM up to 20 mM. However, in LV, OM attenuated the depressant effect of Pi in a concentration-dependent manner.
**Conclusions:** OM increased $Ca^{2+}$ sensitivity in both LA and LV, with the effect more pronounced in LV. Provided that OM slowed $t_{1/2}$ by a greater magnitude in LV than in LA and the compound attenuated the depressant effect of Pi only in LV, we conclude that OM exerts its $Ca^{2+}$-sensitizing effect in cardiac muscle in a MHC isoform-dependent manner. (COI:No)

## 1P-065

### Transcriptome in fast- and slow-twitch fibers of zebrafish

Souhei Sakata, Fumihito Ono (*Dept Physiol, Faculty Med, Osaka Medical College, Japan*)

Skeletal muscle fibers are mainly divided into two different types; fast-twitch fiber and slow-twitch fiber. Fast-twitch fibers generate strong power and are used for bursts of movements such as sprinting, whereas slow-twitch fibers contribute to long-endurance exercises such as distance running. A difference of gene expressions in these two types of fibers associated with myosin activities and metabolism has been reported. Here we performed a comprehensive gene expression analysis using the next-generation sequencing (NGS) technique in adult zebrafish. We used a transgenic line expressing the red-fluorescent protein, mCherry, to identify slow-twitch fibers that forms a thin layer in fish trunk. Our sequencing yielded 4.8 million and 4.6 million reads, and 89.71% and 96.87% reads were mapped to the genome for the fast- and slow-twitch fiber, respectively. Gene ontology analysis showed that genes involved in cellular component organization, cytoskeleton organization and oxidation-reduction process among others were significantly different between two fiber types. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that gene expressions associated with multiple pathways, including cell cycle, regulation of actin cytoskeleton and calcium signaling pathway were significantly different. We found that many genes, including myosin VI and VII, slow myosin heavy chains, myoglobin, ryanodine receptor Ia were specifically expressed in the slow-twitch fiber. On the other hand, genes such as parvalbumins, fast muscle-type troponins or ryanodine receptor III showed strong expression in the fast-twitch fibers. Our NGS data is expected to uncover molecular mechanisms that characterize fast- and slow-twitch fibers. (COI:No)

## 1P-066

### Decreased response of muscle hypertrophy and the expression of muscle atrophy-relating factors in the sarcopenia muscle

Ippei Yamato, Tsuyoshi Fukuzawa, Shuichi Soeda, Tetsuro Tamaki (*Muscle Physiol & Cell Biol Unit, Tokai Univ. Japan*)

The changes of skeletal muscle atrophy-relating genes were examined in the progressing sarcopenia muscle in old rat after the strong stimulation of muscle hypertrophy. For this purpose, the surgical ablation (SA) of the synergistic muscles (gastrocnemius and soleus muscles) was performed on the right hindlimb, and hypertrophic stimulation was added to the plantaris (PLT) muscles using old (over 2.5-years-old, n=9) and normal adult (10-15-week-old, n=9) male Sprague-Dawley rats. Contralateral-left side was preserved as a control. After 10 days of the SA, both side PLT muscles were excised and weighed, then, the progressing sarcopenia was confirmed by the standard co-relation curve of the body-PLT muscle weight, which was obtained by over 500 rats at the left control PLT. Expressions of Myogenin, Atrogin-1 and MURF1 were analyzed by real time qPCR based on the control. Significant increase of Myogenin (2.5-fold) was observed in the adult group, but there were no changes detected in remaining two factors. Thus, this the muscle hypertrophy depending increase of myogenin expression. However, in the old group, quite higher increase (33-fold) of myogenin was detected associated with the slight increase in Atrogin-1 (1.3-fold) and MURF-1 (1.8-fold). Interestingly, the old group showed 28-fold increase of Myogenin even in the contralateral control. These results showed that the sarcopenia muscle basically showed an extremely high level of Myogenin in the resting state, and this was further accelerated by the hypertrophic stimulation. However, this is not the muscle hypertrophy depending increase, because of the following increase of Atrogin-1 and MURF-1. This result further supported by the result of our time-course study that the compensatory muscle hypertrophy did not occur in the sarcopenic muscle in the old rat after 5 weeks. (COI:No)

## 1P-067

### A possible of gastric inhibitory polypeptide in the regulation of skeletal muscle mass

Katsumasa Goto[1, 2], Megumi Aoshima[1], Arisa Hagiwara[1], Tomohiro Yamashita[1], Huascar Pedro Ortuste Quiroga P.[1], Shingo Yokoyama[2] (*[1]Dept Physiol, Grad Sch Health Sci, Toyohashi SOZO Univ, Japan, [2]Dept Health Sci, Toyohashi SOZO Univ*)

Various interorgan communication networks of proteins, peptides, and metabolites have been proposed. A member of incretin gastric inhibitory polypeptide (GIP), which is synthesized in and secreted from K cells, regulates nutrient absorption in a gastrointestinal tract. GIP receptor (GIPR) expresses in not only a gastrointestinal tract but also $\beta$ cells in the pancreas. Recently, the expression of GIP in skeletal muscle cells is also confirmed. However, the interorgan communication network between a gastrointestinal tract and skeletal muscle remains unclear. In the present study, we investigated a physiological role of GIP in the regulation of the skeletal muscle mass using mouse myoblast-derived C2C12 cells. GIPR expressed in both C2C12 undifferentiated myoblasts and differentiated myotubes. Administration of GIP stimulates myogenic differentiation of C2C12 cells. Knockdown of GIPR suppressed the mRNA expression level of MyoD and Pax7 and myogenic differentiation of C2C12 cells. On the other hand, GIPR-knockdown stimulated proliferation of C2C12 myoblasts. Evidences indicates GIPR-associated intracellular signal(s) play a regulatory role in skeletal muscle mass by maintaining myogenic differentiative potential. This study was supported, in part, by KAKENHI (Grant Numbers JP16K13022, JP17K01762, JP18H03160), the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan, and Graduate School of Health Sciences, Toyohashi SOZO University. (COI:No)

## 1P-068

### Diagnosis of stress exhaustion during induction of anti-stress hormones by hypothalamic response to chronic stress using mouse salivary gland microRNAs as biomarkers

Kinji Kurihara (*Div Physiol, Sch of Dent, Meikai Univ, Japan*)

Amylase in saliva is used as a stress marker, but amylase is partially questioned as a stress marker because it is secreted by various stimulations without stress. MicroRNAs are small non-coding RNAs of 18-28 nucleotides. We examined a new diagnostic method of stress exhaustion using microRNAs in salivary glands as bio-markers.

We investigated microRNA expression patterns in the salivary glands of ICR mice and identified 39 microRNAs. The expression patterns of these microRNAs in mice treated with various hormones were analyzed by quantitative real-time PCR.

When adrenaline was administered for 12 days using a micro osmotic pump (Alzet, Model 1002) continuously and the sympathetic nerve alone was enhanced for a long period, the microRNA did not change. It is well known that anti-stress hormones (glucocorticoids) are secreted when the hypothalamus recognizes chronic stress.

When dexamethasone was administered to normal male mice, miR-29b-3p was increased and let-7c-3p was decreased significantly. Dexamethasone administration to castrated mice showed a more significant effect. These results suggest that glucocorticoids have an additive effect when androgens are depleted as PADAM.

Administration of dihydrotestosterone to castrated mice increased miR-21-5p and miR-141-3p in salivary glands, however had no effect for miR-29b-3p and let-7c-3p.

Dexamethasone administration increased miR-16-5p and miR-451a in serum significantly. The change of miRNA in salivary gland by dexamethasone was not correlated to one in serum.

In conclusion, enhancement of sympathetic nerve alone does not change the microRNA pattern in salivary gland. Changes in the expression pattern of miR-21a-5p and miR-141-3p are affected by androgen, whereas let-7c-3p and miR-29b-3p are not. Thus, an increase in the ratio of miR-29b-3p to let-7c-3p is a selective biomarker of stress via the hypothalamus. Furthermore, unlike amylase, it can be distinguished stress exhaustion from sympathetic nerves acceleration.

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## 1P-069

### Neural communication between odontoblasts and pulpal neurons in dentinal pain

Sadao Ohyama[1, 2], Suzuro Hitomi[3], Asuka Higashikawa[1], Wataru Ofusa[1], Haruna Toda[1], Sobhan Ubaidus[1], Maki Kimura[1], Daisuke Nishida[4], Toshihide Mizoguchi[4], Kentaro Ono[3], Yoshiyuki Shibukawa[1] (*[1]Dept Physiol, Tokyo Dent Coll, Japan, [2]Oral surg Tokyo Metro Komagome Hosp, Japan, [3]Dept Physiol, Kyushu Univ Coll, Japan, [4]Oral Health Sci Cent, Tokyo Dent Coll, Japan*)

The teeth are composed of enamel, dentin, cementum and dental pulp. Among dental pain, dentinal pain is caused by the dentinal fluid movement by the various stimuli applied on the dentin surface. Dentinal fluid movement activates mechanosensitive TRP/Piezo channels in odontoblasts, and subsequent intracellular $Ca^{2+}$ signals elicits release of ATP from the pannexin channel. The released ATP activated the $P2X_3$ receptor in pulpal neurons to generate and propagate action potentials to generate dentinal pain (Odontoblast hydrodynamic receptor theory). In the present study, we analyzed rat nociceptive behaviors to examine whether these neural communication between odontoblasts and neurons was necessary to induce dentinal pain or not. We prepared rat model which is enable to induce dentinal pain by dentin exposure on the mandibular incisors (pain group). For the pain group rats, we applied a bonding agent on dentin surface, or administrated antagonists for the piezo channel, $P2X_3$ receptor and pannexin-1 channels. We evaluated nociceptive scores upon cold water stimulation of dentin surface of the incisors. The nociceptive score was significantly increased in the pain group rats, while the scores were decreased in the rat with bonding agent treatment as well as in the rat administrated by the various channel/receptor antagonists. These in Vivo experimental results indicated that neurotransmission between odontoblasts and neurons is necessary for the development of dentinal pain. (COI:No)

## 1P-070

### Behavioral study on nausea induction by serotonin agonists in rats

Jun Sanefuji, Yoshiyuki Hirai, kazunari Hisadome, Mayu Fujita, Shaoyi Su, Nanae Yamazaki, Makoto Funahashi (*Dept. Oral Physiol, Grad Sch Dent, Hokkaido Univ, Japan*)

In previous studies to determine whether nausea is induced by serotonin agonists in rats, conflicting results have been shown (Rudd, 1998; Higgins, 1993; Miller and Nonaka, 1992). To clarify the conflict, we investigated the effect of serotonin agonists as an unconditioned stimulus (US) on the CTA to saccharin. Male Wister rats weighing 200 to 350 g at the start of the study were used. 0.1% saccharin sodium solution was used as conditioned stimulus (CS). 1-phenylbiguanide (1-PBG; 30 mg/kg i.p.), meta-chlorophenylbiguanide (mCPBG; 10 mg/kg i.p.) or 0.3M LiCl (0.1% body weight i.p.) were used as US. The volume of saccharin intake was compared before and after applying the US. CTA experiments were repeated twice in each animal. In the first trial of CTA experiment with 1-PBG or mCPBG, saccharin consumption was slightly decreased but the acquisition of CTA was not statistically significant (n=5). In the animals that once experience LiCl-induced CTA at the first trial, the second trial of 1-PBG injection significantly induced CTA to saccharin (n=5). These results suggest that the serotonergic mechanism for induction of nausea may be boosted by preceding nausea experience. In any case, the nausea-inducing effect of serotonin agonists is not as strong as LiCl, but it was found to be effective enough to induce nausea in rats that once experienced nausea induction. The present study may lead to understanding anticipatory nausea and vomiting during the repetitive chemotherapy. (COI:No)

## 1P-071

### Secretion of amylase and procathepsin B from newly-formed secretory granules in rat parotid acinar cells

Osamu Katsumata-Kato, Megumi Yokoyama, Junko Fujita-Yoshigaki (*Dept Physiol, Nihon Univ Sch Dent at Matsudo, Japan*)

**Purpose:** Secretory granules (SGs) are generated from the Golgi complex. After the generation, newly-formed SGs become a mature SGs by homotypic fusion, acidification, condensation and membrane remodeling. It is generally considered that these process is required to acquire the capacity of stimulus dependent secretion. Here, we examined whether newly-formed SGs have an ability of the secretion.

**Material & Methods:** Isoproterenol (IPR) was injected (5mg/kg) into rat abdominal cavity to deplete pre-existing SGs. Newly-formed SGs were purified by Percoll centrifugation. Acinar cells were dispersed from parotid glands by treatment with collagenase and hyaluronidase. After stimulation with 1 $\mu$M IPR, secretions of amylase and procathepsin B (proCB) into incubation buffer were measured by immunoblot analysis.

**Results & Conclusion:** We observed that newly-formed SGs were generated at 5 hours after injection of IPR. After stimulation with 1 $\mu$M IPR, depletion of newly-formed SGs from parotid gland was observed by electron microscopy. The syntaxin 6 was concentrated in membrane of newly-formed SGs. Because syntaxin 6 is marker of immature SGs, it is supposed the exocytosis of newly-formed SGs. Moreover, secretion of amylase from newly-formed SGs was detected in incubation buffer after stimulation with 1 $\mu$M IPR for 10 min. And proCB was also detected in the buffer. But the matured cathepsin B was not detected because proCB activates after transport to lysosome. Our results suggests that the newly-formed SGs already has an ability of the secretion, and that the aim of membrane remodeling of SGs is the other function except for acquirement of secretion capacity. (COI:No)

## 1P-072

### Evaluation of swallowing function by surface electromyography recorded by handy electromyograph

Taichi Akie[1], Hideaki Seki[2], Hiroko Toshima[3] (*[1]Department of Information and Communication Systems Engineering, Student, Chiba Institute of Technology, [2]Department of Information and Communication Systems Engineering, Chiba Institute of Technology, [3]Department of Food and Nutrition, Toita Wemen's College*)

**Purpose:** Surface electromyography is on of methods for non-invasively evaluating swallowing function. We had already reported swallowing function evaluation method using a 4-channel surface electromyogram. We thought that the electromyograph which we had used was difficult to use for elderly people at home care. Therefore, we have developed a method for recording the surface electromyogram during swallowing using a handy type electromyograph and measuring the swallowing function index automatically.

**Subjects and Method:** Subjects were 12 young healthy male (21.7 ± 0.5 years old). Surface electrode was affixed on the suprahyoid muscle group, a ground electrode was affixed on the seventh cervical spinous process, and a surface electromyogram during swallowing was recorded with Neuropack n1. 3 ml of water, hot water, carbonated water, and jelly-type drink were used as load drink. Using the software developed independently, the amplitude and peak latency of the recorded swallowing surface electromyogram were automatically measured. EMG was recorded 5 times for each beverage, and the average of the 5 measurements was used as a swallowing function index.

**Result:** The average peak latency of all subjects when swallowing cold water, carbonated water, hot water, and jelly was 473.6 ± 164.0, 433.9 ± 193.3, 450.6 ± 208.3, 666.1 ± 194.4 (msec), and latency that induced by jelly-type drink was delayed significantly ($p = 0.016$).

The amplitudes were 31.9 ± 10.9, 31.5 ± 9.1, 34.4 ± 16.0, and 31.2 ± 8.5 ($\mu$V), respectively, and there was no significant difference among the 4 groups.

**Conclusion:** It was found that jelly-type drinks are drinks that require long time for swallowing. We thought this was related to aspiration prevention. Since young people did not find any difference in other drinks, we would like to study the elderly next time. (COI:No)

## 1P-073

### A novel nucleic acid analogue COA-Cl enhances glucose-dependent insulin secretion

Ikuko Tsukamoto[1], Akram Hossain[1], Maki Takata[1], Liu Wenhua[2], Katsuya Hirano[1], Yasuo Kubota[1], Junsuke Igarashi[3], Masaaki Tokuda[4], Ryoji Konishi[1] (*[1]Fac Med, Kagawa Univ. Japan, [2]Chinese Academy of Sciences, [3]Morinomiya Univ. of Med. Sci., [4]International Office, Kagawa Univ.*)

COA-Cl is a synthesized nucleoside analogue with the molecular weight of 284. We previously reported that COA-Cl has angiogenic potency with the promotion of the synthesis and secretion of VEGF. COA-Cl also exhibits neurotrophic/neuroprotective property.
In this study, we explored the effects of COA-Cl on insulin secretion both *in vitro* and *in vivo*. In mouse insulinoma cells (MIN6), COA-Cl enhanced the secretion of insulin only under the high-glucose condition while a typical diabetes therapeutic drug Glibenclamide(SU) enhanced the insulin secretion regardless of glucose concentration. COA-Cl also enhanced the glucose-induced $Ca^{2+}$ influx in MIN6. Further, enhancement of the glucose-dependent insulin secretion was detected with rat pancreatic islet culture.
As for the *in vivo* study, we performed the OGTT (oral glucose tolerance test) using normal rats. Increase in insulin concentration in blood by COA-Cl was observed, however the effects of COA-Cl on blood sugar were unclear. Pharmacodynamics/kinetics and other physiological effects of COA-Cl should be investigated in future. (COI:No)

## 1P-074

### Ni+-sensitive $Ca^{2+}$ channels are involved in the exocytotic secretion from somata/dendrites in vasopressin neurons

Kaori Sato-Numata[1, 2], Tomohiro Numata[1], Yoichi Ueta[3], Yasunobu Okada[4, 5] (*[1]Dept Physiol, Sch Med, Fukuoka Univ, Fukuoka, Japan, [2]Japan Society for the Promotion of Science, Tokyo, Japan, [3]Dept Physiol, Sch Med, Univ Occupat and Environ Health, Kitakyushu, Japan, [4]Dept Physiol, Kyoto Prefect Univ Med, Kyoto, Japan, [5]Natl Inst Physiol Sci, Aichi, Japan*)

Arginine vasopressin (AVP) is an important hormone that maintains plasma osmotic homeostasis. It is synthesized by AVP-producing neurons (AVP neurons) located in the hypothalamus, transported to the axon terminals located in the posterior pituitary gland, and then secreted by exocytosis into the systemic circulation. It has been reported that the $Ca^{2+}$ influx via a voltage-gated $Ca^{2+}$ channel (VGCC) is important for exocytotic secretion from axon terminals. It is known that AVP is secreted not only from the axon terminal but also from the somata/dendrites to the brain stroma. AVP secreted from somata/dendrites was significantly increased by hyperosmotic stimulation. This secretion was almost completely suppressed by an exocytosis inhibitor tetanus toxin. In addition, somato-dendritic AVP secretion was significantly suppressed in the presence of $Ni^{+}$, which is a non-specific $Ca^{2+}$ channel inhibitor. The $Ca^{2+}$ channel currents measured in AVP neurons by the patch-clamp technique were sensitive to $Ni^{+}$. The currents were also suppressed by an N-type VGCC blocker $\omega$-CgTx and a T-type VGCC blocker NNC 55-0396. These results suggest that VGCCs, including T-type and N-type VGCCs, are involved in the mechanism of AVP secretion not only from axon terminals into the systemic circulation but also from somata/dendrites into the brain stroma by hyperosmotic stimulation. (COI:No)

## 1P-075

### Hypothalamic oxytocin is significantly up-regulated after acute osmotic challenge and acute hypovolemia

Hiromichi Ueno[1], Mitsuhiro Yoshimura[1, 3], Kenya Sanada[1, 2], Takashi Maruyama[1], Tetsu Miyamoto[2], Yutaka Otsuji[2], Yoichi Ueta[1] (*[1]Dept physiol, Sangyoika Univ, Fukuoka, Japan, [2]2nd internal medicine, Sangyoika Univ, Fukuoka, Japan, [3]Translational Health Sciences, Bristol Medical School, University of Bristol, UK*)

**Background:** We generated a transgenic rat line that expresses oxytocin (OXT)-monomeric red fluorescent protein 1 (mRFP1) fusion protein to visualize the dynamics of OXT. In this transgenic rat line, hypothalamic OXT can be assessed in diverse physiological and pathophysiological conditions by semi-quantitative fluorometry of mRFP1 fluorescence intensity as a surrogate marker for endogenous OXT. Hypothalamic vasopressin is known to be increased after acute osmotic challenges and acute hypovolemia, whereas the dynamics of OXT after these stimuli are unknown.
**Method:** The OXT-mRFP1 transgenic rat line was used to observe mRFP1 fluorescent intensity in the supraoptic (SON) and paraventricular nuclei (PVN) in perfusion-fixed brains at 0, 3, 6, 12, and 24h after acute osmotic challenge and acute hypovolemia induced by intraperitoneal (i.p.) administration of 3% hypertonic saline (HTN) and polyethylene glycol (PEG), respectively. Rats in the experimental groups, but not the control group, were food and water restricted during the experiment. Fos expression, a marker for neuronal activity, was also assessed by using immunohistochemistry. We also analyzed the gene expression of OXT and mRFP1 in the SON and PVN at 3 and 6h after i.p. administration of HTN and PEG by using in situ hybridization histochemistry.
**Result:** mRFP1 fluorescence intensity in the SON and PVN were significantly increased at 3 and 6h after i.p. administration of HTN and PEG along with robust Fos-like immunoreactivity (co-expression). OXT and mRFP1 gene expression was dramatically increased at 3 and 6h after i.p. administration of HTN and PEG.
**Conclusion:** Acute osmotic challenge and acute hypovolemia induced activation of OXT neurons and increased gene expression of OXT in the SON and PVN. These results suggest that not only endogenous vasopressin, but also endogenous OXT, has one of the key roles for maintaining body fluid homeostasis to cope with hyperosmolality and hypovolemia. (COI:No)

## 1P-076

### Changes in gene expressions of hypothalamic feeding-related neuropeptides in the streptozotocin-induced diabetic rats with variable hyperglycemia and hyperphagia

Satomi Sonoda[1, 2], Kazuhiko Baba[1], Kenya Sanada[1], Kazuaki Nishimura[1], Haruki Nishimura[1], Kentaro Tanaka[1], Mitsuhiro Yoshimura[1, 3], Hiromichi Ueno[1], Takashi Maruyama[1], Yosuke Okada[2], Yoshiya Tanaka[2], Yoichi Ueta[1] (*[1]Dept Physiol, Sch Med, UOEH, Japan, [2]The First Dept of Inter Med, Sch Med, UOEH, Japan, [3]Translational Health Sciences, Sch Med, Univ of Bristol, UK*)

Streptozotocin (STZ), which destroys beta-pancreatic cells, is widely used to develop diabetic animal models. Here, we examined the relationship between expression of the genes encoding hypothalamic feeding-related neuropeptides in the paraventricular nucleus (PVN) and the arcuate nucleus (ARC) and plasma glucose levels in STZ-administered adult male Wistar rats exhibiting variable plasma glucose levels. STZ (80 mg/kg) was administered intraperitoneally (i.p.) in adult male Wistar rats. Rats were divided into 3 groups: PG1 (<300 mg/dl at light period), PG2 (≧300 mg/dl at light period and <200 mg/dl after fasting for dark period) and PG3 (≧300 mg/dl at light period and >200 mg/dl after fasting for dark period). Two weeks after i.p. administration of STZ, they were decapitated after fasting for 12 hours. The gene expressions of proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), neuropeptide Y (NPY), agouti-related protein (AgRP) in the ARC, corticotrophin releasing hormone (CRH), thyrotropin-releasing hormone (TRH) in the PVN were quantified by using in situ hybridization histochemistry. POMC and CART were significantly decreased in PG2 and PG3 compared to PG1. On the other hand, NPY, AgRP and TRH were significantly increased in PG3 but not PG2 compared to PG1. CRH was comparable among all groups. The gene expressions of orexigenic neuropeptides was correlated with the severity of hyperglycemia. In contrast, the gene expressions of anorexigenic neuropeptides such as POMC and CART was significantly decreased, exacerbating hyperglycemia. These results suggest that hyperphagia in STZ-induced diabetic rats may be caused by dynamic regulation of hypothalamic feeding-related neuropeptides associated with plasma glucose levels. (COI:No)

## 1P-077

### Effects of the gene expression of hypothalamic feeding-regulating neuropeptides after being exposed to different gravity in mice via vestibular inputs

Yoichi Ueta[1], Satomi Sonoda[1], Mitsuhiro Yoshimura[1, 2], Kazuhiko Baba[1], Kenya Sanada[1], Kazuaki Nishimura[1], Haruki Nishimura[1], Kentaro Tanaka[1], Hiromichi Ueno[1], Takashi Maruyama[1], Chikara Abe[3], Hironobu Morita[3] (*[1]Dept Physiol, Sch Med, UOEH, Japan, [2]Translational Health Sciences, Sch Med, Univ of Bristol, UK, [3]Dept Physiol, Grad Sch Med, Gifu Univ, Japan*)

The effects of hypergravity on the gene expression of the hypothalamic feeding-related neuropeptides in sham operated and vestibular-lesioned mice were examined by in situ hybridization histochemistry. Vestibular lesion was conducted by a laser. Adult male C57BL/6J mice were received sham operation (Sham) or vestibular lesion (VL) before the experiment. After the recovery, they were divided into 4 groups: Sham-1g, VL-1g, Sham-2g, and VL-2g (n=6 in each) and exposed to 1g or 2g environment with centrifugation of custom-made gondola-type rotating box for 3 days, 2 weeks, and 8 weeks. At the end of the each time point, they were decapitated. The gene expression of the corticotrophin releasing hormone (CRH) in the paraventricular nucleus (PVN), propiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), neuropeptide Y (NPY), agouti-related peptide (AgRP) in the arcuate nucleus (ARC), melanin-concentrating hormone (MCH), and orexin in the lateral hypothalamic area (LHA) were quantified by using in situ hybridization histochemistry. VL did not affect all neuropeptides which we investigated at each time point. CRH in the paraventricular nucleus was increased significantly in Sham but not in VL mice after 3 days of exposure to a 2g environment compared with a 1g environment. Significant decreases in POMC and CART and significant increases in NPY, AgRP and orexin were observed in both Sham and VL mice. After 2 weeks of exposure, CRH and POMC were increased significantly in Sham but not in VL mice. After 8 weeks of exposure, the hypothalamic feeding-related neuropeptides were comparable between Sham and VL mice. These results suggest that the hypothalamic feeding-related neuropeptides may be affected during the exposed duration of hypergravity via vestibular inputs. (COI:No)

## 1P-078

### Size-selective accumulation of MR tracers in the kidney of the mussel *Mytilus galloprovincialis*

Yoshiteru Seo[1], Hidefumi Wakashin[1], Yoshie Imaizumi-Ohashi[1], Mika Yokoi-Hayakawa[1], Eriko Seo[2] (*[1]Dept Regl physiol, Dokkyo Med Univ Scl Med, Tochigi, Japan, [2]Div Marine Life Sci, AORI, Univ Tokyo*)

In order to determine the molecular weight dependency of atrial wall filtration into kidneys of the mussel *Mytilus galloprovincialis*, we applied 5 magnetic resonance (MR) tracers: manganese ion ($Mn^{2+}$; 0.055 kDa), gadolinium ion ($Gd^{3+}$; 0.16 kD), manganese-ethylenediaminetetraacetic acid (MnEDTA; 0.35 kDa), gadolinium-diethylenetriamine pentaacetic acid (GdDTPA; 0.55 kDa), and oligomer-based contrast agent (CH3-DTPA-Gd; 2.1 kDa). After a bolus injection of the MR tracers into the visceral mass, $T_1$-weighted MR imaging ($T_{1w}$-MRI) and the longitudinal relaxation rates ($1/T_1 = R_1$) were measured by 7 T MRI at 20℃. One hr after injection, the MR tracers distributed uniformly in the visceral mass. The increase of $R_1$ of kidney ($R_{1K}$) was detected by group of injection of $Mn^{2+}$ and MnEDTA, and urine concentrations were estimated at 210 and 65 $\mu$M from $R_{1K}$, respectively. No increase of $R_{1K}$ were detected by GdDTPA and CH3-DTPA-Gd injected mussel. When the mussels were additionally incubated in seawater with 10 $\mu$M $MnCl_2$, $R_{1K}$ was increased in the GdDTPA-injected mussel, but not in the $GdCl_3$-injected mussel. Therefore, GdDTPA does not interfere renal accumulation of $Mn^{2+}$, but, $Gd^{3+}$ might have inhibited. Since incubation in seawater with 10 $\mu$M MnEDTA showed no increase in the $R_{1K}$, it is suggested that injected MnEDTA was filtrated as MnEDTA per se, and not likely separated into free $Mn^{2+}$. Thus, we concluded that the molecular weight cut-off (MWCO) of the atrial wall of the *Mytilus galloprovincialis* is around 0.5 kDa, which is almost 1/100 of that for vertebrate animals. In the vertebrate, it is considered that the slit diaphragm formed by nephrin controls the MWCO for filtration by the glomerulus. We are not sure nephrin can cover the wide range of the MWCO. Therefore, it is necessary to investigate the molecular-barrier mechanism in the atrial wall of *Mytilus*. (COI:No)

## 1P-079

### Quantitative analysis of epithelial transport in proximal tubule with mathematical model

Taiki Nishizuka[1], Junichi Taniguchi[2], Akinori Noma[1], Akira Amano[1], Yukiko Himeno[1], Taiki Tahara[1] (*[1]DeptBioinf, Col Life Sci, Ritsumeikan Univ, Shiga, Japan, [2]Mol.Pharmacol. Med. Jichi Med. Univ., Tochigi, Japan*)

Proximal tubule (PT) is known to reabsorb about 2/3 of filtered Na+, K+ and water. This fact raises several questions about the mechanisms underlying their transport across proximal tubular wall. The transcellular reabsorption of Na+ is coupled with partner solutes such as glucose, HCO3-, etc. Total amount of partner solutes in filtrate is at most 40 mM × glomerular filtration rate (GFR), which is not enough to reabsorb the 2/3 of filtered Na+, i.e., 2/3 × 140 mM × GFR. About 2/3 of filtered K+ is also reabsorbed along the PT against the driving force generated by Na+, K+-ATPase in basolateral membrane. These contradictions cannot be explained by assuming only transcellular reabsorption of Na+ and K+. In addition, epithelial reflection coefficients of Na+ and K+ are not very high, though aquaporins, transcellular pathways of water, are highly selective to water. This fact suggested that the water should be reabsorbed via a pathway not very highly selective to water, probably paracellular pathway. In order to reveal the roles and mechanisms of paracellular reabsorption, we built a mathematical PT model that enabled us to analyze the transcellular and paracellular transport separately. Assuming that water passes at tight junction (TJ), our model analysis predicted that high osmolarity in lateral space between neighboring epithelial cells drove much water reabsorption via paracellular TJ water pathway. This water reabsorption was accompanied by K+ solvent drag. In addition to this, the K+ should be electrically reabsorbed at the latter section of PT where luminal potential was positive to interstitium. Paracellular backflow of partner solutes coupled with transcellular Na+ reabsorption accelerated their recycling, which might be important to reabsorb enough amount of Na+. (COI:No)

## 1P-080

### Inhibitory Effect of Acupuncture on Long Latency Reflex in Humans

Akira Nihonmatsu (*Hokkaido College of Oriental Medicine*)

In this study, we examined effects of acupuncture stimulation on short latency reflex (SLR) and long latency reflex (LLR) to determine the site of action of acupuncture stimulation in modulating motor reflexes. Further, we investigated the relationship between changes in LLR and changes in the N20 somatosensory evoked potential (SEP) component induced by acupuncture stimulation and examined the changes in central motor conduction time (CMCT).
Sixteen healthy and right-handed adults (11 males and 5 females; 28.9 ± 6.6 years; upper limb length 54.9 ± 3.2 cm) participated in this study. The experiments were performed under three conditions: (1) control (no acupuncture stimulation), (2) acupuncture stimulation of right-sided Hegu (LI4), and (3) acupuncture stimulation of left-sided LI4. An acupuncture needle (0.18 mm in diameter) was inserted up to a depth of 10 mm at the right- or left-sided LI4. Electrical stimulation was delivered to the median nerve in the right hand joint at a 120% intensity compared with the threshold to produce an M-wave. SLR and LLR were recorded from the opponens pollicis muscle of the right hand. The frequency and amplitude ratio of SLR (latency, approximately 20–30 ms) and LLR (latency, approximately 40–70 ms) were analyzed. We analyzed the mean latency and calculated the CMCT using the mean latencies of LLR, N20, F-wave, and M-wave.The frequency and amplitude ratio of SLR were reduced by the acupuncture stimulation of left- and right-sided LI4, respectively. LLR frequency and amplitude ratio were reduced by acupuncture stimulations on either side. A correlation was observed between changes in the LLR amplitude ratio and changes in the N20 SEP amplitude induced by acupuncture stimulation. No effect of acupuncture stimulation was observed on CMCT. These findings suggest that acupuncture stimulation inhibits motor nerve reflexes via both spinal and supraspinal modulation systems. (COI:No)

## 1P-081

### Characteristics of blood pressure measurement by wrist-cuff automated oscillometric measurement at rest and after exercise in healthy college students

Hiroya Yamazaki, Tatsuya Sato, Nobutoshi Ichise, Yoshinori Terashima, Aoi Kato, Noritsugu Tohse (*Dept Cell Physiol, Sap Med Univ, Japan*)

**Background:** A wrist-cuff automated oscillometric device is portable and useful for self-monitoring blood pressure (BP) home and outdoors when an upper-arm device is not available. However, it remains unclear whether physical activity such as exercise affects the wrist BP measurement or not.
**Methods and Results:** Ninety-seven healthy college students (median age 20 years ranging from 19 to 36 years, 70.1% men) were participated. Rest BP measurement at the sitting position on the wrist measured by wrist-cuff automated oscillometric device (Omron HEM-6183) was compared with BP on upper-arm measured by automated oscillometric device (Omron HEM-7130-HP) and classical auscultatory method. The median BP (IQR) in wrist, upper-arm oscillometric and upper-arm auscultatory method were 109 (100 - 118), 108 (101 - 113) and 109 (104 - 116) mmHg for systolic BP and 66 (61 - 71), 65 (59 - 72) and 68 (64 - 71) mmHg for diastolic BP, respectively. There were no statistical differences among three groups in both systolic BP and diastolic BP. To assess the effect of exercise on wrist BP measurement, BP on the wrist and upper-arm were simultaneously measured with winding oscillometric cuffs on separate arms before and after Master's double two-step exercise test. The ratio of systolic BP just after exercise test to that before exercise on wrist (1.22 ± 0.14) was significantly decreased compared with upper-arm measurement (1.27 ± 0.14), and the difference was significantly correlated with exercise-induced increase in pulse rate (Spearman's rho = 0.23), suggesting the involvement of sympathetic nerve activity.
**Conclusion:** The results suggest that wrist BP measurement at rest by wrist-cuff automated oscillometric device seems generally similar compared with upper-arm BP measurement, while blunted response of exercise-induced BP elevation should be considered in wrist BP measurement. (COI:No)

## 1P-082

### Suppression of the swallowing reflex by stimulation of the pedunculopontine tegmental nucleus

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

It has been reported that the swallowing central pattern generator (CPG) is divided into two parts in the medulla; a dorsal area including the nucleus of the solitary tract and a ventral area corresponding to the reticular formation around the nucleus ambiguus. Morphological study has reported that pedunculopontine tegmental nucleus (PTg) projects to the latter area. It is therefore likely that the PTg is involvement in control of swallowing. This study examines whether the swallowing reflex is modulated by stimulation of the PTg. These experiments were performed on rats anesthetized by urethane (1.3 g/kg, i.p.). Electromyograms were recorded from the mylohyoid muscle to identify swallowing event. The swallowing reflex was evoked by electrical stimulation (0.2 ms duration, 30 Hz) of the superior laryngeal nerve (SLN). Repetitive electrical stimulation applied to the pedunculopontine tegmental nucleus (PTg) (0.2 ms duration, 30 Hz, 150-200 $\mu$A). During recording sessions, the SLN and the PTg were simultaneously stimulated for 10 s. As a control, the SLN was solely stimulated for 10 s twice before and after the simultaneous stimulation. After each experiment, the stimulus sites were checked histologically. The PTg stimulation had suppressive effect on the number of swallowing reflexes. The number of swallows was 8.8 ± 0.9 (mean ± SE) in the pre-control, 4.3 ± 1.0 during Ce stimulation, and 9.6 ± 1.0 in the post-control (n = 6). The onset latency of the first swallow was significantly longer (0.70 ± 0.31 s; mean ± SE, n = 6) than in the pre-control (0.31 ± 0.12 s) or the post-control (0.27 ± 0.18 s). The present study suggests that the RN is involved in the control of swallowing, and that stimulation of the PTg affects the swallowing CPG. (COI:No)

## 1P-083

### Functional recovery by cell grafts of oligodendrocyte progenitor cells to neonatal white matter injury model in rats

Naoki Tajiri[1], Atsunori Hattori[1], Shino Ogawa[1,2], Akimasa Ishida[1], Takeshi Shimizu[1], Hideki Hida[1] (*[1]Dept Neurophysiol & Brain Sci, Grad Sch Med, Nagoya City Univ, Japan, [2]Dept Obstet & Gynecol, Grad Sch Med, Nagoya City Univ, Japan*)

Hypoxia-ischemia (H-I) in preterm infants occasionally results in neonatal white matter injury (NWMI) associated with neurodevelopmental disabilities such as paralysis and cognitive dysfunction. Based on selective vulnerability of late oligodendrocyte progenitor cells (OPCs) to H-I, we made a rat NWMI model that showed hindlimb motor dysfunction without loss of cortical neurons, hypomyelination in the sensorimotor cortex and disturbed cortical motor map in the ipsilateral motor cortex. To find out new effective treatment for NWMI, we are challenging cell therapy to NWMI model using OPCs. In this study, we investigated whether the grafted OPCs can promote motor function in NWMI model. Male rats that received right common carotid artery occlusion followed by 6% hypoxia for 1 hour at P3, were grafted green fluorescent protein (GFP)-positive OPCs ($2.0 \times 10^5$ cells/2 $\mu$l) into the corpus callosum two days later. Three groups were prepared weaning from P25: sham-operated controls, NWMI + non-grafted group, NWMI + OPC-grafted group. As behavioral evaluations, hindlimb retraction, elevated body swing, beam walk ability, rotarod, horizontal ladder tests were performed at 4 and 8 weeks, followed by immunohistochemical investigations. The OPC-grafted group showed better behavioral recovery in hindlimb retraction, elevated body swing, beam walk ability tests compare to the non-grafted NWMI group at both 4 and 8 weeks. The differences in rotarod and horizontal ladder tests were shown at 8 weeks. These data suggest that OPC transplantation during the period of development has a potency to improve deteriorated motor function in the rat NWMI model. Histological evaluations for cell survival and differentiation of grafted OPCs, neurons, astroglial cells, and microglia will be also presented to know the mechanism of OPC effects on developing NWMI model. (COI:No)

## 1P-084

### Influence of lower leg-bath on upright postural sways: comparison of artificial high concentration $CO_2$-water with general tap-water

Noriyuki Yamamoto[1], Tadashi Wada[2], Fumiko Takenoya[3], Masaaki Hashimoto[4] (*[1]Dept Health Sci, Fac Nurs, Jpn Red Cross Hokkaido Coll Nurs, Kitami, Japan, [2]Dept Human Informatics, Fac Sci Engi, Kokushikan Univ, Tokyo, Japan, [3]Dept Exer Physiol, Fac Med, Hoshi Univ, Tokyo, Japan, [4]Lab Physiol, Dept Phys Ther, Fac Med Sci, Teikyo Univ Sci, Tokyo, Japan*)

In this study, the influence of artificial high concentration $CO_2$-water ($CO_2 \geqq 1000$ ppm) lower leg-bath on standing posture sway was investigated. The healthy female college students (n=12) participated in this study. Postural sway was recorded by detecting the body's center of pressure (COP) continuously with a force platform equipped with a data processor. Subjects were requested to stand on the platform with their feet parallel, gazing at a target mark in 2.0 m distance. The body sways of each subject were recorded for 1min, first with the eyes open (EO) and next with eyes closed (EC) conditions. The path length of COP and area of COP trace were registered pre and post lower legs bathing. Each subject immersed both lower legs in tap- and $CO_2$-water (35℃) up to the tibial point for 10 min. The electrocardiogram (ECG) was recorded continuously using a multitelemeter system. Cutaneous blood flow (BF) was measured by laser-Doppler flowmeter in the hand index finger and in the right calf. BF in index finger was not affected whereas BF in calf significantly increased by $CO_2$-water immersion. Heart rate read from ECG did not show any difference between these two water-baths. The path length was significantly decreased by lower legs water immersion in both eye conditions, irrespective of the bath water sort. In the present study, we showed that local water immersion at neutral temperature is able to contribute to a more stable standing posture. Because of ineffective visual conditions, this effect on postural sways might be due to somatosensory modification by the local water-immersion. (COI:No)

## 1P-085

### Characteristics of fine motor performance and factors associated with motor problems in preschoolers with developmental coordination disorders

Misaki Mikami[1,2], Shuhei Koeda[1], Manabu Saito[3], Ayako Osato[3], Takahito Masuda[4], Chihiro Sato[1], Kazuhiko Nakamura[3,5], Junko Yamada[1] (*[1]Grad Sch Health Sci, Hirosaki Univ, Japan, [2]JSPS Research Fellow, [3]Grad Sch Med, Hirosaki Univ, Japan, [4]Fac Edu, Hirosaki Univ, Japan, [5]Research Center for Child Mental Dev, Grad Sch Med, Hirosaki Univ, Japan*)

The main symptom of developmental coordination disorder (DCD) is a motor skill deficit with unknown etiology that significantly interferes with child's activities of everyday living and academic achievements. Poor fine motor skill is one of the most common problems experienced by children with DCD. This study aimed to reveal the characteristics of fine motor performance and the factors associated with fine motor problems in children with DCD. Thirty nine Japanese children aged five years, 18 with DCD and 21 typically developing (TD) were examined. A web camera was positioned above children's hands and recorded during the Movement Assessment Battery for Children 2 (MABC-2) (Age Band 1) posting coins task. Then average speed, acceleration and total trajectory length of children's hand movement were calculated. We also carried out the grip strength test and the finger to nose test. Differences in all scores between the DCD and TD groups were analyzed. In addition, correlation analyses were used between scores on the MABC-2 posting coins task, video tracking data, and other factors. The average speed was significantly slower, and the total trajectory length was longer in the DCD group. They also scored worse on average grip strength and the correct answer rate of the finger to nose test. A significant correlation was found between scores on the MABC-2 posting coins task and average grip strength. These results suggest that children with DCD exhibit slowness and extra movement in fine motor performance, weaker grip strength, and problems in the sense of position and movement. Moreover, overshoot dysmetria appears to point to some degree of dysfunction in the brain. (COI:No)

## 1P-086

### Generation of airway epithelium with CFTR function from iPS cells

Susumu Yoshie[1], Ryosuke Nakamura[2], Daisuke Kobayashi[1], Masao Miyake[1], Koichi Omori[2], Akihiro Hazama[1] (*[1]Dept Cell Integrative Physiol, Grad Sch Med, Fukushima Med Univ, Fukushima, Japan, [2]Dept Otolaryngol, Grad Sch Med, Kyoto Univ, Kyoto, Japan*)

**Background:** Primary airway epithelial cells and experimental animals have been used for cure of airway disease such as cystic fibrosis (CF) caused by mutations of cystic fibrosis transmembrane conductance regulator (CFTR) gene. However, it is not easy to acquire an adequate quantity of cells. Furthermore, CFTR knockout mice cannot mimic human-specific CF phenotype due to species difference. Therefore, airway epithelium from induced pluripotent stem cells (iPS cells) are expected to be a useful cell source for cure of CF disease. The aim of this study is to generate functional airway epithelium with CFTR function.
**Methods:** We generated airway epithelium from iPS cells based on serum-free conditions and air-liquid interface culture. iPS cell-derived airway epithelium was characterized by RT-PCR, immunocytochemistry, HE staining, Scanning Electron Microscope (SEM), ciliary movement, and measurement of CFTR function using yellow fluorescent protein (YFP) molecule sensitive to halide ions.
**Results:** RT-PCR and immunocytochemistry indicated that airway epithelium markers and Cl- channel markers including CFTR were detected in iPS cell-derived airway epithelium. Pseudostratified ciliated columnar epithelium like tracheal epithelium was confirmed by HE staining. Furthermore, SEM analysis indicated that iPS cell-derived airway epithelium had morphologically cilia. Additionally, iPS cell-derived airway epithelium showed the ciliary movement with a ciliary beating frequency of approximately 10 Hz. Finally, the transport function of CFTR was successfully confirmed, judging from the change in fluorescence intensity using YFP molecule.
**Conclusion:** Airway epithelium generated from iPS cells has CFTR function and will be a useful cell source for cure of CF disease, and goblet cell hyperplasia caused by asthma and cigarette smoking. (COI:No)

## 1P-087

### Exercise capacity and intelligence assessed in behavioral tests in adults mice after betamethasone given at 4-day-old infancy

Syunta Maruo, Takayoshi Hosono (*Dept Bio Med Engng, Grad Sch Osaka Electro-Commun Univ*)

**Purpose:** In clinical practice of obstetrics, betamethasone has been administrated to pregnant women with threatened premature delivery to decrease the possibilities of suffering respiratory distress syndrome, intraventricular hemorrhage and periventricular leukomalacia after birth. However long-term side effects of betamethasone on the developmental delay or in intelligence and motor ability has not been clarified. We showed that betamethasone administrated in infancy might deteriorate motor capacity after growth using a small number of rats. In this study we investigated long-term effects of betamethasone in its infancy using mice.
**Method:** We administered betamethasone of 0.5mg/body weight (kg) on 4-day-old mice (group B, n=29) and also established control group (group S, n=27) by giving saline. We performed behavioral tests using 3- to 10-week-old mice in both the groups. We performed suspension test by hooking mice' forelimbs on horizontal bar of 40 cm high and measured suspension time using 3-week-old mice for consecutive five days to test motor ability. We performed step-down type passive avoidance test by measuring staying-time on insulted platform using 5-weeks-old mice after 2-day learning periods to avoid unpleasant electrical shocks. We performed Rotarod test by placing mice on the rotating rod. We removed brains of mice under deep anesthesia, and measured brain sizes.
**Results:** Averaged body weights in B group were significantly smaller in S group. In Rotarod test the mice in B group stayed on the rotating rod significantly shorter than those in S group. There were no significant differences in the results of suspension tests, step-down passive avoidance and the sizes of the brains.
**Conclusion:** Betamethasone administration to mice infants may cause delay in their motor activities after growth. (COI:No)

## 1P-088

### The role of TRPM7 on neuronal cell differentiation using induced pluripotent stem (iPS) cells

Shingo Tsuji, Susumu Yoshie, Akihiro Hazama (*Dept Cell Integrative Physiol, Sch Med, Fukushima Med Univ, Fukushima, Japan*)

**Background:** Transient receptor potential cation channel subfamily M member 7 (TRPM7) is a nonselective cation channel and is expressed in various cells types. TRPM7 has been known to play roles in cell adhesion, directionality, and migration. However, the relationship between differentiation and TRPM7 is not elucidated. In this study, we investigated the role of TRPM7 on neuronal cell differentiation using mouse induced pluripotent stem (iPS) cells and metergoline, which is an activator of TRPM7.
**Methods:** We have generated neuronal cells from iPS cells by stromal cell-derived inducing activity (SDIA) method, with/without metergoline. The differentiated cells from iPS cells were characterized by morphology, Nanog-GFP reporter system, gene expression, and immunoreactivity.
**Results:** While the cells without metergoline showed the typical morphology of neuronal cells, the cells treated with metergoline did not show their morphology and formed the undifferentiated iPS cell colonies. Furthermore, Nanog-GFP reporter system was used to visualize endogenous Nanog (an undifferentiated marker) gene expression. The cells without metergoline did not show the expression of GFP controlled by the Nanog promoter. On the other hand, GFP expression was strongly observed in the cells treated with metergoline. Real-time RT-PCR also showed that the expression levels of undifferentiated markers such as Nanog and Rex1 in the cells treated with metergoline were higher than that of the untreated cells. Additionally, while TuJ1 (a neuronal marker)-positive cells were appeared in the cells without metergoline, those cells were not appeared in the cells treated with metergoline.
**Conclusion:** The activation of TRPM7 is involved in the inhibition of the neural cell differentiation from iPS cells or the maintenance of undifferentiated iPS cells. (COI:No)

## 1P-089

### Cl- channels regulate lipid droplet formation via Rab8a expression during adipocyte differentiation

Kanae Ouchi[1,2], Susumu Yoshie[1], Masao Miyake[1], Akihiro Hazama[1] (*[1]Dept Cellular and Integrative Physiol, Fukushima Med Univ Grad Sch Med, Fukushima, Japan, [2]Dept Judo Therapy, Koriyama Inst Health Sci, Koriyama, Japan*)

Cl- channels have been known to play important roles for the differentiation, cell volume regulation, migration, proliferation, and intracellular acidification. However, whether Cl- channels are involved in adipocyte differentiation from ASCs remains unknown. Thus, we investigated the role of Cl- channels on adipocyte differentiation using adipose tissue-derived stem cells (ASCs) and Cl- channel blocker. We induced rabbit ASCs into adipocytes using Cl- channel blocker. The expression levels of adipocyte markers were no significant difference between the cells treated with a Cl- channel blocker NPPB and untreated cells. However, when the cells were treated with Cl- channel blockers, lipid droplets (LDs) sizes decreased compared with the untreated control. Interestingly, the expression levels of Rab8a, which is known as a regulator of LD fusion, were also decreased in the cells treated with NPPB. Other Cl- channel blockers, DIDS and IAA-94, also inhibited large LDs formation and Rab8a expression.
Additionally, we examined the role of lysosomal acidification on LDs formation, using Bafilomycin. Interestingly, Rab8a expression levels and LDs sizes were significantly decreased in the differentiated cells treated with Bafilomycin. Furthermore, intracellular pH value of the differentiated cells treated with Cl- channel blocker and Bafilomycin were shifted to acidic side. Therefore, it is considered that the lysosomal acidification was also involved in LDs formation. These results demonstrate that Cl- channels do not regulate the adipocyte differentiation, but do regulate the LDs formation via Rab8a expression. (COI:No)

## 1P-090

### New assay system to detect mechanical force in myelinating oligodendrocytes using a tension sensor probe

Takeshi Shimizu[1], Hideji Murakoshi[2,3], Hidetoshi Matsumoto[4], Akimasa Ishida[1], Naoki Tajiri[1], Hideki Hida[1] (*[1]Dept of Neurophysiol and Brain Sci, Grad School of Med Sci, Nagoya City Univ, [2]Supp Cent for Brain Res, Natl Inst for Physiol Sci, [3]Dept of Physiol Sci, Grad Univ for Advanced Studies, [4]Dept of Mater Sci and Engineer, Tokyo Inst of Technol*)

Oligodendrocytes (OLs) form myelin sheath around neuronal axons to express higher brain functions. Although both thick and thin axons exist, the ratio of diameter of axon + myelin to axon diameter (g-ratio) is adjusted to optimum values for each axon, suggesting a factor that controls myelin formation in response to the axon diameter. To clarify the mechanism of the constant g-ratio, we try to investigate mechanical factors depending on its diameter. To visualize the OL generating force during myelination process, a tension sensor based on fluorescence resonance energy transfer (FRET) was used: the efficiency of FRET decreases when the force is applied on it, setting two fluorescent molecules apart from each other.
Artificial polystyrene nanofibers with a diameter similar to the axons are firstly prepared to measure the mechanical force without neuronal activity, extracting a physical factor that is purely dependent on the axon diameter. We found that cultured OL could wrap the myelin sheath around the nanofibers when OLs were cultured on polystyrene nanofibers. The change of FRET intensity in the myelinating OL depending on the nanofiber diameter was also observed in our preliminary experiment. In addition, the focal adhesion morphology could be investigated as the tension sensor is localized at the focal adhesion. Thus, we can examine the correlation among the following four factors using FRET system: axon diameter - OL generating force - focal adhesion morphology - myelin shape, in the same cultured OL cell. (COI:No)

## 1P-091

### A cytosolic N-terminal region of Transmembrane Channel-like protein 1 (TMC1) is cleaved and imported into the nucleus in an overexpression system

Soichiro Yamaguchi[1], Maho Kamino[2], Maho Hamamura[2], Ken-ichi Otsuguro[2]
(*[1]Lab Physiol, Facul Vet Med, Hokkaido Univ, Japan, [2]Lab Pharmaco, Facul Vet Med, Hokkaido Univ, Japan*)

Transmembrane Channel-like protein 1 (TMC1) has been shown to be the pore-forming subunit of Mechano-Electrical Transduction (MET) channel, which transduces mechanical stimuli into electrical signals at the top of stereocilia of hair cells in the inner ear. As an unexpected result, we found that the cytosolic N-terminal regions of mouse TMC1 (mTMC1) which was overexpressed in HEK293 cells were localized in nuclei in a small population of the transfected cells. The accumulation of the N-terminal regions into the nuclei depended on nuclear localization signals within the N-terminal region. Western blot analyses revealed that at least three fragments of the N-terminal region were cleaved and produced from overexpressed mTMC1. Amino acid residues which were required for each cleavage were identified by site-directed mutagenesis approaches. Using a next-generation sequencer, we performed transcriptome analyses of the cells which transiently expressed the most dominant N-terminal fragment from among the three fragments. In comparison with transcriptomes of the cells in which empty vectors were transfected, the overexpression of the N-terminal fragment of mTMC1 slightly altered expression levels of some genes. Although it is still unknown whether these phenomena happen *in vivo*, these results suggest that the cytosolic N-terminal fragment of mTMC1 might work as a transcription factor in some specific conditions. (COI:No)

## 1P-092

### Mitochondrial localization of fluorescent fusion proteins is inhibited by fast folding of fluorescent proteins

Sayaka Kashiwagi, Yoichiro Fujioka, Aya O. Satoh, Aiko Yoshida, Mari Fujioka, Prabha Nepal, Atsushi Tsuzuki, Ozora Aoki, Sarad Paudel, Hitoshi Sasajima, Yusuke Ohba
(*Dept. Cell Physiol., Fac. Med. & Grad. Sch. Med., Hokkaido Univ.*)

Green fluorescent protein (GFP), its variants, and coral fluorescent proteins (FPs) were widely used in the field of cell physiology and cell biology. Once they are fused with organelle targeting and/or retention sequences, the resulting fluorescent fusion proteins (FFPs, or organelle markers) enable us to visualize cellular organelles and their dynamics in living cells. However, such FFPs occasionally display behaviors different from that of the native proteins: i.e. mislocalization due to aggregation, multimerization of FP, or other reasons. Here, we constructed a series of FFPs targeted to different organelles that harbor a variety of FPs with different color spectra and found that FFPs fused with certain types of FPs were localized not only to the mitochondria and endosomes, but also to the cytosol and the nucleus. Such mislocalization was observed for FFPs that included several variants of *Aequorea Victoria* GFP (avGFP) and a monomeric variant of the red FP from *Discosoma* sea anemones (DsRed). Comparison of the amino acid sequences revealed that the FPs in mislocalizing FFPs contain mutations that accelerate protein folding or maturation, indicating that fast folding of FPs might prevent expected localization of FFPs. In fact, reintroduction of amino acid substitutions to make the FP sequences identical to those of wild-type avGFP significantly restored the FFP localization to the expected organelles. In addition, similar amino acid substitutions improved the mitochondrial localization of the pH-sensitive GFP variant pHluorin that was targeted to the mitochondria. This improvement appears to be advantageous for the precise monitoring of mitochondrial pH. Taken together, fast folding of FPs, which is generally thought to be beneficial for fluorescence imaging, might occasionally inhibit the expected localization of FFPs. We therefore propose that the significance of FP selection to maximize FFP function.(COI:No)

## 1P-093

### Research on the role of CFL1-mediated cytoskeleton remodeling in invasion and metastasis of gastric cancer cells

Haibo Wang[1], Tengyang Ni[1], Zewen Chu[1], Masataka Sunagawa[2], Yanqing Liu[1]
(*[1]Medical college, Yangzhou University, Yangzhou, China, [2]Showa University*)

**Objective:** To investigate the role CFL1-mediated cytoskeleton remodeling in invasion and metastasis of gastric cancer cells, and to provide new ideas and experimental basis for research and development of new anti-metastasis therapies and drugs for gastric cancer.
**Method:** The expression of CFL1 in 37 clinical samples was analyzed to find out the difference of CFL1 expression between paracancerous tissue and gastric cancer tissues. Western blot and PCR were used to detect the expression of CFL1 in cells. Cytoskeleton staining combined with immunofluorescence staining was used to analyze the relationship between CFL1 and cytoskeleton activity. TEM and SEM were used to observe the changes of microfilaments on the inner and surface of gastric cancer cells. Lentivirus infection technology is used to overexpress and interfere with the expression of CFL1 in gastric cancer cells. The subcutaneous transplantation model and peritoneal metastasis model of gastric cancer in nude mice were used to detect the invasion and metastasis of gastric cancer cells in vivo. Living imaging system and transwell assay were used to observe the invasion and metastasis of gastric cancer cells in vitro and in vivo.
**Result:** The expression of CFL1 in gastric cancer was significantly higher than that in adjacent tissues. The same results were found in the analysis of gastric mucosal cells and gastric cancer cells; The expression of CFL1 was significantly increased in the cells with active cytoskeleton remodeling; CFL1 plays an important regulatory role in cytoskeleton remodeling. Invasion and metastasis experiments showed that inhibiting CFL1 could significantly inhibit the invasion and metastasis of gastric cancer cells. The same results were obtained by living imaging system experiments of small animals.
**Conclusion:** These findings demonstrate that CFL1 induces invasion and metastasis by promoting cytoskeletal rearrangement. Our results may provide the basis for developing new anticancer drugs to inhibit CFL1. (COI:No)

## 1P-094

### Proliferation and migration of NIH/3T3 are suppressed by cesium

Ziasmin Khatun, Natsumi Nishimura, Daisuke Kobayashi, Akihiro Hazama
(*Dept Cell Integrat Physiol, Grad Sch Med, Fukushima Med Univ, Japan*)

Fibrosis, a common feature of epithelial tissue injury is attributable to the excessive accumulation of extracellular matrix such as collagen, produced by excessive proliferation of fibroblast cells, provoked by injured epithelial cells. Our laboratory previously showed that CsCl inhibited the growth of the human cancer cells by inhibition of the glycolytic pathway. In this study, we investigated the effects of CsCl on fibroblast cell cultures. Mouse fibroblast cells (NIH/3T3) were grown to form a confluent monolayer in 24 well plates, and artificial wounds were scratched, then treated by 1 mM, 3 mM, and 10 mM CsCl. Gap closure was checked before and after treatment using fluorescence microscopy. For cell viability assays, cells were plated at 60 mm dishes at a density of $1 \times 10^5$ cells per dish and cell viability was measured at 0, 24, 48 and 72 h of treatment with 1 mM, 3 mM and 10 mM CsCl using Trypan blue. The treatment of cesium inhibited the migration of fibroblast cells compared with the control and inhibited the migration significantly and a dose-dependent manner. The number of the cells was decreased a dose-dependent manner after treatment, whereas the viability of cells remained almost unchanged. Above these results suggest that CsCl inhibits the migration and proliferation of fibroblast cells. This study provides a possible therapeutic role of CsCl in the treatment of tissue fibrosis. (COI:No)

## 1P-095

### Effects of 405 nm light irradiation by using light emitting diode on HeLa cells

Toshitaka Ikehara[1, 2], Mutsumi Nakahashi[3], Takahiro Emoto[4], Masatake Akutagawa[4], Koichiro Tsuchiya[5], Akira Takahashi[6], Yohsuke Kinouchi[4] (*[1]Dept Phys Therapy, Fac Health Welfare, Tokushima Bunri Univ, [2]Inst Health Sciences, [3]Tokushima Agri Forest Fish Tech Center, [4]Dept Electri Electro Engineer, Grad Sch Sci Tech, Tokushima Univ, [5]Dept Med Pharmacol, Grad Sch Biomed Sci, Tokushima Univ, [6]Dept Med Environ Nutri,Grad Sch Biomed Sci,Tokushima Univ*)

We tested effects of 405 nm wavelength irradiation on cultured HeLa cells. Cells were plated in plastic dish (3cm diameter) and were maintained for 24-48 hours. The cells were irradiated with the light at 146 mW/cm$^2$. Reactive oxygen species (ROS) were monitored by fluorescent probe, dichlorodihydrofluorescein diacetate ($H_2$DCF-DA).We tried to measure the intra- and extracellular ROS accumulation after 385, 395 and 405nm light irradiation for 15 min. The ROS accumulation by these lights irradiation indicated a similar tendency and 405nm light showed relatively high ROS accumulation compared to other 2 lights. Then, I tested further about influences of 405 nm light on the cells. We tried again to measure the intracellular ROS accumulation in the lower glutathione (GSH) cells loaded with 1- Chloro-2, 4 dinitrobenzene (CDNB). GSH and thioredoxin (mentioned below) are known as intracellular physiological scavenger. The ROS accumulation in the low GSH cells obtained by addition of CDNB more rapidly increased compared to normal cells. Moreover, we also irradiated the light to low GSH cells obtained by preincubation with buthionine sulfoximine (BSO) for 24 hr. The light irradiation for more than 1.5 hr significantly induced cell death in the low GSH cells. Then, we tried to measure the intracellular thioredoxin and GSH content. But, GSSG (oxidized GSH) was only slightly increased with the irradiation and thioredoxin content was increased regardless of cell death. These results suggest that the intracellular ROS induced by 405 nm light are containing singlet oxygen ($^1O_2$) and hydroxyradical, and the intracellular glutathione plays an important role of scavenging these ROS. (COI:No)

## 1P-096

### Disrupted feeding rhythms reduced serotonin sensitivity to stress and p-CREB/CREB ratio in hippocampus, leading to time-dependent depressive-like behavior

Atsushi Haraguchi, Miyabi Fukuzawa, Konomi Tamura, Kengo Takahashi, Rina Hirooka, Tomohiro Yamazaki, Shuhei Sato, Shigenobu Shibata (*Lab. of Physiology and Pharmacology, ASE, Waseda Univ, Japan*)

The circadian clock regulates the feeding rhythm, and feeding in turn regulates the phase of peripheral clocks, suggesting a close interaction between the circadian clock and feeding. Moreover, the circadian clock system also affects mood. We have indicated that disrupted feeding rhythm, like night eating syndrome (NES), increased 5-HIAA/5-HT in hippocampus, and then caused depressive-like behavior in the beginning of inactive period. However, we could not clear why disrupted feeding rhythm caused time-dependent depressive-like behavior. Therefore, to understand the mechanism by which disrupted feeding rhythm caused time-dependent depression-like behavior, we treated NES group with 5-HT receptor agonists and antagonists, and examined p-CREB/CREB ratio in hippocampus as marker of depressive-like behavior. We prepared two groups, control and NES groups. A normal diet (ND) with ad lib-feeding was given to mice in the control group, and a high-fat diet for 5-minute during the inactive period under ND ad lib-feeding was given to mice in the NES group. We injected 5-HT receptor agonists or antagonists to mice 30 min before a forced swimming test at ZT 1, after adaptation to each feeding protocol for 4 weeks. As the result, immobility time in the NES group treated with 5-HT1B agonists was significant shorter than those in the NES group injected with saline. Moreover, we found that at p-CREB/CREB ratio in hippocampus of NES group was lower at ZT 1 than that of control group at ZT 1, and similar values in both groups at ZT 13. Currently, we are measuring the active rate of some signal kinase related in 5-HT1B and CREB/BDNF signal. These results indicated that disrupted feeding rhythms reduced serotonin sensitivity and lowered ratio of p-CREB/CREB in hippocampus, leading to time-dependent depressive-like behavior. (COI:No)

## 1P-097

### Artificial organic arsenic compounds promote the expression of Tau protein in Neuro2a cells

Tomoyuki Masuda[1], Junko Itoh[1], Mioko Iseki[1], Yuka Sugawara[1], Yasushi Tomidokoro[1], Naruhiko Sahara[2], Takeshi Ikeuchi[3], Kazuhiro Ishii[1], Akira Tamaoka[1] (*[1]Fac Med, Univ Tsukuba, Japan, [2]Nat Inst Radiol Sci, Japan, [3]Brain Res Inst, Niigata Univ, Japan*)

In 2003, health hazards occurred in Kamisu City, Ibaraki Prefecture by well water contaminated with a high concentration of organic arsenic compound diphenylarsinic acid (DPAA). Arsenic is known as one of the metalloid elements and exists mainly in the form of both organic and inorganic arsenic compounds in nature. However, DPAA detected from well water is an artificial organic arsenic compound that would not exist in nature. Due to the health hazards of environmental arsenics, patients exposed to DPAA mainly exhibited neurological symptoms. When arsenic compounds in the environment enter the body via drinking water, they are known to pass through the blood-brain barrier and reach the brain. This suggests long-term intake of DPAA via well water caused the accumulation of DPAA in the brains of patients, resulting in these health hazards. Tau protein is known as an indicator of neurodegeneration. In the pathological condition, Tau is over-phosphorylated, causing neurodegeneration. In this study, we focused on changes in the amount of total and phosphorylated Tau proteins in cultured neurons after the addition of DPAA, and investigated the possibility that DPAA may be involved in the promotion of neurodegeneration. We used N2a cells (a cell line derived from mouse neuroblastoma) in which human Tau was overexpressed. DPAA was added at the concentrations of 0, 0.1, 1.0, 10, and 50 ($\mu$g/ml), and cultured for 48 hours after addition. Cells and medium were collected after culture, and the amounts of total and phosphorylated Tau proteins were determined by Western blotting. As a result, the both intracellular Tau proteins in the N2a-MAPT cells were significantly increased compared to those in the control. Furthermore, the amount of total extracellular Tau also increased significantly compared to the control. These results suggest that DPAA may be involved in the mechanism of neurodegeneration. (COI:No)

## 1P-098

### One-week-galvanic vestibular stimulation improves arterial pressure control at the onset of postural change

Kunihiko Tanaka[1], Akihiro Sugiura[2] (*[1]Grad Sch Health Med, Gifu Univ Med Sci, Japan, [2]Dept Radiotech, Gifu Univ Med Sci, Japan*)

The vestibular system consists of otolith and semicircular canals. It has been known that the otolith stimulation modifies the sympathetic nerve activity. Previously, we demonstrated that the otolith organs are playing an important role for controlling arterial pressure (AP) at the onset of head-up tilt (HUT). Furthermore, sub-somatosensory galvanic vestibular stimulation (GVS) arguments AP response at the onset of HUT. In the present study, we examined chronic effects of sub-somatosensory GVS for AP control.12 healthy subjects were recruited. AP was measured continuously, and the posture was changed from supine position to 60 degrees of HUT. Center of gravity (CoG) was measured with eye-open and -close. Romberg ratio was analyzed. For 6 subjects, sub-somatosensory GVS of white noise was applied 10 minutes per day for 7 days. The amplitude of GVS was set and monitored at 0.1 mA-lower than somatosensory threshold. At 8th day, AP during postural change and CoG were measured again for the all-12 subjects. The subjects without GVS, both AP change at the onset of HUT and Romberg ratio of total trajectory length in CoG did not change significantly, compared to those of the first day. However, AP at the onset of HUT was significantly higher in the subjects who had GVS, compared to that before GVS. Romberg ratio was significantly smaller than that before GVS. The changes were maintained in 9th and 10th days. Thus, one-week-GVS improves both posture and AP control, and the change was maintained at least for 3 days. (COI:No)

## 1P-099

### Effects of acceleration (+Gz) on blood pressure and biomarker in anesthetized rats

Megumi Tandai-Hiruma[1], Satoshi Maruyama[2], Yasunaga Shiraishi[3], Masanori Fujita[3] (*[1]Dept Physiol, Natl Def Med Coll, Tokorozawa, Japan, [2]Aero Med Lab, JASDF, Tachikawa, Japan, [3]Div Environ Med, Natl Def Med Coll, Tokorozawa, Japan*)

**Introduction:** Acceleration (+Gz) occurs during fighter aircraft maneuvers. It is well known that the +Gz causes loss of consciousness. We previously reported the effect of the +Gz on blood pressure in urethane or pentobarbital anesthetized rats. The purpose of this study is to confirm the effect of the +Gz on blood pressure and to estimate the biomarker of +Gz exposure in rats with the anesthesia recommended in recent years.
**Methods:** We used 12-week-old male Sprague-Dawley rats. Rats were randomly allocated to a +2, +3, +4 and +5 Gz exposure groups. All rats were intraperitoneally anesthetized with a combination of ketamine (75mg/kg) and xylazine (10mg/kg). 30min after the anesthesia, the rat was exposed to the +Gz load for 2 min using the animal centrifuge system (arm radius 1.15m, Tomy Seiko Co. Ltd., and Bio Research Center Co. Ltd.). Arterial pressure (AP) and central venous pressure (CVP) were continuously recorded during +Gz exposure. Blood was sampled before +Gz exposure (20 min after the intraperitoneal injection) and after +Gz exposure (40 min and 70 min after the intraperitoneal injection).
**Results:** In +2 Gz exposure groups, AP rapidly decreased but gradually recovered during the load. In +3 or higher Gz exposure group, AP rapidly decreased but little recovery was seen. AP and CVP were decreased during +Gz exposure and the recovery of AP during +Gz exposure was affected by the strength of the load. The value of blood glucose and lactic acid went up after the +Gz exposure.
**Conclusion:** In our experimental protocol, the blood pressure regulation during +Gz exposure was disturbed depending on the strength of the load. The value of blood glucose and lactic acid were indicated as biomarkers of +Gz exposure. (COI:No)

## 1P-100

### Anorectic action of estrogen replacement is not modified by gonadotropin releasing hormone (GnRH) and gonadotropins in ovariectomized rats

Ayana Izumi, Kurumi Iida, Rie Shiroi, Yume Mori, Keiko Morimoto, Akira Takamata (*Nara women's University, Japan*)

Estrogen replacement in ovariectomized rats reduces food intake specifically during the light phase. It suggests that estrogen exerts an anorectic action by modifying circadian feeding rhythm. Ovariectomy, however, elevates the levels of gonadotropin releasing hormone (GnRH) and gonadotropins, such as luteinizing hormone (LH) and follicle stimulating hormone (FSH) via the negative feedback system of the hypothalamo-pituitary-gonadal axis. To elucidate whether GnRH and/or gonadotropins are involved in the anorectic action of estrogen replacement in ovariectomized rats, we examined the effect of GnRH antagonism on feeding behavior in female rats. Wistar female rats ware divided into ovary-intact (SHAM) groups and ovariectomized (OVX) groups. One week after surgery, rats in the SHAM groups were implanted subcutaneously with an empty silicon capsule, and rats in the OVX groups were implanted a silicon capsule containing cholesterol (Veh) or 17$\beta$ estradiol (E2). And, rats in each group were injected subcutaneously with either 1 mg/kg degarelix, a GnRH antagonist, or the same amount of vehicle (5% mannitol). They were provided free access to water and a standard chow. Both endogenous and exogenous estrogens attenuated daily food intake, especially food intake during the light phase, and body weight gain, regardless of the levels of GnRH and gonadotropins. In contrast, GnRH antagonism with degarelix unaltered food intake and weight gain in both the estrogen replete and depleted conditions. The results indicate that GnRH and/or gonadotropins do not a role in the regulation of food intake, suggest that effect of estrogen replacement on food intake behavior in ovariectomized rats is not modified by the elevated GnRH or gonadotrophins. (COI:No)

## 1P-101

### Mild stress influences estrogen-induced enhancement of sucrose intake in ovariectomized rats

Kurumi Iida, Ayana Izumi, Rie Shiroi, Yume Mori, Keiko Morimoto, Akira Takamata (*Nara Women's Univ, Japan*)

Intake of palatable food or drink is regulated by the hedonic feeding mechanism, and is influenced by many factors, such as physiological and emotional/psychological conditions. We previously found that 17$\beta$-estradiol (E2) replacement in ovariectomized rats enhanced progressive increase in 10% sucrose solution, indicating that E2 increases preference for sweet taste. Furthermore, chronic stress is also known to affect the feeding behavior. In the present study, we hypothesized that the E2-induced enhancement of sucrose intake is influenced by mild stress with isolation rearing. Seven-week-old female Wistar rats were ovariectomized, and were implanted subcutaneously with a silicon capsule containing either E2 (E2 group) or cholesterol (Veh group). After the surgery, rats of each group were reared in a normal condition (control rearing), in which rats were singly housed in a transparent plastic cage with a wire mesh lid, so they can see and hear the neighbor rats, or an isolated condition (isolation rearing), in which rats were singly housed in the same cage, which was separated by the thick walls, ceiling and floor, so they cannot see or hear the neighbor rats. Then, rats were allowed access to 10% sucrose in addition to standard chows and water for 14 days. On the first day, sucrose solution intake was not different among the groups. Sucrose solution intake gradually increased with time in all groups. However, the increase in sucrose solution intake over the 14 days was greater in the E2-control group than the other groups. Therefore, in the last week, sucrose solution intake in the control rearing was greater in the E2 group than the Veh group, whereas the intake in the isolation rearing was not different between the E2 and the Veh groups. These results suggest that isolation stress attenuates the E2-induced enhancement of sucrose intake in rats. (COI:No)

## 1P-102

### Complaxity of cardiorespiratory responses to dexmedetomidine in adult rats

Yoichiro Kitajima, Chikako Saiki, Nana Sato, Ryoji Ide, Yukari Nagakura, Toshio Imai (*Dept physiol, Sch of Life Dent. at Tokyo, The Nippon Dent. Univ, Japan*)

**Introduction:** A sedative drug dexmedetomidine (DEX) is known to have depressive effect on respiration less apparent than that of circulation. In this study, we examined in adult rats the effect of DEX on the cardiorespiratory indices, including the arterial blood gases.
**Materials and methods:** Adult Wistar rats (n = 18) had been received cannulation in the tail artery and vein under isoflurane anesthesia. After recovery, they were divided into three groups and Administered one of the three drugs; vehicle (normal saline, NS group), 5$\mu$g/kg DEX (Low group), and 50$\mu$g/kg DEX (High group). We measured respiratory indices; i.e. respiratory rate ($f_R$), tidal volume ($V_T$), minute ventilation ($V'_E$), mean inspiratory flow ($V_T/T_I$), arterial blood gases ($PaO_2$, $PaCO_2$, and pH), and circulatory indices; i.e. heart rate (HR) and mean blood pressure (MBP).
**Results:** Compared to NS group, High and Low group animals significantly decreased $f_R$ and increased $V_T$, and maintained $V_T/T_I$ with DEX administrations. Mean $f_R$ was lower in High group than that in Low group, and significant decrease in $V'_E$ was observed only in High group. $PaO_2$ decreased in Low and High groups, but increase in $PaCO_2$ and decrease in pH was significant only in High group. Compared to NS group, High and Low group animals decreased HR (-27 and -17 %, respectively) and increased MBP (+52 and +30 %, respectively) immediately after DEX administration. However, the increase in Low group was only temporary and became lower than that in NS group several minute after DEX administration. In contrast, increased MBP in High group was not coming down during the measurement.
**Conclusion:** Our results suggest that DEX overload causes hypoventilation and paradoxical circulatory changes (hypertension and bradycardia), which could be factors to disrupt functional cooperation between respiration and circulation. (COI:No)

## 1P-103

### Contribution of oxytocin to the anti-stress effect of the Kampo medicine Kamikihito -Part II-

Mana Tsukada, Hideshi Ikemoto, Naoki Adachi, Yasunori Takayama, Takayuki Okumo, Wakako Yogi, Mami Kato, Tadashi Hisamitsu, Masataka Sunagawa (*Dept Physiol, Grad Sch Med, Showa Univ, Japan*)

**Purpose:** *Kamikihito* (KKT) is a Kampo medicine (Japanese traditional herbal medicine) that is administered to patients with psychological symptoms, such as anxiety, depression and sleeplessness. Oxytocin, a hypothalamic nonapeptide, is linked to increased levels of social interaction, well-being and anti-stress effects. We have already reported that KKT exerts regulatory effects on stress responses under acute stress conditions. We therefore investigated whether or not oxytocin contributes to the mechanism underlying such an effect.

**Methods and Results:** Male Wistar rats were divided into control, acute stress (Stress), and KKT (300 mg/kg/day po)-treated acute stress (KKT+Stress) groups. Rats in the Stress and KKT+Stress groups were exposed to a 90-min restraint stress procedure involving novel physical stress. During the stress loading, the cerebrospinal fluid (CSF) was collected using microdialysis. Liquid chromatography tandem-mass spectrometry (LC-MS/MS) were used to monitor the oxytocin secretion in the CSF. In the Stress and KKT+Stress groups, the CSF oxytocin levels were increased during stress loading. At 30 and 60 min after stress loading, the levels in the Stress group were decreased; however, those in the KKT+Stress group remained higher than before stress loading. Furthermore, the anxiety-like behavior immediately after the acute stress loading was examined using an open field test. As a result, the total moved distance in the Stress group significantly decreased; however, the decrease was significantly inhibited in the KKT+Stress group. Moreover, the effect of KKT was obstructed by the pre-administration of the oxytocin receptor antagonist (L-368,899 hydrochloride; 10 mg/kg, intraperitoneal).

**Conclusion:** These results suggested that KKT has anti-stress activities and that increased oxytocin secretion may be a mechanism underlying this phenomenon. (COI:No)

## 1P-104

### Chronic diazepam administration impaired hippocampal CA3 LTP and spine morphology in aged mice

Tomonori Furukawa[1], Yoshikazu Nikaido[2], Shuji Shimoyama[1], Yoshiki Ogata[1], Shinya Ueno[1,3] (*[1]Dept Neurophysiol, Grad Sch Med, Hirosaki Univ, Japan, [2]Dept Anesthesiol, Grad Sch Med, Hirosaki Univ, Japan, [3]Research Center for Child Mental Development, Grad Sch Med, Hirosaki Univ, Japan*)

Diazepam (DZP, a benzodiazepine), which bind with high affinity to gamma-aminobutyric acid type A receptors (GABAA-Rs) and potentiate the effects of GABA, is widely prescribed for anxiety, epileptic discharge, insomnia, muscle-relaxing, and anti-convulsants. However, the long-term use of DZP is limited due to adverse effects such as tolerance, dependence, withdrawal effects, and impairments in cognition and learning. Additionally, some clinical reports have shown that chronic DZP treatment increases the risk of dementing disorder in the elderly. Several studies reported that chronic DZP administration could affected neuronal activity of hippocampus, dendritic structure, and cognitive performance. However, effects of chronic DZP administration on aged mice regarding cognitive function is still incompletely understood. In the current study, to investigate the effect of chronic DZP administration on cognitive function, we performed behavioral test, morphological analysis for dendritic spine density, and hippocampal long-term potentiation (LTP) assay in both young (8 weeks-old) and aged (12 months-old) mice. DZP was administered chronically by Alzet mini-osmotic pumps. Morris water maze test was used for spatial learning and memory evaluation. To visualize dendritic spines and analyze spine density, lucifer yellow was injected into hippocampal neurons. The retrieval performance was impaired by chronic DZP administration in aged mice but not in young mice. LTP was attenuated by DZP administration in CA1 of young mice and CA3 of aged mice. The spine density of hippocampal neuron was decreased by chronic DZP administration in CA1 of both young and aged mice and CA3 of aged mice. These results suggested that the effects of chronic DZP were different between young and aged mice. The impairment of retrieval performance induced by chronic DZP administration was likely to be affected by attenuation of LTP and decrease of dendritic spine density in hippocampal neurons. (COI:No)

## 1P-105

### Salicylate-induced changes of the responses to the downward FM sounds in AI and DC field of guinea pigs observed by optical recording

Yutaka Hosokawa [1], Syunji Sugimoto[2] (*[1]Dept Systems Physiol, Grad Sch Med, Okinawa Univ, Japan, [2]Dept of Comp, Sci and Eng, Grad Sch Eng, Toyohashi Univ. of Technology, Toyohashi*)

The influence of salicylate on the responses to the downward FM sounds in the primary auditory cortex (AI) and DC field of the guinea pig were 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 downward FM sounds (the linear sweep: the start and end frequency, 16 and 0.5 kHz in 16-64 ms duration) and tones (0.5-16 kHz, 200 ms duration) at 55-85 dB SPL were recorded from the AI and DC field on both sides before (control) and 0.5-2 hours after the intraperitoneal injection of 300 mg/kg salicylate. When the sound pressure level is high, the active-spots to the downward FM sounds were appeared at the 16kHz-frequency band (FB) in the AI and DC field with and without salicylate injection. On the lower sound pressure, the active-spots were separately appeared in the lower FB of the each field at an hour after salicylate injection. The active-spots positions were separated longer when the sound pressure was lower. These results show that the responses to the high frequency sound were suppressed by the salicylate injection and the active-spots to the downward FM sounds appeared at the lower FB. In other words, these results suggest the threshold increase of the high FB after salicylate injection. Similar results were reported in the rat auditory cortex (Jiang et al., 2017). (COI:No)

## 1P-106

### Participation of GABAergic mechanisms in the reduction of noradrenaline release in the median preoptic nucleus caused by volume expansion in rats

Junichi Tanaka[1,2], Makoto Takahashi [1,2], Akihiko Ushigome[3], Ken Fujisawa[2], Shinichiro Takahashi [1], Yasushi Hayashi [4] (*[1]Dept Disability Sci, Grad Sch Edu, Naruto Univ Edu, Japan, [2]Sci Sch Edu, Joint Grad Sch, Hyogo Univ Teach Educ, Japan, [3]Faculty Human Care, Teikyo Heisei Univ, Japan, [4]Dept Food & Human Nurt, Notre Dame Seishin Univ, Japan*)

In urethane-anesthetized rats, microdialysis was employed to investigate whether $\gamma$-aminobutyric acid (GABA) receptor mechanisms are implicated in the modulation of noradrenaline (NA) release in the median preoptic nucleus (MnPO) in response to changes in extracellular fluid volume. In intact animals, volume expansion (VE) elicited by intravenous infusion of 4% Ficoll (1% body wt, 0.4 ml/min) significantly decreased the release of NA in the MnPO that accompanied an elevation (18 ± 3 mmHg of baseline at 20 min after the start of the Ficoll infusion) in mean arterial pressure (MAP). The VE-induced decrease in the NA release in the MnPO was significantly attenuated by perfusion with either the $GABA_A$ receptor antagonist bicuculline (10 $\mu$M) or the $GABA_B$ receptor antagonist phaclofen (10 $\mu$M) through a microdialysis probe. The amount of the antagonist effects was much greater in the bicuculline-treated group than in the phaclofen-treated group. In bilaterally-vagotomized rats, no significant changes in the NA release were observed. These results demonstrate the contribution of the GABAergic system to the regulation of NA release in the MnPO by neural inputs from the peripheral baroreceptors, and imply that the GABAergic inhibitory action may be mediated through $GABA_A$ receptors rather than $GABA_B$ receptors in the MnPO. (COI:No)

## 1P-107

### Physiological effects in CNS and the autonomic nervous system by listening two kinds of classical music

Mitsuyuki Ichinose, Yutarou Kurisawa (*Dept BiolSci, Sci & Eng, Iwate Univ, Morioka, Japan*)

To investigate physiological effects by listening classical music, especially so-called $\alpha$-music, we measured EEG and the autonomic nervous system by measuring heart rate and heart rate variability. Psychological conditions were monitored by describing two subjective psychological tests, i.e. the multiple mood scale (MMS) and the General Arousal Checklist (GACL). Subjects were young healthy 3 students. The subjects listened the 2 kinds of classic music selected by commercial CD. Both music belongs to relaxation music. One kind is calm and feel comfortable but another kind is exciting music or excitement. By listening the music, amplitude of $\alpha$ and $\delta$ band in frequency analysis (mFFT) of EEG was changed. Heart rate was reduced. The sympathetic nervous activity indicated by LF/HF was reduced. These data indicated that listening the music suppressed the sympathetic nervous activity. The parasympathetic nervous activity indicated by HF was contrarily enhanced. Respiratory interval between inhalation and expiration was reduced during listening the music, the reduction size was depended on kinds of music and the subjects. The data indicate that the autonomic nervous activities in heart activity and respiration was influenced by the kinds of music. MMS in each subject was different by two kinds of music. GACL in each subject was also different by two kinds of music. These data suggested that listening the classic music, especially so-called $\alpha$-music, rested general brain activity recorded by EEG, namely leading to relaxation in the central nervous activity and the autonomic nervous activity, and comfortable feelings in the multiple mood scale and arousal level. However, based on the results of the central and autonomic nervous activities and psychological effects, responses to two kinds of music were different depending on the subjects. (COI:No)

## 1P-108

### The oculomotor foraging task: a novel behavioral paradigm to evaluate working memory capacity and utility

Ryo Sawagashira, Masaki Tanaka (*Dept Physiol, Grad Sch Med, Hokkaido Univ, Japan*)

Working memory deficits are commonly observed in various neurodegenerative disorders, whereas quantitative evaluations of these deficits are usually difficult in clinical cases. Therefore, developing the above assessments is in urgent need. Recent studies in experimental animals use low-dose ketamine (NMDA receptor antagonist) to disrupt working memory partly mimicking pathophysiology of schizophrenia. Here, we developed a novel behavioral paradigm to assess memory capacity and utility, and adapted it to monkeys with and without ketamine administration.

In the oculomotor foraging task, the animals were presented with 15 identical objects (0.6° white squares, > 4° apart each other) on the screen. One of the objects was associated with a liquid reward, and monkeys were trained to search for the target by making sequential saccades for up to 6 seconds. We assume that the rate of revisiting movements to the same objects may depend on the amount of memory capacity and utility. We constructed the "foraging model" incorporating the following three parameters: 1) memory capacity, 2) exploratory rate and 3) memory decay, which could explain several features of behavioral data. When we fit the data obtained from three monkeys with those expected from the model, the goodness-of-fit evaluated by coefficient of determination was always greater than 0.88. The resultant estimates of memory capacity ranged from 7-11 items, exploratory rate 18-28% and the memory decay 2-5 chronologic items in memory storage. Following the systemic administration of ketamine (≤ 1.5 mg/kg), all these parameters dramatically changed. In contrast, systemic administration of medetomidine ($\alpha$2 adrenoceptor agonist, 10 $\mu$g/kg) reduced saccade velocity, but failed to alter the model parameters. Thus, our oculomotor paradigm and the foraging model appear to be useful to quantitatively evaluate working memory capacity and utility, which might be applicable to clinical testing in neurodegenerative disorders in future studies. (COI:No)

## 1P-109

### Neural correlates of task performance in the mouse anterior cingulate cortex

Hiroshi Nishimaru, Riga Sachu, Yusaku Takamura, Jumpei Matsumoto, Taketoshi Ono, Hisao Nishijo (*System. Emotional Sci., Univ. Toyama, Toyama, Japan*)

Deciding when to start and when to stop a certain behavior is crucial for the animal to survive. The medial prefrontal cortex (mPFC) is implied in such decision making and controlling the behavior based on a external and internal cues including reward signals. However, how the mPFC dynamically codes the transition between active and inactive states still remains unclear. Among the mPFC areas, the anterior cingulate cortex (ACC) has been shown to be involved in a wide range of functions including motor control, emotion and motivation.
In this study, we investigated how the information for initiation and termination of the reward-seeking behavior is coded in the ACC by examining the neuronal signals in mice performing a stop-and-go task on a spherical treadmill.
Three male mice were trained to perform a self-paced locomotor task with their head fixed and to run on the spherical treadmill to obtain a reward. In a single trial of this task, the mouse can start running voluntarily but when it stopped, a visual stimulus was presented, and then it had to start running again within 2 seconds to obtain sucrose/water solution as a reward. After 1-2 weeks of training to (90-100 trials per day), the success rate of the task exceeded 60% but the interval between trials varied from several seconds to several minutes. In 423 sorted single units in the ACC recorded by micro-drive tetrode arrary, the majority of them (273 units, 65 %) showed activity related to the task. Among those task-related units, more than half of them (144 units, 53%) increased their firing rate when the mice were running while 24% (66 units) of them were active during the interval. These results indicate that the ACC neurons encode information related to active/inactive states of the animal during goal directed behaviors.
(COI:No)

## 1P-110

### Brain activity measurement during art works viewing using NIRS

Mariko Yamagishi[1,2] ([1]*Grad Sch HHS, Kyoto Univ, Japan,* [2]*Tenri Health Care Univ, Japan*)

In this study, we examined the emotion-induced brain activity when a paired person appreciates art works consisted of various combinations of sounds and lights, using a 2-channel type wearable NIRS (near infrared spectroscopy), HOT-1000 (Hitachi High Technologies Corporation). Compared with PET, fMRI, and other multi channels NIRS using for emotion research, HOT-1000 is a very small, lightweight, and low restraint measuring device that allows natural art appreciation and free action. In this experiment, we created a cylindrical 3D art work with light and sound as motifs, and asked healthy and familiar pair subjects to enter into the work. During the subjects appreciating changes in lights and sounds, we measured and evaluated the brain activity obtained using HOT-1000. We also evaluated effects of oral communication with each other on the appreciative comprehension. As a result, it was suggested that the brain becomes more active by talking with others during appreciation. On the other hand, several problems required to be solved were remained to detect subtle changes in the brain activity induced by changes in lights and sounds. Some of them were caused by the environmental and device-induced anxieties.
(COI:No)

## 1P-111

### The role of dopamine D1 receptor on the whole brain activity and on the motor function

Makoto Osanai[1,2], Hiroki Tanihira[2], Ryo Inagaki[1], Satomi Kikuta[2,3], Toshikuni Sasaoka[4], Atsushi Nambu[5,6] ([1]*Osaka Univ Grad Sch Med, Japan,* [2]*Tohoku Univ Grad Sch Med, Japan,* [3]*Primate Res Inst, Kyoto Univ, Japan,* [4]*Brain Res Inst. Niigata Univ, Japan,* [5]*Div Syst Neurophysiol, Natl Inst Physiol Sci, Japan,* [6]*Dept Physiol Sci, SOKENDAI, Japan*)

Dopamine (DA) is one of the most important neurotransmitters in the central nervous system, especially in the basal ganglia, and is believed to modulate the neuronal activities. In the striatum, direct pathway neurons express dopamine D1 receptors (D1R), whereas indirect pathway neurons express dopamine D2 receptors (D2R). It is thought that this dichotomy contributes to the distinct roles for direct and indirect pathway neurons of the striatum. However, how DA signals modulate whole brain activities and how those affect behavioral properties has not been well explored.
To reveal the role of D1R, we conducted motor performance tests on D1R conditional knock-down (D1R-KD) mice. All behavioral tests indicated that the mice exhibited impaired motor abilities when D1R expression was suppressed. Next, we conducted the whole brain activity mapping in D1R-KD mice by quantitative activation-induced manganese enhanced MRI (qAIM-MRI; Kikuta et al., 2015). qAIM-MRI is based on the use of $Mn^{2+}$, which entered through $Ca^{2+}$ channels in activated neurons, as a marker of neuronal activities. After intraperitoneal injection of $MnCl_2$, whole brain activities were measured by MRI. The results suggested that reduced D1R expression led to increase activity in the nucleus accumbens, hippocampus, and thalamus of D1R knock-down mice compared to normal conditions.
(COI:No)

## 1P-112

### What determines the *set value* (i.e., 37) of our core body temperature?

Yuki Yoshimura[1], Kazuomi Nakamura[2], Tatsuo Watanabe[1] ([1]*Div. Integr Physiol, Sch. Med, Fac. Med, Tottori Univ, Japan,* [2]*Div. Pathol Bioche, Sch. Life Science, Fac. Med, Tottori Univ, Japan*)

**Purpose:** Core body temperature (*Tc*) of homeothermal animals is set at around 37℃ ; however, little is known about what determines the *set value* (i.e., 37) of *Tc*. In this study, we examined the possibility that the *Tc* in pregnant mice establishes the *set value* of the *Tc* in their offspring.
**Method:** To this end, we cultured fertilized mice embryos *in vitro* at 37℃ or 38℃, which is 1℃ warmer than common embryo culture temperature, from pronuclear embryo to blastocyst. Thereafter, we transferred these blastocysts into uteri of pseudo-pregnant mice. In 9-weeks old male offspring, the *Tc* was measured using telemetry system. In addition, hypothalamus of those animals was subjected to RNA-Seq analysis and real-time RT-PCR.
**Results and Discussion:** The *Tc* of mice derived from 38℃ cultured embryos (38℃-group) was significantly lower than that of the control mice (37℃-group). However, there were no significant differences in body weight and physical activity. The RNA-Seq analysis and real-time RT-PCR revealed that expressions of Insulin-like growth factor (Igf-1) and Igf-binding protein 2 (Igfbp2) in the hypothalamus of 38℃-group were significantly higher than those of the control mice. Igf-1 and Igfbp2 were also expressed in the liver; however, there were no significant differences between the two groups. Judging from the previous reports, it seems likely that Igf-1 increases the *Tc* thorough its action in the hypothalamus, and that this effect is being inhibited by Igfbp2. In order to examine whether upregulation of Igfbp2 is responsible for the hypothermia seen in the 38℃-group, we have just started the new study, in which we are planning to analyze the *Tc* of the forebrain-specific Igfbp2 KO mice.
(COI:No)

## 1P-113

### Zymosan-induced fever and brain prostaglandin $E_2$ production in mice are mediated by cyclooxygenase-2 expressed in brain endothelial cells

Hiromu Kitagawa (*Dept Physiol, Grad Sch Eng, Osaka Inst Technol, Japan*)

**Purpose:** Inflammation is associated with fever and hyperalgesia. These reactions involve the centrally produced prostaglandin $E_2$ ($PGE_2$) as a common mediator. In this study we examined the mechanism of fever and central $PGE_2$ production in a mouse model of peripheral inflammation.
**Methods:** Mice were subcutaneously injected with zymosan at the plantar of hind paw. PBS was injected as the control. In some experiments, celecoxib, a cyclooxygenase-2 (COX-2) inhibitor, was administered intraperitonealy just after zymosan injection. Abdominal temperature was recorded under free moving state. A separate group of mice were euthanized 3 h after zymosan injection and blood was removed by cardiac perfusion. Their brains were sampled for $PGE_2$ analysis, immunological detection of COX-2, and ex vivo analysis of $PGE_2$ production.
**Results:** Body temperature was elevated from 3 h to 9 h after zymosan injection. Celecoxib significantly suppressed zymosan-induced rise in body temperature. Zymosan injection significantly elevated $PGE_2$ contents of brain tissue compared to PBS. Zymosan induced COX-2 expression in blood vessels, especially in endothelial cells, rather than brain parenchyma as revealed by immunohistochemistry and western blot. Isolated subarachnoidal blood vessels and brain parenchyma were separately incubated in HEPES-Ringer solution and $PGE_2$ released into the incubation medium was measured. Blood vessels produced more $PGE_2$ than brain parenchyma from both zymosan and PBS injected mice. Furthermore, blood vessels from zymosan injected mice produced more $PGE_2$ than those from PBS-injected mice. These results indicate that fever and central production of $PGE_2$ during zymosan-induced peripheral inflammation are mediated by COX-2 expressed in brain endothelial cells.
(COI:No)

## 1P-114

### The nutrition and food intake of Japanese 5-years autism spectrum disorder children

Shuhei Koeda[1], Kanae Akiyama[1], Manabu Saito[2], Misaki Mikami[2], Tamaki Mikami[2], Chihiro Sato[1], Kazuhiko Nakamura[2], Junko Yamada[1] ([1]*Hirosaki Univ Grad Sch Health Sci, Japan,* [2]*Grad Sch Med, Hirosaki Univ, Japan*)

The children with developmental disorders are at risk of having an unbalanced diet because of sensory problems, such as hyperesthesia. The current study investigated the food and nutrition intake of Japanese autism spectrum disorder (ASD) children.
Subjects were 994 children who underwent the Hirosaki Five-Year-Old Children Developmental Health Check-up. We investigated children's nutrition status using the Brief-type Self-administered Diet History Questionnaire 3 years (BDHQ3y). We divided children into three groups of ASD group, development disorder (DD) group, typically development (TD) group by the each diagnosis. The intergroup comparison of the volume of the food and nutrition at each group was also achieved with the one-way ANOVA (Bonferroni correction was used as post-hoc analysis). Data were analyzed with the SPSS version 26.0. Probability values $p<0.05$ were considered statistically significant.
ANOVA revealed statistically significant difference in the K, Vitamin K, Foric acid, Cryptoxanthin ($F=3.0\text{-}5.4$, $p<0.05$). The result of post-hoc analysis, the ASD group had fewer intakes of vitamin K, natto and many intakes of the vegetable juice than TD group ($p<0.05$).
As a result of investigation, it was thought that the deficiency of vitamin K of ASD children was caused by poor intake of the natto. There are many children who don't like food of a strong smell such as the fermented food in the ASD children. Influence of the hyperesthesia is thought about for this behavior. Additionally, it was known to the ASD children that intestinal bacterial flora had many evil bacteria, and deficiency of vitamin K was thought about in one of the causes. And the excessive intake of the vegetable juice seems to be associated with vegetables phobia. A protector might give it vegetable juice as a substitute of the vegetables phobia of the ASD children.
(COI:No)

## 1P-115

### Evaluation of postprandial sleepiness by event-related potential P300

Shota Satake[1], Hideaki Seki[2], Hiroko Toshima[3] (*[1]Department of Information and Communication Systems Engineering, Student, Chiba Institute of Technology, [2]Department of Information and Communication Systems Engineering, Chiba Institute of Technology, [3]Department of Food and Nutrition, Toita Wemen's College*)

**Purpose:** People may feel strong sleepiness after lunch. We thought that this drowsiness was due to circadian rhythm, but we couldn't deny the influence of the meal content of lunch. We thought that if we were able to reduce afternoon drowsiness, we could improve afternoon work efficiency. We wanted to find an objective and easy-to-measure sleepiness index in order to examine the content of meals to reduce sleepiness after lunch. In this research, we focused on the latency of the event-related potential P300, the response time at the time of P300 recording, and the power in the $\alpha$ band of EEG.
**Subjents & Method:** Subjects were 10 young healthy male(21.5 ± 0.7years old). Electroencephalograms were recorded using Polymate mini (Miyuki Giken). The related electrodes were attached at Fz, Cz, and Pz of the international 10-20 method and the insensitive electrode was attached binaural combined electrode. We have developed our own software for summing and analyzing evoked potential and for frequency analysis of the power of $\alpha$ band of EEG. We used high-carbo diet (carbohydrate 97.8 g) and low-carbo diet (46.8 g) as load diets to induce postprandial sleepiness.
**Results:** The P300 latency, response time, and $\alpha$-band power before loading of the high-carbo group and the low-carbo group were (378.7 ± 53.2msec, 272.5 ± 47msec, 15.2 ± 1.2) and (384.0 ± 81.6msec, 291.6 ± 24.5msec, 16.0 ± 1.4). There was no significant difference between the two groups. After meal-loading thye were (444.7 ± 98.7msec, 289.7 ± 54.9msec, 15.8 ± 2.1), (341.3 ± 44.1msec, 303.8 ± 31.6msec, 16.6 ± 2.5). Latency of P300 was significantly delyed in the high-carbogroup (p= 0.041).
**Conclusion:** We thought that latency of P300 could be applied as a non-invasive objective sleepiness index. (COI:No)

## 1P-116

### Effect of salt intake on gastric rhythm recorded by electrogastrogram

Kazuya Yamamoto[1], Hideaki Seki[2], Hiroko Toshima[3] (*[1]Department of Information and Communication Systems Engineering, Student, Chiba Institute of Technology, [2]Department of Information and Communication Systems Engineering, Chiba Institute of Technology, [3]Department of Food and Nutrition, Toita Wemen's College*)

**Purpose:** It has been pointed out that there are 3cpm and 6cpm automatic movements in electrogastrograms. We have found that the powers of these two types of movement alternate with a regular rhythm. We reported that this rhythmic alternation appears only at night when parasympathetic function is dominant, the middle disappears, and is affected by the contents of dinner. The previous report suggested that gastric rhythm was influenced by the amount of supper salt, so this time we examined the rhythm at night after ingesting two test meals with significantly different salinity.
**Subjects & Method:** The subjects were 5 young healthy men (21.7 ± 0.5 years old). Electrodes (Nihon Kohden, Vitrode V) were affixed at two locations across the epigastric region, the ground electrode was affixed under the left clavicle, and an electrogastrogram was recorded on a portable data recorder (Biolog 2000, S & ME). After starting the recording of electrogastrogram at 19:00, he took a loaded meal, and finished recording at 7:00 the next morning. In a subject, two kinds of load diets, a high salt diet (8.9 g salt) and a low salt diet (5.1 g salt) were loaded on different days. The electrogastrogram was frequency analyzed with an originally developed software.
**Results:** In all subjects, a clear rhythm was observed in the 6 cpm power / 3 cpm curve at night after a low salt diet, with periods of 0.067 cpm and 0.033 cpm. Only a small rhythm of 0.067 cpm was observed at night after a high salt diet, and the 0.033 cpm swell disappeared.
**Discussion**: The disappearance of rhythm appeared after 5 hours after dinner, and it was thought that the rhythm disappeared due to the influence of the supper contents that caused some liquid change. (COI:No)

## 1P-117

### Gene expression of hypothalamic feeding related peptides and neuroendocrine responses in an experimental allergic encephalomyelitis rat

Kentaro Tanaka[1, 2], Mitsuhiro Yoshimura[1, 3], Kazuhiko Baba[1], Kenya Sanada[1], Haruki Nishimura[1], Kazuaki Nishimura[1], Satomi Sonoda[1], Hiromichi Ueno[1], Reiko Saito[2], Takashi Maruyama[1], Tatsushi Onaka[4], Koichi Kusuhara[2], Yoichi Ueta[1] (*[1]Dept Physiol, Sch of Med, UOEH, Kitakyushu, Japan, [2]Dept Pediatr, Sch of Med, UOEH, Kitakyushu, Japan, [3]Dept Translational Health Sciences, Sch of Med, Bristol, UK, [4]Dept Physiol, Sch of Med, Jichi, Shimono, Japan*)

Experimental allergic encephalomyelitis (EAE) is a model of human multiple sclerosis (MS). MS shows various clinical symptoms, including inflammatory anorexia. In the present study, we examined the changes in body weight and food intake, the expression of hypothalamic feeding-related peptides and neuroendocrine responses such as the hypothalamo-neurohypophysial hormones (arginine vasopressin (AVP) and oxytocin) and the hypothalamo-pituitary adrenal (HPA) axis in EAE rats. The weight gain and cumulative food intake in EAE rats were significantly lower than those in controls. Gene expression of the hypothalamic orexigenic peptides (NPY, AgRP) in EAE rats were significantly increased at day 12 and day 18 after their immunization compared with controls. The gene expression of the hypothalamic anorectic peptides (POMC, CART) in EAE rats were significantly decreased at day 12 and day 18 after their immunization compared with controls. The gene expression of oxytocin but not AVP in the supraoptic nucleus and the whole paraventricular nucleus (PVN) of the hypothalamus of EAE rats was significantly increased, along with elevation of plasma oxytocin but not AVP at day 12 and day 18 after their immunization compared with controls. At day 12 after immunization, CRH gene expression was downregulated and AVP gene expression was upregulated in the parvocellular division of the PVN compared with controls. Furthermore, gene expression of POMC in the anterior pituitary was significantly increased, along with elevation of plasma corticosterone at day 6, 12 and 18 after immunization compared with controls. These results suggest that inflammatory anorexia in an EAE model induced by immunization may be associated with dysregulation of hypothalamic feeding-related peptide gene expressions and activation of both oxytocinergic pathways and HPA axis via AVP but not CRH. (COI:No)

## 1P-118

### The spatial and time-dependent changes of various miRNAs expression in the ischemic rat brain

Kana Sugimoto, Chihpin Yang, Yukie Murata, Rina Gono, Yuichiro Hirata, Teiji Harada, Yohei Miyashita, Kazuma Higashisaka, Kazuo Harada, Ryuichi Katada, Hiroshi Matsumoto (*Dept Leg Med, Grad Sch Med, Osaka Univ, Japan*)

Stroke is one of the leading causes of morbidity and disability in the world. However, the therapeutic strategies for the treatment of this disease remain limited. MicroRNAs (miRNAs) are small non-coding RNAs that are the key mediators for post-transcriptional gene silencing, and achieved by binding to the 3'untranslated regions of their target mRNAs. In the brain, miRNAs likely contribute to most, if not all, neuroinflammatory processes, including the generation of reactive oxygen species, apoptosis, loss of blood-brain barrier integrity, leukocyte migration and activation, and edema. However, miRNA-mediated gene regulation in ischemic stroke and neuroinflammation have not been fully explored. In the present study, we evaluated the spatial and time-dependent changes of various miRNAs expression on a transient middle cerebral artery occlusion (tMCAO) model of rats. Rats subjected to tMCAO were deeply anesthetized with carbon dioxide at 1, 3, and 7 days postreperfusion (dpr). Tissues from the cerebral cortex representing the three regions: the ischemic core, the peri-infarct tissue and the contralateral cortex were dissected out, which 750 miRNAs were analyzed using TaqMan Array MicroRNA Cards. The expressions of mmu-miR-449a-5p, -34b-3p, -434-5p, -598-3p, and -126a-3p greatly changed in the peri-infarct tissue at 7 dpr, and mmu-miR-199a-3p, -155-5p, -21a-5p -376b-5p, 206-3p, and -137-3p in the core region at 7 dpr. These changes may be correlated with the appearance of activated astrocytes in the peri-infarct tissue and activated microglia/macrophages in the ischemic core. Thus, we should elucidate the roles of these changed miRNAs in the ischemic stroke, suggesting that it can be explored as a potential therapeutic option. (COI:No)

## 1P-119

### The influence of KATP channel dysfunction on mitochondria

Hiroki Takanari[1], Satoru Hashimoto[2] (*[1]pLED, Tokushima Univ, Japan, [2]RIEM, Nagoya Univ, Japan*)

**Background:** The ATP sensitive potassium channel (KATP channel) exists on mitochondrial inner membrane (MIM) to maintain respiratory function of mitochondria via stabilizing MIM polarity. We explored the influence of gain-of-function mutation of KATP channel on mitochondrial function.
**Methods:** Human fibroblasts (hFBs) from healthy volunteer and the patient with gain-of-function mutation on ABCC9 gene, which encodes regulatory subunit of KATP channel, were subjected to the experiments. The ATP production in each cell was evaluated using IntraCellular ATP assay kit (Toyo-b-net). Mitochondrial respiratory function was analyzed using extracellular flux analyzer, XFe24 (Agilent Technologies), by estimating oxygen consumption rate (OCR). The membrane potential of MIM was evaluated by fluorescent imaging of TMRE.
**Results:** The IntraCellular ATP assay revealed that total ATP production at baseline was not changed by KATP channel dysfunction. There was no significant difference in baseline OCR between wild type and mutated hFBs, either. However, maximum OCR measured in the presence of 2 μM FCCP was significantly decreased in hFBs with mutated KATP channel compared with wild type hFBs, suggesting that the reserved respiratory function of mitochondria was suppressed by KATP dysfunction. Fluorescent microscopy showed that the membrane potential of MIM was lower in hFBs with KATP channel mutation than in wild type hFBs, suggesting that KATP channel dysfunction caused depolarization of MIM.
**Conclusion:** Gain-of-function mutation of KATP channel caused the impairment of reserved respiratory function of mitochondria via depolarization of mitochondrial inner membrane. (COI:No)

## 1P-120

### Visualization of epileptogenic activities in human hippocampal slices ex vivo

Hiroki Kitaura[1], Masafumi Fukuda[2], Yukihiko Fujii[3], Akiyoshi Kakita[1] (*[1]Dept Pathol, Brain Res Inst, Niigata Univ, Japan, [2]Dept Neurosurgery, Nishi-Niigata Chuo Hosp, [3]Dept Neurosurgery, Brain Res Inst, Niigata Univ*)

**Introduction:** Mesial temporal lobe epilepsy (MTLE) is the most frequent focal epileptic syndrome in adults, and the majority of seizures originate primarily from the hippocampus. The resected hippocampal tissue often shows severe neuronal loss as that referred to hippocampal sclerosis (HS). Accordingly, there is a paradox between the clinical and pathological features: why should epilepsy be derived from such degenerated tissue? Here we investigated epileptiform activities ex vivo using living hippocampal tissue taken from patients with MTLE.
**Methods:** We prepared acute brain slices from patients with MTLE within 45 min after resection, and optical imaging or local field potential recordings (LFP) was performed ex vivo. We also used a brain block corresponding to the mirror surface of each slice and performed histopathological examination.
**Results:** We revealed that epileptiform activities developed from the subiculum, regardless of the existence of HS. We found spontaneous rhythmic activities in the subiculum and detected discrete component of high frequency oscillations (HFO), a clinical biomarker of the ECoG suggesting the epileptogenic regions. Immunohistochemistry of the HS tissue revealed loss of inwardly rectifying K+ channel 4.1 (Kir 4.1) in astrocytes in the subiculum, indicating failure of the extracellular K+ buffering and possible association with neuronal hyperexcitability.
**Conclusion:** These results indicate that pathophysiological alterations involving the subiculum could be responsible for epileptogenesis in patients with MTLE. (COI:No)

## 1P-121

### Immunohistochemical analysis of Experimental Autoimmune cardiomyopathy Model (EAM) in Nonhuman Primates

Takaya Yamasaki[1,2], Shunya Nakayama[1,2], Hiroshi Koie[1], Chungyu Pai[1,2], Yasuyo Ito-Fujishiro[1,2], Kiichi Kanayama[1], Yoshiko Munesue[2], Tadashi Sankai[2], Yasuhiro Yasutomi[2], Naohide Ageyama[2] (*[1]Laboratory of Veterinary Physiology, Nihon Univ, Japan, [2]Tsukuba Primate Research Center, NIBIOHN, Ibaraki, Japan*)

Cardiac disease, especially myocarditis and cardiomyopathy are the leading cause of human morbidity and mortality. It has been suggested that a part of dilated cardiomyopathy and myocarditis is associated with autoimmune abnormality. Further, the rodent model of experimental autoimmune myocarditis (EAM) has been reported that the T-cell immune system leading main cause of autoimmune in cardiomyocytes. These from, we have been developing EAM model using cynomolgus monkeys, which are closely related to humans, for model of dilated cardiomyopathy. In this study, we aimed to analyze the immune response in autoimmune myocarditis with immunohistochemical method using EAM model of cynomolgus monkeys. We used 5 healthy monkeys which kept same environment in TPRC. We injected to immunize intradermal on medial femoral with plain myosin, and induced autoimmune myocarditis. We performed cardio-specific examinations including echocardiography, ECG, chest X-ray and blood tests. After several weeks, we conducted immunohistochemical and histopathological examinations. As a result, histopathological analysis revealed interstitial fibrosis, complicated myocardium and infiltrated lymphocytes such as dilated cardiomyopathy. And other medical exam revealed remarkable augment of cardiac hormone, decrease of ejection fraction, abnormal ECG and regurgitation of cardiac valves. Those findings provided the diagnosis of heart failure that mimic the human myocarditis. Immunopathologically, infiltrated lymphocytes in cardiac tissues were mainly CD3 and CD4 positive cells, and some infiltrated cells were stained with CD68. In addition, a part of injured cardiomyocytes expressed IL-1$\beta$. From these, it was suggested that EAM model of cynomolgus monkeys also lead the T-cell immune response. Moreover, these results suggested that the expression of IL-1$\beta$ and CD68+ macrophage were related to mechanism of myocardial injury. In conclusion, EAM models of cynomolgus monkeys could be a mimicked model of human myocarditis and it is ideal models that elucidate the pathophysiological mechanism of myocarditis and cardiomyopathy. (COI:No)

## 1P-122

### Involvement of $A_{2A}$ and $A_{2B}$ adenosine receptors in physiological and pathophysiological functions in pancreas

Mikio Hayashi (*Dept Cell Physiol, Inst Biomed Sci, Kansai Med Univ, Hirakata, Japan*)

**Introduction:** Adenosine is considered to play a role in acini-to-duct signaling in the exocrine pancreas. $A_{2B}$ adenosine receptors is involved in anion secretion in a human pancreatic adenocarcinoma cell line. However, the molecular basis of functional adenosine receptors in the exocrine pancreas remain inconclusive.
**Objectives:** The present study focused on $A_{2A}$ and $A_{2B}$ adenosine receptors involved in physiological and pathophysiological functions in the pancreas.
**Methods:** Immunohistochemical analysis was performed in the rat, mouse, and guinea pig pancreas. The secretory rate and concentration of $HCO_3^-$ in pancreatic juice from the rat pancreas were measured. *In silico* analysis was performed of data from The Cancer Genome Atlas database.
**Results:** The $A_{2A}$ adenosine receptor colocalized with ezrin, an A-kinase anchoring protein, in the luminal membrane of duct cells in the mouse and guinea pig pancreas. The $A_{2A}$ adenosine receptor agonist CGS 21680 stimulated $HCO_3^-$-rich fluid secretion from the rat pancreas. Low mRNA expression of *ADORA2A*, which encodes the $A_{2A}$ adenosine receptor, was associated with poor prognosis of human pancreatic adenocarcinoma patients. In contrast, high expression of *ADORA2B* was associated with poor prognosis.
**Conclusion:** These results indicate that $A_{2A}$ adenosine receptors may be, at least in part, involved in exocrine secretion of pancreatic duct cells via acini-to-duct signaling. The adenosine receptors may be a potential therapeutic target for cancer as well as exocrine dysfunctions of the pancreas. (COI:No)

## 1P-123

### Evaluation of surgical incision-induced tissue swelling and Fos-like immunoreactivity in the spinal cord, hypothalamus, thalamus, and amygdala of Trpv1 knockout and Trpv4 knockout mice

Yasuhito Motojima[1,2], Mitsuhiro Yoshimura[3,4], Yoichi Ueta[3], Akinori Sakai[1] (*[1]Dept Orthopaedic Surgery, Sch Med, UOEH, Japan, [2]Medical Department, Oita HCC, Japan, [3]Dept Physiology, Sch Med, UOEH, Japan, [4]Translational Health Science, Bristol Med Sch, Bristol Univ, UK*)

Pain management is a major concern regarding the treatment of postoperative patients. Transient receptor potential (TRP) channels are considered to be new therapeutic targets for pain control. However, details of pain pathways which are involved in TRP channels remain unclear. We evaluated tissue swelling and the number of Fos-like immunoreactive (Fos-LI) positive cells in the dorsal horn of the spinal cord, paraventricular nucleus of the hypothalamus (PVN), paraventricular nucleus of the thalamus (PVT), and central amygdala (CeA) in wild-type (WT), Trpv1 knockout (Trpv1-/-), and Trpv4 knockout (Trpv4-/-) mice after surgical incision. Mice were divided into four groups: WT control, WT incision, Trpv1-/- incision, and Trpv4-/- incision. Mice were anesthetized, and only those in the incision groups received a surgical incision to their right plantar hind paw. Changes in paw diameter and in the number of Fos-LI positive cells in the dorsal spinal cord, PVN, PVT, and CeA were evaluated 2 hours after the incision. There was no statistically significant difference in the paw diameter among incision groups. Fos-LI positive cells in laminae I-II of the dorsal spinal cord and PVN was significantly increased in all incision groups compared to the WT control group. A significant increase in Fos-LI positive cells was also observed in laminae III-IV of the dorsal spinal cord in Trpv1-/- and Trpv4-/- incision groups compared with the WT incision group. There was no significant difference in Fos-LI positive cells in lamina V of the dorsal spinal cord, PVT, and CeA in Trpv1-/- and Trpv4-/- incision groups compared with the WT incision group. Our results indicate that Trpv1 and Trpv4 might be involved in pain pathways in laminae III-IV of the dorsal spinal cord. (COI:No)

## 1P-124

### Examination of relationship between occlusal contact state and static posture control function: Comparison of trampoline gymnasts and healthy subjects

Mutsumi Takahashi[1], Yogetsu Bando[2,3], Takuya Fukui[4,5], Akiko Maruyama[4,6], Masaaki Sugita[7], Kojun Tsuji[1], Yoshihide Satoh[1] (*[1]Dept Physiol, Nippon Dent Univ, Niigata, Japan, [2]Grad Sch of Health and Sport Sci, Nippon Sport Sci Univ, Tokyo, Japan, [3]BANDO Dental Clinic, Ishikawa, Japan, [4]Facul of Health and Human Sci, Kanazawa Gakuin Univ, Ishikawa, Japan, [5]JGA, Tra, Commit, [6]JGA, Tra, Reinforce, [7]Facul of Sport Sci, Nippon Sport Sci Univ, Tokyo, Japan*)

The aim of this study was to evaluate the relationship between occlusal state and static posture control function in trampoline gymnast. The subjects were 12 male trampoline gymnasts (19.7 ± 1.4 years) and 11 healthy men (20.5 ± 1.1 years). Dental Prescale was used to evaluate the occlusal state, and the right-and-left difference of the occlusal contact area (occlusal stability) was determined. A single-layer mouthguard was fabricated using a 3.0-mm-thick-sheet. The static posture control function was evaluated by a gravity center fluctuation meter, and the area enclosed by the trajectory of gravity center fluctuation (ENV-AREA) as the size of center of gravity sway and locus length per unit area (LNG/E-AREA) as fineness of attitude control were used for analysis. The measurement were performed when wearing and not wearing the mouthguard (wearing-MG/no-MG). The correlations between the occlusal stability and ENV-AREA, or LNG/E-AREA were analyzed. The difference of ENV-AREA or LNG/E-AREA depending on wearing-MG or no-MG was analyzed. In the healthy men, a significant positive correlation was found between the occlusal stability and ENV-AREA, and a negative correlation was found between the occlusal stability and LNG/E-AREA. However, no significant correlation was found in trampoline gymnasts. ENV-AREA was smaller in trampoline gymnasts and healthy men when wearing-MG than when no-MG. LNG/E-AREA was greater in trampoline gymnasts when wearing-MG than when no-MG. There were difference between trampoline gymnasts and healthy men in ENV-AREA and LNG/E-AREA when wearing-MG. The relationship between occlusal state and static posture control differed depending on the target, and it was clarified that the effect of the equalization of occlusal contact by wearing a mouthguard on static posture control function was more prominent in trampoline gymnasts than in healthy men. This work was supported by JSPS KAKENHI Grant Number JP18K09668. (COI:No)

## 1P-125

### Effects of Exercise Load when Walking in Water on Respiratory Muscle Strength in Elderly Men

Yoshihiro Yamashina[1], Kazuyuki Tabira[2], Hiroki Aoyama[1], Hirofumi Hori[1], Emiko Morita[1], Nami Sakagami[1], Tomoko Hirayama[1] (*[1]Dept Physical Therapy, Aino Univ, Osaka, Japan, [2]Dept Physical Therapy, Kio Univ, Osaka, Japan*)

**Purpose:** The purpose of the present study was to investigate the effects of exercise load when walking in water on respiratory muscle fatigue in elderly men.
**Methods:** Eight healthy elderly men (age 67.2 ± 2.6 years)participated in 15-min walking trials in water at 40%, 50%, and 60% of their predicted maximum heart rate. We measured respiratory muscle strength by evaluation of maximum inspiratory and expiratory pressure (PImax, and PEmax, respectively) before and after walking trials. PImax and PEmax were evaluated using a sthenometer attached to the spirometer (AAM337, Minato, Osaka, Japan) and were considered the surrogate indices of inspiratory and expiratory muscle strength, respectively. The percent changes in PImax and PEmax following the walking in water (Δ%PImax, Δ%PEmax) were calculated.
**Results:** After walking at 60% of the predicted maximum heart rate, PEmax decreased significantly than before walking trials ($p<0.05$); however, no changes in PEmax were observed under the other load conditions. The Δ%PEmax after walking at 60% of the predicted maximum heart rate was significantly greater than those in the other load conditions. The PImax did not change under all exercise load conditions.
**Conclusions:** We demonstrated that, in elderly men, a greater expiratory muscle fatigue was induced by walking in water at a 60% load of the predicted maximum heart rate. Our results indicate that walking in water may be an effective and preferable option for strengthening expiratory muscles in the setting of cardiopulmonary training or respiratory rehabilitation. (COI:No)

## 1P-126

### Characteristics of the burst generating networks released by disinhibition in the spinal cord of the neonatal rat

Shu Takahashi, Makito Iizuka, Yoshihiro Mikami, Masahiko Izumizaki (*Dept Physiol, Showa Univ Sch Med, Tokyo, Japan*)

Disinhibition of the spinal cord caused spontaneous seizure-like motor activity. Previous study suggested that the disinhibition could cause burst activities in the phrenic nerve, and this propriospinal network could be harnessed to allow diaphragm function after spinal cord injury. However, the characteristics of this network has not been fully clarified. In the present study, to characterize this network, the isolated spinal cord from neonatal rats at P0-3 was cut into blocks with three spinal segments, i.e., C3-5, T6-8, L2-4 and L6-S2, and motor activities were recorded from C4, T7, L3 and S1 ventral roots (VRs). Under control conditions, there were few spontaneous activities in all four VRs. When 10 μM strychnine, a broad antagonist of glycine and $GABA_A$ receptors, was applied, spontaneous burst activities were observed in all VRs. This result suggests the burst generating network (BGN) exists in each segment. Next, using the whole spinal cord preparations from C3 to S2 segments, effects of local disinhibition in the C3-5 segments on the BGNs in other segments were examined. Under control conditions, spontaneous activities frequently occurred in all VRs, and those amplitudes and durations were fluctuated. When strychnine was locally applied to the C3-5 segments, the large spontaneous burst activities concurrently occurred not only in the C4VR but in all other VRs. The occurrence frequency was much higher than that in the block preparations. The electrical stimulation of the C4 dorsal root evoked reflex response in the C4VR and weak reflex response in other VRs under control conditions. Under the local disinhibition in C3-5 segments, same stimulation caused burst activity in all VRs recorded. These results suggest that the BGNs are strongly connected to each other, and it would be difficult to drive BGN individually. (COI:No)

## 1P-127

### Involvement of the Phox2b-positive neurons located in the dorsal medulla in the sucking rhythm generation

Makito Iizuka[1], Keiko Ikeda[2], Hiroyuki Igarashi[3], Kazuto Kobayashi[4], Hiroshi Onimaru[1], Masahiko Izumizaki[1] (*[1]Dept Physiol, Showa Univ Sch Med, Tokyo, Japan, [2]Dept Physiol, Int Univ Health and Welfare, Chiba, Japan, [3]Dept Physiol Pharmacol, Schulich Sch Med Dent, Robarts Res Inst, Western Univ, Canada, [4]Dept Molecular Genetics, Inst Biomed Sci, Fukushima Med Univ Sch Med, Fukushima, Japan*)

We developed a transgenic (Tg) rat in which Phox2b-positive neurons expressed one of channelrhodopsin variants; ChRFR(C167A), and found that the photo-stimulation of the Phox2b-positive neurons from the dorsal skull causes sucking movement in this Tg neonatal rat at free-moving conscious condition. Furthermore, the local photo-stimulation of the dorsal region of the medulla near the solitary nucleus, where the Phox2b-positive neurons are clustered, caused the sucking motor activity in the phrenic and hypoglossal nerves in the isolated brainstem spinal cord preparations from the Tg neonatal rats. In the present study, to examine the role of the Phox2b-positive neurons in the sucking rhythm generation, the whole cell recordings were obtained from cells in the dorsal medulla, and pattern of activity during the sucking rhythm was examined. The recorded cell was stained with 0.2% Neurobiotin for later visualization. Phox2b immunostaining was also carried out to check whether the recorded cell was Phox2B-positive or -negative. Many of Phox2b-positive neurons were depolarized by photo-stimulation, but did not fire in phase with the sucking rhythm. Many of neurons that showed the sucking rhythmic firing were Phox2b-negative. However, a few neurons that fired synchronous with the sucking motor bursts were Phox2b-positive. It has been shown that the Phox2b-positive neurons are glutamatergic. To examine the involvement of the inhibitory neurons in the sucking rhythm generation, a broad antagonist of the glycine and GABAA receptors, 10 μM strychnine was applied. Under strychnine, photo-stimulation still could evoke the sucking motor bursts without any apparent effects on the rhythm frequency, although the seizure-like activity often obscured the sucking activity. The present results suggest that the sucking generators consist from excitatory interneuronal networks, and the Phox2b-positive neurons firing in phase with the sucking motor bursts could be constituents of the sucking rhythm generator itself.

(COI:No)

## 1P-128

### Application of unsupervised machine learning to analysis of large scale, multi-dimensional neuronal data

Yoshiya Matsuzaka[1], Teruaki Watabe[2], Junko Kawakami[2], Kenji Hoshi[2] (*[1]Div Neurosci, Sch Med, Tohoku Med Pharm Univ, [2]Div Med Pharm Info Sci, Grad Sch Pharm, Tohoku Med Pharm Univ*)

Recent neuroscience studies requires the analysis of increasingly large scale of simultaneously recorded neuronal population and the number of features obtained for individual neurons including morphological, immunohistochemical and functional properties. Yet the conventional analysis protocol depends on criteria subjectively set by human scientists, which leaves open the possibility of overlooking either important features or relation between features. In the present study, we examined the efficacy of unsupervised machine leaning in objectively categorizing neuronal data each of which is characterized by complex features. Two Japanese macaques were trained to perform a two choice arm reaching task in which a color cue instructed the monkeys either to reach to or away from the spatial cue which subsequently appeared either on the left or the right of the working space. The neuronal data were recorded from the posterior medial prefrontal cortex (pmPFC), presupplementary and supplementary motor areas (preSMA and SMA, respectively). Each neuron was characterized by eleven features that were defined by their temporal profile of spiking activity and the areas where they were recorded. The multidimensional data of 492 neurons were categorized by Kohonen's self-organizing map (SOM). The SOM successfully identified several neuronal clusters that were either common across or specific to respective cortical area. Also, it objectively measured and visualized the degree of similarities as well as dissimilarities between the identified neuronal clusters. Finally, we examined how the neuronal clusters were reorganized across different task conditions. Our preliminary results indicated that machine learning is a viable data-mining tool for data that is growing both in the size of neuronal population and complexity in future neuroscience studies.

(COI:No)

## 1P-129

### Assessment of actin cytoskeleton by fluorescent polarization microscopy

Yohei Kanemura[1, 2], Hiroki Takanari[2] (*[1]Mec sci, Fac Sci Tec, Tokushima Univ, Japan, [2]Institute of Post-LED Photonics*)

**Background:** Cytoskeleton including actin plays an important role in maintaining cell morphology and its movement. In the research field of mechanobiology, proper assessment of the cytoskeletal response against the external force is important. Subcellular distribution of actin can be observed by fluorescent microscopy. However, the microstructure or dynamic changes of cytoskeleton due to mechano-stress can hardly be observed with an ordinary optical microscope. Polarization microscopy is a technique that is generally used in physical property analysis. It is also possible to analyze stress applied to a substance by polarization analysis. We propose a new method for measuring the mechano-stress on the cytoskeleton by polarization microscopy. To achieve this issue, we first confirmed that the fluorescence-labeled actin fiber could be observed by polarization microscopy.
**Methods & Results:** A custom-made polarization microscope was established to observe fluorescence polarization. A polarizer was placed immediately after the light source and an analyzer was placed immediately before the detector. Actin cytoskeleton in HeLa cells fixed with 4% paraformaldehyde was labeled with rhodamine by immunostaining method, and placed on the microscopic stage. The changes in the intensity of detection light was acquired while rotating the polarizer, then the fluorescence intensity of rhodamine-labeled actin fiber was periodically changed. The time phase of the detection light change was shifted between the vertical fiber and the horizontal fiber with respect to the microscopic field, suggesting that this was not an artifact, but a change in the detected light accompanying the polarizer rotation. However, the polarization extinction ratio due to polarizer rotation was only 3.5, it was thought that improvements of the polarizer and optical system were still necessary.
**Conclusion:** Fluorescent polarization microscope was established, and polarization dependency of fluorescent labeled actin fiber was confirmed. Fluorescent polarization microscopy could be a powerful tool for analyzing the microstructure of actin cytoskeleton.

(COI:No)

## 1P-130

### Analysis of the function of *bcl-7* in the LIN-28 / let-7 pathway

Luna Izuhara[1], Sawako Yoshina [1], Sayaka Higuchi[2], Yuji Suehiro[1], Shohei Mitani[1, 2] (*[1]Dept Physiol, TWMU, Tokyo, Japan, [2]TIIMS, TWMU, Tokyo, Japan*)

The human BCL7 gene family functions as a tumor suppressor, and is involved in cancer development and progression. However, there are still many unclear points about the mechanisms. We previously reported that the C. elegans *bcl-7* gene, which is the only homolog of the BCL7 gene family, is involved in the regulation of self-renewal ability as stem cells, and acts in the Wnt signaling pathway. However, because several phenotypes of the *bcl-7* mutants are different from those of mutants for Wnt signaling, there is a possibility that pathways other than Wnt also work with BCL-7.
We previously reported that the C. elegans *bcl-7* mutant influences the development of seam cells, which have both self-renewal potential and differentiation capability. So we sought the relationship between *bcl-7* and the LIN-28 / let-7 pathway that regulates the seam cell differentiation.
In this study, using RNA interference (RNAi) experiments, we investigated the relationship between *bcl-7* and the LIN-28 / let-7 pathway. We found that *bcl-7* RNAi alleviated the sterile phenotype of the *lin-28* mutants, while *lin-28* RNAi did not drastically affect that of *bcl-7* mutants. Also, *bcl-7* RNAi alleviated the lethal phenotype of the *let-7* mutants.
Next, we investigated the relationship between *bcl-7* and regulation of *let-7* expression. When the expression level of *bcl-7* decreased, the expression stage of *let-7*, which is a representative factor, became earlier. And this effect is inherited through generations.
We investigated whther the transgenerational inheritance of parental phenotypes are involved in epigenetic modifications. Now, we found that *bcl-7* may regulate histone methylation.
In the future, we will further clarify the relationship between maintaining the undifferentiated state of stem cells and functions of *bcl-7*, *lin-28* and *let-7* genes.

(COI:No)

## 1P-131

### The participation experience to Integrated Laboratory Practice: we have decided the experimental issues by ourselves

Akinaga Kusano[1], Takeshi Sakamoto[10], Miyu Mori[2], Yuki Yoshida[3], Hayata Kimura[4], Shinnosuke Tomita[5], Miho Sakuma[6], Hiromu Ito[3], Risa Tanaka[7], Naoki Hashimoto[8], Michinari Kawahara[2], Noriko Mukai[2], Iori Nishida[9], Mion Horie[7], Shota Sagasaki[4] (*[1]Faculty of medicine, Oita university, Japan, [2]Gifu University Faculty of Medicine, [3]University of Fukui Faculty of Medicine, [4]Chubu University College of Life and Health Sciences, [5]Fujita Health University Faculty of Medicine, [6]Tokyo Women's Medical University Faculty of Medicine, [7]National Defense Medical College, [8]Hyogo College of Medicine, [9]Akita University Faculty of Medicine, [10]Kindai University Faculty of Medicine*)

**Purpose:** Participation to Integrated Laboratory Practice (August, 2019 at Department of Physiology Gifu University).
**Methods & Results:** The participants were from many different Universities with year 1-6, who were divided into 3 groups. After reading a case sheet describing haemorrhagic shock, group discussion was followed. After deciding the experiments design, day 2-4 was for the experiments though it was modified after observing/analyzing the results. Main theme was oxygen and blood pressure (group A), hypertonic solution on shock state (group B), and plasm outflow to the third space during shock (group C). Monitored issues were blood pressure, ECG, electrolytes, hematocrit, et ctr. At day 5, the results was presented. The organizer behaved as a tutor (not giving any answer, but questions), The ethics committee made a round. At final presentation (September), new search results and/or histological analysis were added.
**Conclusion:** We were happy to decide the experimental protocol by ourselves. The group discussion was useful/stimulus for the junior students. We all appreciate this project, laboratory experiments without guidebook.

(COI:No)

## 1P-132

### Concentrations of blood lactic acid and blood glucose levels during incremental and decremental treadmill exercise

Tadashi Saitoh, Kyuichi Niizeki (*Dept Bio-Systems, Grad Sch Sci Eng, Yamagata Univ, Japan*)

The aim of this study was to investigate changes in concentrations of blood lactic acid and blood glucose levels during incremental and decremental exercise. One subject who exercised regularly participated in this study. The protocol of incremental exercise constituted of 5 sets of running between 8 km/h and 16 km/h on a treadmill, with an increase of 2 km/h after each set. The decremental exercise protocol was the reverse of the incremental exercise protocol. There was also a 2 min rest period before and after the exercise test and between the sets. During rest, concentrations of blood lactic acid and blood glucose levels were measured using blood obtained from pricking the subject's fingertip. Heart rate was calculated during the exercise test using electrocardiography. Each exercise test was conducted 7 times in the total study period. The concentrations of blood lactic acid after the subject exercises at speeds over lactic threshold level in incremental exercise were significantly higher than that in decremental one. However, the blood glucose level and heart rate were not significantly different between incremental and decremental exercises. These results suggest that the effect of a warm-up exercise is greatly reflected in the concentration of blood lactic acid.

(COI:No)

# Poster Presentations

## Day 2

（March 18, 14:20～15:20）

| | |
|---|---|
| **2P-001～2P-018** | Ion Channel・Receptor（2） |
| **2P-019～2P-033** | Heart・Circulation（2） |
| **2P-034～2P-051** | Neuron・Synapse（2） |
| **2P-052～2P-064** | Sensory Function（2） |
| **2P-065～2P-074** | Behavior Science・Biorhythm（2） |
| **2P-075～2P-078** | Neurochemistry（2） |
| **2P-079～2P-084** | Autonomic Nervous（2） |
| **2P-085～2P-092** | Muscle Physiology（2） |
| **2P-093～2P-098** | Oral Physiology（2） |
| **2P-099～2P-104** | Endocrinology（2） |
| **2P-105～2P-107** | Kidney・Urination（2） |
| **2P-108～2P-114** | Motor Function（2） |
| **2P-115～2P-119** | Reproduction |
| **2P-120～2P-123** | Development・Growth・Aging（2） |
| **2P-124～2P-127** | Digestion・Absorption |
| **2P-128～2P-139** | Cell Physiology・Molecular Physiology（2） |
| **2P-140～2P-145** | Environmental Physiology（2） |
| **2P-146～2P-148** | Drug Actions（2） |
| **2P-149～2P-158** | CNS Function（2） |
| **2P-159～2P-167** | Nutrition・Metabolism・Thermoregulation（2） |
| **2P-168～2P-175** | Pathophysiology（2） |
| **2P-176～2P-178** | Physical Fitness・Sports Medicine（2） |
| **2P-179～2P-181** | Blood |
| **2P-182～2P-184** | Respiration（2） |
| **2P-185～2P-187** | Study Methodology（2） |
| **2P-188～2P-190** | Others（2） |

## 2P-001

### Expression of Mechanosensitive Ion Channel in Osteoblast

Sayoko Nagai[1,2], Haruna Toda[1], Sadao Ooyama[1], Wataru Oofusa[1], Asuka Higashikawa[1], Maki Kimura[1], Yoshiyuki Shibukawa[1], Akira Katakura[2] (*[1]Dept Physiol, Tokyo Dent Coll, Japan, [2]Oral Pathobiological Sci Surg, Tokyo Dent Coll, Japan*)

**Introduction:** Mechanical stress is one of the important regulatory factors to regulate bone homeostasis. Although it has been reported that application of stress stimulation to osteoblasts elicits an increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$), their detailed mechanism of the mechanosensitive processes remains unclear. We thus investigated the biophysical and pharmacological properties of direct mechanical stimulation-induced $[Ca^{2+}]_i$ response in osteoblasts.
**Method:** Mouse osteoblast-like cells, MC3T3-E1, were cultured for 12 to 24 h in 5% $CO_2$ at 37℃, and loaded with $Ca^{2+}$ fluorescent indicator fura-2/AM for 1 h. The standard extracellular solution was Krebs solution, and we measured $[Ca^{2+}]_i$ responses during plasma membrane stretch with hypotonic extracellular solution or direct mechanical stimulation with a glass micropipette, we utilized several mechanosensitive ion channel blockers, $Gd^{3+}$, GsMTx4, RN1734, HC030031, and clemizole. To activate Piezo1 channel, we applied Yoda1 to the osteoblasts.
**Result:** An application of hypotonic Krebs solution and Yoda1 solution increased $[Ca^{2+}]_i$ in the osteoblasts. When direct mechanical stimulation was applied, $[Ca^{2+}]_i$ was increased and not showed significant desensitizing effects. Extracellular $Gd^{3+}$, GsMTx4 and RN1734 reversibly inhibited mechanical stimulation-induced $[Ca^{2+}]_i$ increases. When the concentration of $Gd^{3+}$ or GsMTx4 were changed, changing in the increases of $[Ca^{2+}]_i$ was observed in their dose-dependent manner.
**Discussion:** $Gd^{3+}$, GsMTx4 and RN1734 are inhibitors of mechanosensitive ion channels, and significantly suppressed $[Ca^{2+}]_i$ increases induced by direct mechanical stimulation to the osteoblasts. Mechanosensitive ion channels might be involved in the mechanosensitive processes of osteoblasts.
Yoda1 is an activator of Piezo1 channel while GsMTx4 is an antagonist for it. Piezo1 channel thus might be the one of the functional mechanosensitive ion channel in osteoblast. (COI:No)

## 2P-002

### Basal ciliary beating enhanced by the temperature-dependent $Ca^{2+}$ entry in airway ciliary cells of Hochu-ekki-to (TJ-41) treated mice

Riko Ikeda (*Dept Mol Physiol, Col Pharm Sci, Ritsumeikan Univ, Japan*)

The effects of Hochu-ekki-to (TJ-41, Bu Zhang Yi Qi Tang), a Chinese traditional medicine, on airway ciliary beatings were examined. The ciliary beat frequency (CBF) and ciliary bend angle (CBA) were measured in airway ciliary cells isolated from lungs using a video microscopy equipped with a high-speed camera. In the test mice, TJ-41-containing water was administered for 4-6 weeks (1.8g/kg/day), while in the control mice, only water was administered. The basal CBF and CBA of the TJ-41 treated mice were enhanced compared with the control mice. In the control mice, the basal CBF was maintained by both cAMP and $Ca^{2+}$ signals whereas, in TJ-41 treated mice, the basal CBF was enhanced and maintained by $Ca^{2+}$ signal, not cAMP signal. An $[Ca^{2+}]_i$ increased by TJ-41 treatment inhibited a cAMP accumulation by activating PDE1A in airway ciliary cells. The concentration response studies of acetylcholine and ionomycin exhibited that TJ-41 treatment shifted the CBF concentration-response curves to lower concentrations. TJ-41 treatment enhanced CBF, CBD and $[Ca^{2+}]_i$ stimulated by temperature. TRPV4 is expressed in airway ciliary cells judging from RT-PCR. We are now on studying the possibility that in airway ciliary cells, TJ-41 treatment stimulates TRPV4 expression and activity, leading to enhancement of CBF, CBD and $[Ca^{2+}]_i$ under the basal condition. (COI:No)

## 2P-003

### The analysis of novel ATP release channel in the mitochondria

Toshiyuki Ishii, Takumi Akagi, Makoto Kaneda (*Dept. Physiol., Nippon Med. Sch., Tokyo, Japan*)

Adenosine triphosphate (ATP) plays important roles for P2X receptors-mediated signal transmission in sensory system, especially taste and pain sensation. In retina, we have previously identified the localization of seven subtypes of P2X receptors, and shown that these P2X receptors modulate visual information. However, the origin of ATP as a neurotransmitter in the retina has not been well elucidated. Recently, ATP release through calcium homeostasis modulator (CALHM) channels has been reported in taste buds. We have examined the expression level of mRNA of CALHM1, CALHM2, and CALHM3, and found that signals of CALHM2 were dominantly expressed in the retina. Therefore, in the present study, we investigated whether the CALHM2 channel-mediated ATP release can occur in the retina by morphological and physiological methods. The immunoreactivity of CALHM2 was detected in the outer plexiform layer and the inner plexiform layer, the synaptic layers of the retina. In the outer plexiform layer, strong immunoreactivity was detected at the terminals of photoreceptors. At the terminals of photoreceptors, the immunoreactivity for CALHM2 was co-localized with the immunoreactivity for COXIV, a marker of mitochondria. The immunoreactivity of CALHM2 was detected in mitochondria of the rod spherules and the cone pedicles at the ultrastructural level. In HEK293T cells, the immunoreactivity for CALHM2 was also co-localized with the immunoreactivity for COXIV. An overexpression of CALHM2 in HEK293T cells increased the ATP concentration of the extracellular solution. On the other hand, knocking down of CALHM2 in HEK293T cells reduced the ATP concentration of the extracellular solution. These results support the hypothesis that CALHM2 contributes ATP release from mitochondria, and the ATP release via CALHM2 might be involved in the modulation of visual information. (COI:No)

## 2P-004

### "Knock-off" and "lock-in" of the polyamine block by K+ ions determine the position along the voltage axis of the current-voltage relationship of the Kir2.1 inward rectifier

Keiko Yanagi-Ishihara (*Dept Physiol, Kurume Univ Sch Med, Kurume, Japan*)

Kir2.1 conductance declines steeply with membrane depolarization (i.e., inward rectification) due to voltage-dependent pore blockades by intracellular cations, chiefly spermine (polyamine). It has been suggested that multiple $K^+$ binding sites exist along the long pore of Kir2.1, and that the negatively charged residues located in the central cavity and in the cytoplasmic pore contribute to the polyamine binding. Importantly, the voltage dependence of the block causing the inward rectification shifts along the voltage axis with the change in $E_K$ when extracellular $K^+$ concentration ($[K^+]_{out}$) is altered, as if the block is coupled to the $K^+$ flow through the channel. However, it has been reported that the voltage-dependence does not shift when intracellular $K^+$ concentration ($[K^+]_{in}$) is changed. Here, we examined the effects of intracellular $K^+$ and $Na^+$ on the voltage dependence of the spermine block to explore the mechanism of the $E_K$ dependence of the inward rectification. When the effects of $[K^+]_{in}$ on the spermine block were examined by replacing $K^+$ with $Na^+$, the effects of the pore block by intracellular $Na^+$ overlapped with the effects of $[K^+]_{in}$ on the spermine block. When $[K^+]_{in}$ was altered without any cation substitution, the "genuine" voltage dependence of the spermine block evaluated by its unblocking rate shifted along the voltage axis by the amount of the change in $E_K$. When $[K^+]_{in}$ was altered by replacing $K^+$ with $Na^+$, the shift became smaller than the change in $E_K$ as $[K^+]_{in}$ was lowered and $[Na^+]_{in}$ was increased. From these results, we propose that the polyamine at the blocking site is "knocked-off" by $K^+$ ion(s) coming from the extracellular solution (Hille & Schwarz, 1978), and is "locked-in" by $K^+$ ion(s) entering the pore from the intracellular solution and preventing the bound polyamine from dissociating. (COI:No)

## 2P-005

### ANO1 inhibition by the extracts from *Glechoma hederacea*

Yasunori Takayama, Mami Kato, Mana Tsukada, Naoki Adachi, Hideshi Ikemoto, Masataka Sunagawa (*Dept Physiol, Showa Univ Sch Med, Japan*)

Anoctamin 1 (ANO1, also called TMEM16A) is a calcium-activated chloride channel expressed in primary sensory neurons of both trigeminal and dorsal root ganglions. Increases in the intracellular free calcium activates ANO1 and the generated chloride efflux evokes a neuronal excitation in primary sensory neurons. ANO1 activity enhances the physiological functions of calcium-permeable channels (ex. TRP channel) and G protein-coupled receptors, which are involved in acute and inflammatory pain sensations. Therefore, ANO1 inhibition would be an intriguing way to reduce pain in some clinical situations. It has been recently reported that ANO1 is inhibited by many plant-derived natural compounds, including tannic acid, polyphenols and menthol. Especially, the menthol, a major component in mint (*Menthae herba*), has a stronger inhibitory effect on ANO1 current compared with other natural compounds. Here, we focused on menthol-containing plant species, and found that the extracts from Glechoma hederacea herb (*Glechomae herba*) also inhibited ANO1 current induced by intracellular free calcium. In preparation of the extracts, we grinded the dried *Glechomae herba* and boiled it at 60 ℃ for 1 hr. After centrifugation and filtration of supernatant, we made the stock powder by evaporation and freeze dehydration. Finally, we applied the diluted solution to HEK293T cells expressing mouse ANO1 in whole-cell patch-clamp recording. The ANO1 currents were inhibited by the *Glechomae herba*-contained solution. This fact indicates a possibility that *Glechomae herba* reduces pain sensation. Currently, this plant is not socially used well in worldwide. However, our study suggests that *Glechomae herba* could be a natural medicine to reduce pain, for instance oral pain sensation. (COI:No)

## 2P-006

### Effects of kampo medicine containing ginger on TRPV1-ANO1 interaction

Mami Kato, Yasunori Takayama, Mana Tsukada, Masataka Sunagawa (*Dept Physiol, Sch Med, Showa Univ, Japan*)

Daikenchuto (TJ-100) is a kampo medicine, one of the Japanese traditional herbal medicines, composed of the extracts from ginger (*Zingiber officinale*), ginseng (*Panax ginseng*), Japanese zanthoxylum peel (*Zanthoxylum piperitum*) and maltose. The component percentages are 50, 30 and 20 %, respectively. TJ-100 is widely used in clinical situations to improve the intestinal motility and blood flow. It has been reported that TJ-100 inhibits a postoperative adhesion formation and paralytic ileus. TJ-100 contains some pungent components including hydroxy-α-sanshool and 6-shogaol, which are agonists of a capsaicin receptor, transient receptor potential vanilloid 1 (TRPV1) channel. Activation of TRPV1 induces the releases of calcitonin gene related peptide (CGRP) and substance P. These neuropeptides enhance vasodilation followed by the increase in intestinal blood flow. In this sturdy, we focused on anoctamin 1 (ANO1), a calcium-activated chlorine channel. Previous report suggests that ANO1 promotes neural excitation through the interaction with TRPV1. However, TJ-100 effect on ANO1 is unknown. Here, we show that TJ-100 (100 μg/mL) inhibits ANO1 current induced by 100 nM intracellular free calcium. We investigated that in HEK293T cells expressing mouse TRPV1 (mTRPV1) and mouse ANO1 (mANO1), or cells expressing either mTRPV1 or mANO1. Interestingly, TJ-100 induced the large currents dependent on TRPV1-ANO1 interaction, although TJ-100 activated mTRPV1 and inhibited mANO1. These results indicate that TRPV1-ANO1 interaction evokes the nervous excitation in the oral administration of TJ-100. This could be a novel molecular mechanism to explain the pharmacological effects of TJ-100 in enteric canal. (COI:No)

## 2P-007

### Phospholipid transport mechanism of transmembrane protein 16F

Takahiro Shimizu, Kanon Shirai, Syota Nabeshima, Takuto Fujii, Hideki Sakai (*Dept Pharm Physiol, Faculty Pharm Sci, Univ Toyama, Japan*)

Transmembrane protein 16 (TMEM16) family is composed of ten isoforms. Among them, TMEM16A and 16B have been demonstrated to function as $Ca^{2+}$-activated $Cl^-$ channels. On the other hand, TMEM16C, D, F, G, and J have been reported to show $Ca^{2+}$-activated phospholipid scramblase activities. Especially, TMEM16F has a pivotal role in phosphatidylserine exposure during blood coagulant. Its mutation causes Scott syndrome showing a blood clotting disorder. We found previously that human TMEM16F exhibits not only phospholipid scramblase activities but also $Ca^{2+}$-activated $Cl^-$ channel activities with low $Ca^{2+}$ sensitivity. In the present study, we therefore investigated how these functions are correlated in HEK293T cells in which human TMEM16F is exogenously expressed. In flow cytometry using annexin V-phycoerythrin (PE), $Cl^-$ channel blockers, which inhibited TMEM16F-dependent $Cl^-$ currents, significantly decreased $Ca^{2+}$-triggered phospholipid scramblase activities. The $Cl^-$ currents measured by whole-cell patch-clamp recordings were closely correlated with the scramblase activities in some TMEM16F mutants. In tail current analysis, the mutants having larger scramblase activities exhibited slower tail currents, suggesting that increased gating frequency of TMEM16F channels enhanced their phospholipid transports. Phospholipid scramblase activities were temperature-dependent, and they were decreased at low temperature. These results suggest that structural changes during TMEM16F channel gating are essential for the scramblase activities. (COI:No)

## 2P-008

### Implications of the mechanosensitivity of TRPC6 channel in bone marrow stromal cell cycle progression

Jun Ichikawa, Ryuji Inoue (*Dept Physiol, Fukuoka Univ Sch Med, Japan*)

Several lines of evidence suggest that bone marrow stromal cells (BMSCs) are mechanosensitive. However, how this property operates in physiological settings and what molecular mechanism is involved therein remain entirely unclear. We therefore investigated the mechanical responses of BMSCs in some detail by using a digital $Ca^{2+}$ imaging technique. Sole application of 2, 4, 6-trinitrophenol (TNP), a cell membrane-bulging agent, did not induce discernible $Ca^{2+}$ responses. However, this agent potentiated $Ca^{2+}$ elevations in BSMCs elicited by UTP, an agonist of the $P2Y_2$ receptor. A similar phenomenon is known in vascular smooth muscle cells, where synergistic activation of TRPC6 channel was shown to occur by simultaneous receptor and mechanical stimulations.* Consistent with this mechanism, siRNA knockdown of TRPC6 expression abrogated the potentiation of UTP-induced $Ca^{2+}$ elevations in BMSCs by TNP. The flow cytometry analysis in BMSCs indicated that siRNA knockdown of TRPC6 arrests the cell cycle at the $G_2$/M phase. Furthermore, the cell cycle-specific arrest by synchronizing agents showed a heightened potentiation of UTP-induced $Ca^{2+}$ responses by TNP in the M phase compared with the other cell cycle phases. Collectively, these results suggest that the mechanosensitivity of TRPC6 may play a facilitatory role for the $G_2$/M progression of proliferating BMSCs.

*Inoue R. *et al*., *Circ.Res.* 104:1399-1409, 2009 (COI:No)

## 2P-009

### Analysis of the molecular mechanism underlying ER $Ca^{2+}$ sensor STIM1-dependent suppression of $Ca_V1.2$ channel activity

Takuro Numaga-Tomita, Hiroki Takahashi, Mitsuhiko Yamada (*Dept Mol Pharma, Sch Med Health Sci, Shinshu Univ, Japan*)

Activation of phospholipase C-coupled cell surface receptors evoked intracellular $Ca^{2+}$ increase via phosphatidylinositol 4, 5-bisphophate hydrolysis. $Ca^{2+}$ release from $Ca^{2+}$ store, endoplasmic reticulum (ER) by inositol 1, 4, 5-trisphosphate receptors causes the reduction of a $Ca^{2+}$ content in ER. ER-resident $Ca^{2+}$ sensor STIM1 senses this reduction, activates Orai1 channels at the plasma membrane, and induces store-operated $Ca^{2+}$ entry. It is known that STIM1 activation upon store depletion also suppresses the activity of $Ca_V1.2$ L-type voltage-dependent $Ca^{2+}$ channels at the plasma membrane through a largely unknown molecular mechanism. In this study, we measured the effect of STIM1 on recombinant $Ca_V1.2$ channel currents expressed in tsA201 cells in the whole-cell configuration of the patch-clamp technique. Co-expression of STIM1 significantly suppressed the $Ca_V1.2$ current. Under this condition, the expression of channel subunits were not reduced as assessed with wester blotting whereas $Ca_V1.2$ gating currents were greatly suppressed, indicating that STIM1 inhibited the membrane expression of $Ca_V1.2$ . The STIM1-dependent suppression of $Ca_V1.2$ current was abrogated by the deletion of distal C-terminus (DCT) of $Ca_V1.2$. Endogenous STIM1 in tsA201 cells also significantly suppressed $Ca_V1.2$ channel currents when activated by thapsigargin without altering their steady-state inactivation or activation. These data suggest that STIM1 induces the endocytosis of $Ca_V1.2$ via interaction with DCT, thereby reducing the number of $Ca_V1.2$ channels and the amount of their currents upon store-depletion. (COI:No)

## 2P-010

### The $NH_2$-terminal region is the key for the differential drug sensitivity of TRPM7 and TRPM6

Hana Inoue[1], Takashi Murayama[2], Takuya Kobayashi[2], Ryo Mizumoto[1], Utako Yokoyama[1] ([1]*Dept Physiol, Tokyo Med Univ, Tokyo, Japan,* [2]*Dept Pharmacol, Juntendo Univ Sch Med, Tokyo, Japan*)

TRPM7 and its closest homologue TRPM6 are $Ca^{2+}/Mg^{2+}$-permeable cation channels with an alpha-kinase domain at their COOH-terminal. It has been reported that TRPM7 and TRPM6 are differentially affected by 2-aminoethyl diphenylborinate (2-APB) which inhibits TRPM7, but activates TRPM6. To elucidate the underlying mechanism of its different action, we investigated the effect of 2-APB using various truncated and chimeric channels. We first tested the effect of 2-APB on the kinase domain truncated TRPM7 (TRPM7-ΔK). TRPM7-ΔK current was significantly inhibited by 200 $\mu$M of 2-APB (201.9 ± 27.1 pA/pF and 42.8 ± 7.57 pA/pF, before and after 2-APB application, respectively, n = 5), suggesting that the kinase domain is not the target of 2-APB. Truncation of $NH_2$-terminal region (NTR, 1-644) of TRPM7 impaired the expression, therefore, we constructed a chimeric channel consisted of the NTR of TRPM6 (1-636) and the TRPM7 transmembrane domain with its kinase domain (645-1863). The chimeric channel was expressed on the plasmamembrane, though the current was strongly inhibited by intracellular free $Mg^{2+}$ with an $IC_{50}$ value of 11 $\mu$M, compared to TRPM7-wild type ($IC_{50}$ 346 $\mu$M). Interestingly, the chimeric channel was not inhibited by 2-APB (57.3 ± 4.7 pA/pF and 60.3 ± 4.7 pA/pF, before and after 2-APB application, respectively, n = 5). These results suggest that the NTR of TRPM7 is involved in the differential effects of 2-APB. (COI:No)

## 2P-011

### Three zebrafish ROMK channels show different pharmacological properties

Yuriko Takeda[1], Fumihito Ono[1], Koichi Nakajo[1,2] ([1]*Dept Physiol, Osaka Med College,* [2]*Div Integrative Physiol, Jichi Med Univ*)

Potassium ion homeostasis is important in all organisms. To maintain its concentration at the appropriate level, potassium excretion at the distal nephron is essential, for which ROMK channels are the main pathway. They are also responsible for recycling potassium ions to help $Na^+$-$K^+$-$2Cl^-$ cotransporter NKCC2. The genetic defect of either of ROMK or NKCC2 can cause Bartter syndrome, which exhibits secondary hyperaldosteronism. Fish also need to maintain the potassium ion homeostasis by excreting potassium ions through the kidney. Distinct from mammals, fish also utilize the gill for potassium excretion. Zebrafish possess seven ROMK genes in the genome (kcnj1a.1~6 and kcnj1b). We previously isolated all seven genes from kidney and gill of zebrafish and confirmed that three of them (kcnj1a.1, kcnj1a.2, and kcnj1b) were functional as ROMK channels. We also identified that they have different sensitivity against extracellular barium: kcnj1b is the most sensitive to barium and kcnj1a.2 is the least sensitive to barium. This is partially due to the variety of amino acid side chains at the end of pore helix. In this study, we examined the recently-developed ROMK-specific inhibitor VU591. Interestingly, only kcnj1a.1 was sensitive to VU591. VU591 is supposed to bind deep in the pore and presumably interact with Val168 and Asn171 (Swale et al. Biophys J. 2015). All three zebrafish ROMK have different amino acid residues at 168 and 171; Leu and Asn in kcnj1a.1, Leu and Ser in kcnj1a.2, Ala and Asn in kcnj1b. We conclude that the different sensitivity to VU591 is due to the variation of amino acid residues in the deep pore region, as opposed to the case of barium sensitivity in which the shallower pore region may be more responsible. (COI:No)

## 2P-012

### Investigation of the Analgesic Effect of Saliva of Mosquito with a Patch-Clamp Method

Tianbang Li (*Cell Signaling, NIPS, Japan*)

Various tropical diseases are transmitted via mosquito bites, which are barely sensed by human beings. The painless blood feeding of mosquito enlightens the development of bionic microneedles, which could be applied for diabetes treatment, neonatal care and acupuncture therapy. It is believed that there are two reasons for humans to be not able to sense the ache of puncture: mechanically, the subtle mosquito proboscis could efficiently avoid the pain spots of the skin and reduce the resistive force. On the other hand, biochemically, the saliva of mosquito which reaches free nerve endings might contain some compounds which possess analgesic effects. However, the later mechanism is poorly understood. To address the question of the analgesic effect of mosquito saliva, we investigated whether it affects human nociceptors. Transient Receptor Potential (TRP) V1 and TRPA1 play crucial roles in perception of painful stimuli. Expressing either TRP channel in HEK293 cells and then performing a whole-cell patch-clamp recording provided us a promising in vitro model to analyze the analgesic effect of saliva of Culex pipiens pallens. Surprisingly, 20-fold diluted mosquito saliva sample suppressed human TRPV1 currents but not human TRPA1 currents. Moreover, such an analgesic effect was abolished upon heating the saliva sample to 95oC for 20 minutes, indicating that peptides might be involved in this suppressing. We plan to not only repeat electrophysiology experiments in mouse dorsal root ganglia neurons but also carry out behavioral experiments in a mouse model. Mass spectrometric method will be also employed to identify which ingredient of mosquito saliva plays the major role in analgesia. Taken together, this work might shed light upon the mechanism of the anesthetic effect of mosquito saliva, which could offer support for developing bionic microneedles to achieve painless injection. (COI:No)

## 2P-013

### Possible involvement of transient receptor potential canonical channel in ectopic activity of pulmonary vein cardiomyocytes of guinea-pig

Xinya Mi[1], Wei-Guang Ding[1], Akiko Kojima[2], Hiroshi Matsuura[1] (*[1]Dept Physiol, Shiga Univ Med Sci, Otsu, Japan, [2]Dept Anesthesiol, Shiga Univ Med Sci, Otus, Japan*)

Pulmonary veins contain a myocardial layer, and its ectopic activity propagates into the left atrium and underlies atrial arrhythmias such as atrial fibrillation. The ectopic activity in pulmonary vein cardiomyocyte (PVC) appears to arise from multiple ionic mechanisms. The present study was designed to examine the possible involvement of transient receptor potential canonical 3 (TRPC3) channel in the electrical activity of PVC, enzymatically isolated from guinea-pig heart/pulmonary vein preparations. Spontaneous action potential was observed in 40.2% of PVC during exposure to normal Tyrode solution, and firing frequency in these spontaneously active PVC was 120.0 ± 26.7 /min (n = 6), which was significantly reduced by the selective TRPC3 blocker Pyr-3 at 0.5 μM (24.0 ± 10.1 /min, 80.8 ± 10.7% reduction). The $\beta$-adrenergic agonist isoproterenol (100 nM) markedly increased the firing rate to 195.5 ± 14.0 /min, which was also significantly reduced by the concomitant presence of 0.5 μM Pyr-3 (92.5 ± 23.9 /min, 7.0. ± 8.5% reduction). Western blot experiments using anti-TRPC3 antibody detected most abundant immunoreactive band at ~85 kDa, which is close to the expected molecular mass for the TRPC3. Immunocytochemistry experiments using anti-TRPC3 antibody detected the expression of TRPC 3, predominantly in the transverse tubule. These results strongly suggest that TRPC3 channel is expressed in PVC and functionally contributes to ectopic electrical activity of PVC in guinea-pig. (COI:No)

## 2P-014

### Involvement of the C-terminal domain in cell surface expression and G-protein coupling of mGluR6

Atsushi Shimohata[1], Dilip Rai[1, 2], Takumi Akagi[1], Toshiyuki Ishii[1], Mie Gangi[1], Takuma Maruyama[1], Yuko Kiyama[1], Ikuo Ogiwara[1], Makoto Kaneda[1] (*[1]Dept Physiol, Nippon Med Sch, Japan, [2]Visual Funct Sec, Depat Rehabil Sens Funct, Nat Rehabil Center, Japan*)

Metabotropic glutamate receptor 6, mGluR6, is expressed in the dendritic tips of retinal ON-bipolar cells and activates G-protein-dependent signaling cascades upon binding to glutamate released from photoreceptors. We herein investigated the role of the mGluR6 C-terminal domain (CTD) (residues 840-871) in cell surface localization and G-protein coupling using immunocytochemical, biochemical, and electrophysiological approaches involving 293T cells and primary hippocampal neurons. We tested C-terminally truncated mGluR6, and showed that the removal of up to residue 858 did not affect surface expression or glutamate-induced G-protein-mediated responses, while a 15-amino acid deletion (Δ 857-871) impaired these functions. However, a 21-amino acid deletion (Δ 851-871) restored surface expression and glutamate-dependent responses, which were again attenuated when the entire CTD was removed. We investigated other mGluR6 deletions, in which amino acids were sequentially removed from the N-terminal side of CTD, and showed that mGluR6 surface localization was unaffected until the entire CTD was deleted. We demonstrated that mGluR6 with CTD consisting of only a middle segment (851-856) was deficient in surface expression. These results suggest that a single histidine or lysine residue at the N or C terminus of CTD is sufficient for mGluR6 surface localization and receptor function, whereas mGluR6 CTD may contain regulatory elements for intracellular trafficking and signaling. (COI:No)

## 2P-015

### $Ca^{2+}$ dependent inactivation of Cav1.2 channel induced by two molecules of calmodulin

Etsuko Minobe[1], Jianjun Xu[1], Masayuki Mori X.[2], Masaki Kameyama[1] (*[1]Dept Physiol, Grad Sch Med & Dent Sci, Kagoshima Univ, Japan, [2]Dept Chem, Sch Med, UOEH, Japan*)

Calmodulin (CaM) performs critical regulation of Cav1.2 $Ca^{2+}$ channels. $Ca^{2+}$-free CaM preassociates dynamically with the channels, and subsequent $Ca^{2+}$ influx drives conformational changes that induce the channel regulation. Several conformations of the channel-CaM complex have been reported, but it remains to be controversial. We have previously reported that the wildtype Cav1.2 channel requires CaM and ATP to induce its activity. The model, based on our experiments, consists of two CaM-binding sites in the channel each for $Ca^{2+}$-dependent facilitation and inactivation. In this study, we have investigated the conformation of Cav1.2 channel in the inactivated state by recording the activity of carboxyl-terminal (CT) deleted channel liked to CaM (CaM-linked channel) and amino-terminal (NT) deleted CaM-linked channel (delN-CaM-linked channel) in the inside-out mode of patch-clamp technique. We found that the CaM-linked channel maintained the activity in the presence of ATP, and that CaM-dependent inactivation (CaMDI) could be isolated from $Ca^{2+}$-dependent inactivation (CDI). The CaM-linked channel showed both CDI and CaMDI, while the delN-CaM-linked channel showed only CaMDI but not CDI. GST-pulldown assay was performed in different $Ca^{2+}$ and CaM concentrations to explore the interaction NT and CT peptide with CaM. The results suggested that the inactivation induced by one CaM linked with the channel may require NT of the channel. The inactivation induced by more than one CaMs may not require NT, but involve CT of the channel. Thus, there might be two types of the conformation of $Ca^{2+}$/CaM-dependent inactivation of Cav1.2 channel. (COI:No)

## 2P-016

### Expression of $Ca^{2+}$ activated $K^+$ Channels in Human Cementoblast

Satomi Kamata[1], Asuka Higashikawa[2], Maki Kimura[2], Sadao Oyama[2], Wataru Ofusa[2], Haruna Toda[2], Yoshiyuki Shibukawa[2], Shuichiro Yamashita[1] (*[1]Dept Removable Partial Prosthodont, Tokyo Dent Coll, [2]Dept Physiol, Tokyo Dent Coll*)

**Purpose:** The function of the cementum is to provide an attachment site for collagen fibers that connect to and fix the alveolar bone teeth. Cementum is formed by cementoblasts. Transmembrane signaling associated with cell membrane ion transport regulates various physiological processes in the cell, but the detailed properties of cementoblast ion signals have not yet been clarified. The purpose of this study was to investigate the biophysical and pharmacological properties of currents generated by ion channels expressed in human cementoblasts (HCEM).
**Materials & Methods:** We measured ionic currents using conventional whole-cell patch-clamp recording. Krebs solution was used as a standard extracellular solution (standard-ECS). Standard intracellular solution (standard-ICS) was composed as followings (in mM) ;140 KCl, 10 NaCl and 10 HEPES. we prepared Cs-ICS solution by equimolary replacing $K^+$ in the ICS with $Cs^+$. We used non-selective $K^+$ channel blocker TEA and $Ca^{2+}$ -activated $K^+$ channel blocker IbTX, apamin and TRAM-34 to examine pharmacological properties of the ionic current recorded.
**Results & Conclusion:** Depolarizing voltage steps from holding potential of -70 mV with 10 mV increments evoked outward currents under the ECS/ICS condition. When the same depolarization stimulation was applied to the cells with Cs-ICS, the outward currents almost disappeared. When TEA and IbTX were administered under the ECS/ICS, the current densities of the outward currents significantly and reversibly decreased. However, apamin and TRAM-34 had no effects on the current density of the outward currents. These results suggested that HCEM expresses large-conducutance $Ca^{2+}$-activated $K^+$ channels. (COI:No)

## 2P-017

### Single-Molecule Fluctuations and Conformational Changes of the Human Transient Receptor Potential Vanilloid 1 (TRPV1) Channel Recorded using Diffracted X-ray Tracking

Hirofumi Shimizu[1], Takuya Kobayashi[2], Masayuki Iwamoto[3], Kentaro Kajiwara[4], Nagomi Kurebayashi[2], Haruo Ogawa[5], Takashi Murayama[2] (*[1]Dept Integ Sys Phisiol, Univ Fukui Fac Med Sci, Japan, [2]Dept Cell Mol Pharm Juntendo Univ Grad Sch Med, Japan, [3]Dept Mol Neurosci, Univ Fukui Fac Med Sci, Japan, [4]SPring-8/JASRI, Japan, [5]IQB, The Univ of Tokyo, Japan*)

The transient receptor potential vanilloid 1 (TRPV1) channel senses various stimuli, such as vanilloid, heat, and pain. Recent progress in the structural analysis of the channel has revealed an important clue for understanding the structure-function relationship. We applied diffracted X-ray tracking to the human TRPV1 channel to reveal/investigate further the molecular mechanisms underlying the activations. The purified channels were fixed on the plate at the extracellular side, while the gold nanocrystals were attached to the other side as a probe. The diffraction spots from the crystals were tracked on the plane of the 2D X-ray detector at the synchrotron facility, which was then translated into the motions of the channels. We have introduced a toroidal mirror and high-speed 2D X-ray detector at the BL28B2 beamline at Spring-8, which can achieve high temporal (sub-millisecond) and spatial (~0.1°) resolutions to track the motions. In the presence of capsaicin, the enhancement of the global fluctuations and twisting motions along the axis of the ion permeation pathway were observed, which was suppressed by its antagonist. In contrast, only small fluctuations were observed in the absence of capsaicin. These results suggested that the observed large fluctuations and twisting motions were involved in the gating motions of the TRPV1 channels. In this presentation, we will discuss the detailed behavior of the TRPV1 channels with respect to activations, and the advantages of our method for revealing the dynamic properties of proteins. (COI:No)

## 2P-018

### Establishment of a method measuring membrane potential in phagosomes

Yoshifumi Okochi[1], Hidekazu Tsutsui[2], Yasushi Okamura[1] (*[1]Integrative Physiol, Grad Sch Med, Osaka Univ, Japan, [2]Japan Advanced Institute of Science and Technology*)

Recently, several ion channels functioning in endomembrane system have been identified. Since one of these channels is a voltage-dependent ion channel, a possibility arose that membrane potential in endomembrane system could change. However, it is still lack the method measuring membrane potential change in this system. To challenge this problem, we set out the establishment of a method measuring membrane potential in endomembrane system. We chose phagosome, a vesicle separated from plasma membrane, formed when phagocytes eat pathogens and dead cells. We have reported previously that we succeeded visualization of phagosomal membrane potential in RAW264.7 macrophage cell line by using FRET-based voltage probe Merm2 and phagosomal membrane potential hyperpolarizes with maturation of phagosome, comparing to cell membrane. However, absolute value of membrane potential of phagosomes remains unknown. In this study, we measured fluorescence intensity of Merm2 in cell membrane of RAW cells by setting reference voltage using patch clamp technique thereby calibrating fluorescence intensity of RAW cells. We found that cell membrane potential of RAW cells was roughly estimated to hyperpolarize from -30 mV to -80 mV when these cells were stimulated with ATP. We are now trying to estimate phagosomal membrane potential of isolated phagosomes using the same method.
What is the biological significance of hyperpolarized phagosomal membrane? In general, hyperpolarization of membrane can facilitate $Ca^{2+}$ flux by increase of driving force. Since phagosome is known to supply $Ca^{2+}$ to cytoplasm through ion channels, it is assumed that hyperpolarization in phagosomal membrane is required for facilitation of $Ca^{2+}$ flux into cytoplasm. We are trying to manipulate phagosomal membrane potential by optogenetic and pharmacological techniques to understand significance of hyperpolarization in phagosomes. (COI:No)

## 2P-019

### Contractility assessment of engineered heart tissue using human iPS cell-derived cardiomyocytes

Daiju Yamazaki (*Div of Pharmacol, NIHS, Japan*)

A number of anticancer agents manifest cardiotoxicity including left ventricular dysfunction and congestive heart failure as side effects. Doxorubicin (DOX) is among the most effective and widely used anticancer agents in the clinic. Although DOX are applied to several types of cancer, it causes a dose-related cardiotoxicity that can lead to heart failure in a subset of patients. In the research and development period at Pharmaceutical companies, if anticancer agents are evaluated for side effects risk such as heart failure before clinical and established for initial dosage, it is possible to reduce the risk in the clinical. However, there is no in vitro contractility assay for detecting cardiotoxicity by anticancer agents.
Here, we produced engineered heart tissue (EHT) using human induced pluripotent stem cell derived cardiomyocytes (hiPS-CMs) and developed contractility assay for evaluating contractile dysfunction to DOX. First, we examined gene expression analysis for maturation of hiPS-CMs. 4-weeks cultured EHTs increased cardiomyocytes maturation marker including *MYH7, TNNI3* and *KCNJ2* compared with unloaded and 1-week cultured EHTs. It was suggested that EHTs matured hiPS-CMs. Next, we investigated $\beta$-adrenergic receptor responses with isoproterenol as an activator and propranolol as a blocker. Isoproterenol showed dose-dependent positive chronotropic and inotropic action and propranolol showed dose-dependent negative chronotropic and inotropic action. These responses were similar with human heart. Finally, we chronically applied DOX to EHTs. Low dose of DOX (0.1 μM) increased beat rate in a time-dependent manner. Middle and high dose (0.3 and 1 μM) of DOX increased beat rate until 48h and impaired contractility in a time- and dose-dependent manner. It is suggested that EHTs may be useful tool for detecting cardiotoxicity by anticancer agents. (COI:No)

## 2P-020

### Vascular gap junctions contribute to pial artery dilation response to somatosensory stimulation in isoflurane-anesthetized rat cortex

Nobuhiro Watanabe[1], Satoshi Sasaki[1,2], Kazuto Masamoto[1,2,3], Harumi Hotta[1]
([1]*Dept Auton Neurosci, Tokyo Metropol Inst Gerontol, Japan,* [2]*Grad Sch Info Eng, Univ Electro-Commun, Japan,* [3]*CNBE, Univ Electro-Commun, Japan*)

Somatosensory stimulation increases cerebral blood flow (CBF) in the representative regions of the somatosensory cortex, accompanying with cerebral arterial dilation. Such stimulation-induced arterial dilation is believed to propagate through the vascular endothelium from the cerebral parenchyma to the cortical surface. Vascular gap junctions transmit vasodilation signals in vitro, but its role in vascular regulation in vivo remains unclear. The present study aimed to investigate whether vascular gap junctions contribute to the regulation of the pial and penetrating arterial tones during somatosensory stimulation. Male Wistar rats were artificially ventilated under isoflurane anesthesia. For somatosensory stimulation, the left forepaw was electrically stimulated. Rhodamine-labeled Ficoll was intravenously administered to fluorescently label the vasculature. The artery in the forelimb area of the right cortex was imaged via a cranial window using two-photon microscopy, and the diameter was measured. In separate experiments, CBF was measured using a laser speckle flowmeter, and the somatosensory-evoked potentials were recorded in the right somatosensory cortex. To block vascular gap junctions, carbenoxolone (CBX) was intravenously administered. The forepaw stimulation increased the diameter of the pial artery by 7.0% and of the penetrating artery by 5.0% of the pre-stimulus diameter, without changing the systemic arterial blood pressure. The stimulation increased regional CBF by 24.1% of the pre-stimulus levels, and induced somatosensory-evoked potentials. Following CBX administration, the pial artery dilation response was reduced to 3.2%, however the extent of penetrating artery dilation or of CBF increase was not affected. Further, CBX did not alter on the resting level of the blood pressure or the magnitude of somatosensory-evoked potentials, indicating that CBX does not influence the neural functions but presumably affects the cerebrovascular responses. These results suggest that vascular gap junctions, possibly on the endothelium, contribute to pial artery dilation but not to penetrating artery dilation during somatosensory stimulation. (COI:No)

## 2P-021

### Candidate genes that contribute to oxygen sensitivity were identified from rat ductus arteriosus and pulmonary arteriolae

Takako Yokota, Toru Akaike, Susumu Minamisawa (*Dept. of Cell Physiology, The Jikei University School of Medicine, Tokyo, Japan*)

**Background:** Oxygen sensitivity plays an important role in vascular tone. In ductus arteriosus (DA) closure, the increase in oxygen concentration inactivates $K_V$ and $K_{ATP}$ channels, which leads to depolarization and enhances $Ca^{2+}$ intake. In contrast, the reaction of pulmonary arteriolae (PA) to oxygen is opposite to DA, and high sensitivity to oxygen allows PA to dilate soon after birth. The specific genes that contribute to this difference remain unknown, however. Therefore, to identify the oxygen-sensing mechanism, we sought to clarify the differential gene expression pattern in DA and PA before and after birth.
**Aim:** The aim of this study is to identify the genes in rat DA and PA that contribute to oxygen sensitivity.
**Methods and Results:** Using Agilent SurePrint G3 Rat GE 8X60K, V2 Microarrays (Agilent®), we compared the gene expression profiling in DA and PA of Wistar rats before (on embryonic day 21: e21) and after birth (on day 2: d2). We found 19 genes in which the expression levels increased in DA and decreased in PA after birth (d2DA/e21DA>2.0 and d2PA/e21PA<-2.0) and 21 genes in which the expression levels were vice versa. Among these 40 genes that exhibited an opposite expression pattern in DA and PA, 15 are already demonstrated to express in smooth muscles. We therefore considered these 15 genes to have a high probability of being involved in vessel closure as a reaction to the increase in oxygen concentration. In addition, referencing a database about their expected oxygen sensitivity, the functional closure mechanism and the epidemiological characteristics of PDA, we selected 5 genes (*dgkg, scara5, abcc8, nr4a1, kcne3*) as important factors for DA and PA to develop oxygen sensitivity.
**Conclusions:** We identified five candidate genes that may contribute strongly to oxygen sensitivity in rat DA and PA. (COI:No)

## 2P-022

### Gene Expression Profiling of the Sinoatrial Node in Mice: the Effect of Endurance Exercise

Seiya Miyazaki[1], Motohiro Mizuguchi[1], Tomoaki Ishida[1], Tomoe Ueyama[2], Takahiro Sogo[2], Shu Nakao[1,2], Teruhisa Kawamura[1,2] ([1]*Dept. of Biomed. Sci., Col. of Life Sci., Ritsumeikan Univ.,* [2]*Global Innov. Res. Org. Ritsumeikan Univ.*)

Chronic endurance training causes slowed resting heart rate known as exercise-induced bradycardia. Severe bradycardia leads to syncope, sudden cardiac death, and necessity of pacemaker implantation. Our previous studies demonstrated that exercise-induced bradycardia is caused by dysfunction of the sinoatrial node (SAN), the primary pacemaking site of the heart, through ion channel remodeling. However, it remains elusive which type of stimuli from exercise directly affect the expression and function of cardiac ion channels in SAN. We here investigate exercise-induced changes in the gene expression profile of the SAN that may cause resting bradycardia using a mouse model. Mice were subjected to high-intensity forced swimming twice a day and 6 days per week for 4 weeks. The swimming sessions resulted in significantly lower resting heart rate in mice than in sedentary mice. To address the global gene expression profile in the SAN, we next performed RNA isolation from the SAN tissues dissected based on the anatomical landmarks. Total RNA samples pooled from three individual mouse SANs were preamplified and used to generate a cDNA library for RNA-sequencing. Transcriptome analysis revealed downregulation of 4,306 genes in the trained SAN. Gene set enrichment analysis showed that significantly reduced expression levels of genes enriched in $Ca^{2+}$ regulation-, ion channel-, impulse conduction-, autonomic receptor-related gene sets in trained SAN, suggesting dysfunction of the pacemaker activity. GO analysis demonstrated that exercise-induced downregulation of lipid metabolic process and immune system process was observed, while there were upregulated genes annotated in GO terms associated with muscle contraction, hypertrophy, oxidative phosphorylation and mitochondrial function. Although further functional analysis is required, our results suggest that chronic exercise stimulates adoptive response of the myocardium to increased hemodynamic stress, and it also downregulates cardiac ion channels and autonomic regulators through metabolic remodeling in the SAN, leading to bradycardia. (COI:No)

## 2P-023

### The arterial baroreflex suppresses psychological distress evoked by social exclusion

Tsubasa Izaki, kei Ishii, Ryota Asahara, Hidehiko Komine (*AIST, AHFRC, Tsukuba, Japan*)

Social exclusion (neglect or rejection by others) evokes psychological distress termed "social pain". A previous study reported that hypertension attenuated sensitivity to social pain. We thus hypothesized that an afferent input of the arterial baroreflex attenuates social pain. To test the hypothesis, we investigated the influence of the arterial baroreflex on social pain evoked by experimentally-induced social exclusion in human. The arterial baroreflex was induced by neck chamber technic (negative pressure (-30-20 mmHg) was applied within a chamber wrapped around the neck). Experimentally-induced social exclusion was evoked by the cyberball task. The cyberball task is a virtual ball tossing game among three players (one subject and two computer players). Social exclusion was simulated by reducing the ratio of throws to a subject (<10%). Subjects receive a ball in the first half of the task, but there are only throws among computer players in the second half of the task. Neck suction was applied at the time when throws among computer players were performed in the second half of the task. Heart rate was noninvasively measured three-lead electrocardiogram. Subjects played two kind of the cyberball task: applying negative pressure during the task (the baroreflex condition) and not applying negative pressure (the control condition). After playing each condition, social pain was assessed by the need-threat scale (self-reported scale for assessing social pain). In the baroreflex condition, HR dropped in the second half of the cyberball task compared to in the first half of the task. Social pain score was lower in the baroreflex condition than in the control condition (baroreflex: 14.47 ± 1.94, control: 15.75 ± 2.02, $p<.05\pm$). These findings suggest that the arterial baroreflex suppress social pain evoked by social exclusion. (COI:No)

## 2P-024

### Effects of NADPH oxidase (NOX) 4 on single cell mechanics in mouse ventricular cardiomyocytes

Keiko Kaihara[1], Keiji Naruse[1], Gentaro Iribe[2] ([1]*Dept Cardio Physiol, Grad Sch Med, Okayama Univ, Japan,* [2]*Dept Physiol, Asahikawa Med Univ, Japan*)

Reactive oxygen species (ROS) plays important physiological roles in the cardiovascular system. We have previously reported that myocardial stretch increases ROS derived from NADPH oxidase (NOX)2 to stimulate the sarcoplasmic reticulum (SR) to increase $Ca^{2+}$ spark rate and increases cellular contractility. However, behavior of NOX4-derived ROS in this response is unknown. In the present study, we investigated the role of NOX4 on acute stretch-induced ROS, $Ca^{2+}$ sparks and cellular mechanics.
Ventricular cells were enzymatically isolated from either 8～12 week old mice (WT) or NOX4 knock out (KO) mice hearts. Isolated mouse ventricular myocytes were exposed to 8～10% axial stretch using computer-controlled piezo-manipulated carbon fibers, attached to both cell ends. Net ROS production and $Ca^{2+}$ sparks were studied using DCF and flou-4, respectively. In single cell mechanics study, slopes of end-systolic force-length relation curves was measured as an index of cellular contractility.
Genetic NOX4 deletion (NOX4 KO) or pharmacological NOX4 inhibition by GKT136901 abolished stretch-induced increase in ROS production and reduced cellular contractility. However, deletion or inhibition of NOX4 did not affect stretch-induced increase in $Ca^{2+}$ spark rate. The results suggest that role of NOX4-derived ROS in the response to myocardial stretch is different from that of NOX2. (COI:No)

## 2P-025

### Anti-IL6 receptor antibody may worsen cardiomyopathy related to laminopathy

Megumi Kato[1], Mizuyo Kojima[2], Kaori Yamashita[1], Eiji Wada[1], Yukiko Hayashi[1]
(*[1]Dept Pathophysiol, Tokyo Medical Univ, Japan, [2]Diversity Promotion Center, Tokyo Medical University, Japan*)

**Purpose:** Mutations in LMNA which encodes A-type lamins can cause several human diseases including fatal cardiomyopathy with conduction defects. Activation of MAPK pathways was reported to link Lmna-related cardiomyopathy. Interleukin-6 (IL-6) is a multi-functional cytokine and known to promote fibrosis in heart. In addition, the IL-6 receptor antibody (MR16-1) was reported to suppress inflammation after myocardial infarction, and improved cardiac remodeling in mice. In this study, we investigated the roles of IL-6 in Lmna-related cardiomyopathy and the therapeutic
effects of MR16-1.
**Methods:** We used Lmna p.H222P knock-in mice (H222P) and C57BL/6J mice as control (WT). We performed qPCR to check gene expression, western blotting to measure protein levels of IL-6 signaling pathway, and histological analyses by Masson's trichrome staining.
**Results:** mRNA of IL-6 was significantly increased in H222P heart. After MR16-1 treatment, mRNA of IL-6 in H222P heart was decreased, whereas mRNA of collagen1 $\alpha$ 1, and TGF $\beta$ 2 was increased. Furthermore MR16-1 enhanced phosphorylation of ERK1/2 and increased fibrosis.
**Conclusions:** These results suggest bloking IL-6 receptor may worsen Lmna-related cardiomyopathy. (COI:No)

## 2P-026

### Experimental and theoretical study on mechanism of $Ca^{2+}$ and $Na^{+}$ permeation in $Ca_V1.3$ L-type calcium channels

Futoshi Toyoda[1], Akinori Noma[2], Ding Wei-Guang[1], Hiroshi Matsuura[1]
(*[1]Dept Physiol, Shiga Univ Med Sci, Japan, [2]Dept Bioinformatics, Col Life Sci, Ritsumeikan Univ*)

We have reported that $Ca_V1.3$ L-type calcium channel (LTCC) is responsible for the generation of the sustained inward $Na^+$ current ($I_{st}$) in cardiac pacemaker cells. However, it remains unclear how $Ca_V1.3$ could mediate both $Ca^{2+}$ and $Na^+$ currents. Here, the competitive permeation of $Ca^{2+}$ and $Na^+$ was examined in LTCCs in mouse sinoatrial node (SAN) cells and $Ca_V1.3$ channels in heterologous system. In SAN cells, L-type $Ca^{2+}$ current ($I_{Ca,L}$) typically elicited at -10 mV was gradually decreased and mostly disappeared as the external $Ca^{2+}$ concentration ($[Ca^{2+}]_o$) was lowered from 1.8 to 0.01 mM. Further reduction in the $[Ca^{2+}]_o$ ($\leq 1\,\mu$M) conversely induced an inward current, indicating a switch of conducting ion through LTCC from $Ca^{2+}$ to $Na^+$. These responses are characteristic of an anomalous mole-fraction dependence of LTCCs reported previously. On the other hand, the persistent inward current at -60 mV, assumed to be $I_{st}$, was gradually increased by lowering $[Ca^{2+}]_o$, consistent with differential responses between $I_{Ca,L}$ and $I_{st}$ to the external $Ca^{2+}$ in our previous study. Reconstituted $Ca_V1.3$ channels successfully recapitulated $I_{Ca,L}$ at the physiological $[Ca^{2+}]_o$ of 1.8 mM, which was typically manifested at -10 mV but also evident at -60 mV as a sustained inward current similar to $I_{st}$ in SAN cells. Importantly, $Ca_V1.3$ also reproduced the distinct response of $I_{Ca,L}$ and $I_{st}$ to lowering $[Ca^{2+}]_o$, suggesting both currents in SAN cells are attributable to the permeability property of $Ca_V1.3$ channels. Theoretical analysis using Almers & McCleskey model well explained the experimental observation of anomalous mole-fraction dependence of $I_{Ca,L}$ as well as the generation of $I_{st}$ through $Ca_V1.3$, but predicted the presence of a large $Na^+$ conducting mode with higher $Ca^{2+}$ sensitivity under extremely low $[Ca^{2+}]_o$ condition. (COI:No)

## 2P-027

### Dopaminergic system is involved in cardiovascular responses induced by lateral habenula activation in rats

Yuma Sato[1,2], Tri Doan Huu[1,3], Masayuki Matsumoto[4,5], Tadachika Koganezawa[1,5]
(*[1]Dept Physiol, Fac Med, Univ Tsukuba, Ibaraki, Japan, [2]Master's Program in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan, [3]Doctoral Program in Biomedical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan, [4]Department of Cognitive and Behavioral Neuroscience, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, [5]Transborder Medical Research Center, University of Tsukuba, Tsukuba, Ibaraki, Japan*)

The habenula is a pair of small nuclei which is located above the thalamus and divided into the medial and lateral nuclei (LHb). It is known that exogenous stress events activate LHb neurons, which in turn inhibit dopamine neurons in the midbrain. Although the stress events also induce various autonomic physiological responses such as changes of blood pressure and heart rate, little is known about whether and how the LHb regulates the cardiovascular responses to the stress event. To understand the roles of the LHb and dopaminergic system in the cardiovascular responses to stress events, here we investigated the effect of the electrical stimulation of the LHb on blood pressure and heart rate with a dopaminergic receptor blocker. We used Wistar male rats that were anesthetized by urethane (1 g/kg, *i.p.*). Arterial pressure was recorded from the femoral artery. Heart rate was analyzed from R-R intervals of the electrocardiograph. The LHb was electrically stimulated with a coaxial electrode (300 μA, 0.5 ms duration, 100 Hz, for 10 s). The LHb stimulation-induced cardiovascular responses were observed before and after administrating clozapine (1 mg/kg, *i.v.*), a non-selective dopamine receptor antagonist. As a result, electrical stimulation of the LHb significantly increased the mean arterial pressure and decreased heart rate. In the presence of the clozapine, these cardiovascular changes induced by electrical stimulation to the LHb were significantly attenuated. These results suggest that the activation of LHb neurons produces the pressor response and bradycardia by affecting the autonomic nervous system, and that the effect of the LHb activation on the stress-induced cardiovascular responses is mediated by the dopaminergic system. (COI:No)

## 2P-028

### Ionic mechanisms of pacemaker activity in HL-1 mouse cardiomyocytes

Yuichi Kuda[1], Yasutaka Kurata[1], Takayuki Ikeda[2], Mamoru Tanida[1], Tsumoto Kunichika[1], Toshishige Shibamoto[1], Hideto Yonekura[2]
(*[1]Dept Physiol [2] Sch Med, Kanazawa Med Univ, Japan, [2]Dept Biochem [2] Sch Med, Kanazawa Med Univ, Japan*)

**Aim:** HL-1 mouse atrial myocytes, often used for systematic electrophysiological studies, exhibit automaticity and thus would be useful for investigating ionic and dynamical mechanisms of pacemaker activity. Therefore, we determined the ionic mechanism of automaticity in HL-1 cells.
**Methods:** We measured membrane action potential, sarcolemmal ionic currents, and intracellular $Ca^{2+}$ concentration changes in HL-1 cells by whole-cell patch clamp methods and fluorescence imaging of $Ca^{2+}$ and membrane potential. Roles of the inward rectifier $K^+$ channel current ($I_{K1}$), hyperpolarization-activated cation channel current ($I_f$)and $Ca^{2+}$ releases from the sarcoplasmic reticulum (SR) in automaticity were determined by using $Ba^{2+}$, ivabradine and caffeine to cause $I_{K1}$ block, $I_f$ block and SR $Ca^{2+}$ depletion, respectively.
**Results:** There exist both pacemaking and non-pacemaking cells. Larger $I_{K1}$ was detected in non-automatic cells by whole-cell patch clamp. By the patch clamp and fluorescence imaging of membrane potential, automaticity was promoted by Ba-induced block of $I_{K1}$. In the absence of $Ba^{2+}$, caffeine attenuated $Ca^{2+}$ transient, slowed pacemaking, and abolished automaticity. In contrast, Ba-induced automaticity did not disappear with co-administration of caffeine or ivabradine.
**Conclusions:** The density of $I_{K1}$ was considered to be an important factor that determines whether pacemaker activity emerge or not. Our findings suggest that the mechanisms of pacemaker activity in HL-1 cells vary depending on the $I_{K1}$ density: SR $Ca^{2+}$ release-mediated pacemaking and membrane clock-dependent ($I_f$-independent) pacemaking occur at higher and lower $I_{K1}$, respectively. (COI:No)

## 2P-029

### Serotonin uptake via plasma membrane monoamine transporter during ischemia-reperfusion in the heart

Takashi Sonobe, Tsuyoshi Akiyama, James Pearson
(*Dept Cardiac Physiol, Natl Cereb Cardiovasc Ctr Res Inst, Suita, Japan*)

Serotonin (5-HT) accumulates in the heart during ischemia-reperfusion. The accumulated 5-HT is considered to be taken up into cells and then metabolized with producing 5-hydroxyindole acetic acid (5-HIAA) and hydrogen peroxide, which worsens the myocardial injury.
Our previous results suggested the contribution of 5-HT transporters, which were resistant to fluoxetine (selective serotonin reuptake inhibitor) to take up 5-HT from the myocardial interstitial space during ischemia-reperfusion.
Therefore, the aim of this study was to investigate contribution of plasma membrane monoamine transporter (PMAT) to the 5-HT uptake in the heart.
By means of microdialysis technique to the heart of anesthetized rats, we monitored myocardial interstitial levels of 5-HT and 5-HIAA during coronary occlusion followed by reperfusion period. We also examined the effects of local administration of a PMAT inhibitor, decynium-22 on the changes in 5-HT and 5-HIAA level.
Baseline dialysate 5-HT concentration significantly increased and 5-HIAA concentration decreased by decynium-22. Dialysate 5-HT concentration further increased during the coronary occlusion and then decreased after the reperfusion, while 5-HIAA increased after the reperfusion. In the presence of decynium-22, the decrease in 5-HT and the increase in 5-HIAA during the reperfusion period were significantly prevented. These results indicated that 5-HT uptake/5-HIAA production was interrupted by the inhibition of PMAT.
In conclusion, PMAT regulates basal level of interstitial 5-HT and contributes to take up 5-HT into cells during reperfusion after ischemia in the heart. (COI:No)

## 2P-030

### Counteractive effects of daily exercise on stress-induced alteration of NTS transcriptome in rats

Hidefumi Waki[1], Makoto Suzuki[1], Keisuke Tomita[2], Ko Yamanaka[1], Kei Tsukioka[1], Sabine Gouraud[3]
(*[1]Grad Sch Health & Sports Sci, Juntendo Univ, Japan, [2]Faculty of Med Sci, Teikyo Univ of Sci, Japan, [3]Dept of Biol, Faculty of Sci, Ochanomizu Univ, Japan*)

**Background:** We have reported that chronic restraint stress increased resting blood pressure (BP), whereas daily exercise exhibited anti-hypertensive effects. Since the nucleus tractus solitarius (NTS) plays important roles in regulating BP, we hypothesized that the NTS is involved in mechanisms underlying both stress-induced hypertension and exercise-dependent anti-hypertensive effects. In this study, we tested this hypothesis by transcriptome analysis at the level of the NTS.
**Method:** Eighteen Wistar male rats (Four-week-old) were divided into three groups (n=6 each): control (Co); chronic restraint stress (ST); and ST + voluntary exercise (SE) groups. ST was produced by one hour-immobilization per day for 3 weeks (5 days per week). After the end of intervention period, PCR array (RT2 Profiler PCR Array) targeting genes related to neuronal functions was used to screen for differentially expressed NTS genes among three groups.
**Results and Discussion:** Six genes in the NTS were identified as differentially expressed genes between Co and ST groups, however only two genes were found as differentially expressed between Co and SE groups, suggesting that exercise modifies stress-induced alteration of gene expression profiles in the NTS. Such modified genes by exercise include dopamine receptor D1 (Drd1) and neuropeptide Y receptor type 5 (Npy5r), both of them are known to modulate neuronal functions.
**Conclusion:** These results suggest that altered gene expression of the NTS might be involved in the mechanism underlying both stress-induced high BP and the preventive effect of exercise. Functional roles of identified genes in the NTS need to be elucidated. (COI:No)

## 2P-031

### Effects of warming or cooling of the forearm on brachial artery endothelial function during leg cycling exercise in humans

Yoshiyuki Fukuba[1], Marina Morimoto[1], Saki Namura[1], Kohei Miura[1], Masako Endo Y.[1], Anna Oue[2], Hideaki Kashima[1] (*[1]Dept Exerc Sci Physiol, Pref Univ Hiroshima, Japan, [2]Toyo Univ*)

It is well recognized that leg cycling exercise induces endothelial adaptation of the conduit artery in an inactive limb (i.e., brachial artery; BA) beyond the active lower limbs. Such beneficial effects on the endothelium may be because of exercise-induced elevated shear stress (SS) due to increased blood flow to the BA. Modulation of the SS profiles, such as the pattern of antegrade and retrograde SS, is especially important for vascular adaptation. Warming of the forearm is often used as an experimental manipulation to modulate the SS profile in the BA, thereby improving flow-mediated dilation (FMD) via shear-dependent mechanisms. Therefore, the aim of this study was to examine the effect of modulating the SS profile using forearm warming and cooling on the subsequent endothelial function in the BA during exercise. The 12 healthy young subjects immersed their right forearm into water at either 15 ℃ or 42 ℃ during 60-min leg cycling exercise at heart rate of 120–130 bpm. A control trial was the same exercise without water immersion. The endothelial function in the right BA was evaluated by means of FMD (%) before (baseline) and after the exercise. In addition, the shear rate (SR, an estimate of SS) profiles before, during, and after the exercise were simultaneously evaluated. After the exercise, there was no significant change in the FMD the warm immersion and control trials. However, in the cool immersion trial, the post-exercise FMD at 60 min decreased from baseline significantly and was lower than the warm and control trials. Concomitant forearm cooling during leg exercise attenuates post-exercise endothelial function and it is associated with modulation of shear patterns. It is concluded that the modulation of shear patterns in the BA during exercise appears to be associated with subsequent endothelial function. [supported by JSPS-KAKENHI (#17K01616)] (COI:No)

## 2P-032

### The cardiac cycle reconstructed using the human ventricular cell (HuVEC) model

Sayaka Niwa (*Dept LifeSci, Grad Sch LifeSci, Ritsumei Univ, Japan*)

We aim at developing the human ventricular cell (HuVEC) model (Himeno et al. 2015). This study asked whether the force of contraction of the HuVEC model well reproduces the intraventricular pressure to perform the pumping function of a ventricle. Toward this end, we applied the Laplace equation to convert the developed tension of HuVEC model to the ventricular pressure. The blood circulation (total volume = ~5800 ml) was composed by connecting the Laplace ventricle to an afterload, a systemic vascular resistance, a preload, a sinus volume and Laplace atrium. A common rate of shortening of the sarcomere length (dX/dt in the NL model) was determined between the myofilament kinetics and the volume change of the Laplace ventricle at each cycle of numerical integration of the model. The Bernoulli's equation was used for blood pressure with the total pressure E, lateral pressure P and the dynamic pressure v with the blood density $\rho$.

$E = P + (\rho \times v^2) / 2$

When the HuVEC model was paced with an interval of 800 ms, the major feature of a cardiac cycle was reproduced, such as the ejection fraction, intraventricular pressure, blood pressure in the afterload, and volume changes in each compartments. It was suggested that the HuVEC model meets main requirements well for driving the blood circulation. Limitations of the model will be discussed in this presentation. (COI:No)

## 2P-033

### Controlling development of phase-2 early afterdepolarizations in human ventricular myocytes of lomg QT syndromes: A theoretical study using bifurcation analyses of two mathematical models

Yasutaka Kurata[1], Kunichika Tsumoto[1], Ichiro Hisatome[2], Yuichi Kuda[1], Mamoru Tanida[1] (*[1]Dept Physiol 2, Sch Med, Kanazawa Med Univ, Japan, [2]Dept Regener Med Therapeut, Grad Sch Med Sci, Tottori Univ, Japan*)

**Aim:** Early afterdepolarization (EAD) causes ventricular arrhythmias in long QT syndrome (LQTS). The aim of this study was to provide a theoretical background for controlling EAD formation in LQTS via slow-fast decomposition analysis based on bifurcation theory.

**Methods:** Assuming inhibition of delayed-rectifier $K^+$ channel currents (slow component $I_{Ks}$ for LQT1 and rapid component $I_{Kr}$ for LQT2), we used LQT1 and LQT2 versions of human ventricular myocyte models (Kurata et al, 2005; ten Tusscher-Panfilov, 2006), which can reproduce EADs during $\beta$-adrenergic stimulation and bradycardia, respectively. Bifurcation diagrams for a fast subsystem were constructed as functions of a slow variable, e.g., activation gating variable of $I_{Ks}$ (xs), $Ca^{2+}$-dependent inactivation gating variable of L-type $Ca^{2+}$ channel current $I_{CaL}$ ($f_{Ca}$), or $Ca^{2+}$ concentration in the sarcoplasmic reticulum (SR). Parameter-dependent shifts in Hopf, saddle-node and period-doubling bifurcation points were determined as functions of parameters such as maximum conductance of $I_{Kr}$ ($g_{Kr}$), $I_{Ks}$ ($g_{Ks}$) and $I_{CaL}$ ($g_{CaL}$).

**Results and Discussion:** In the Kurata et al model, with increasing $xs^2$ or decreasing $f_{Ca}$, an equilibrium point (EP) at depolarized potential was destabilized via a Hopf bifurcation (HB); as $g_{Kr}$ decreased, HB points shifted toward higher $xs^2$ or lower $f_{Ca}$, leading to transient trapping of full system trajectories around the stable EP, i.e., EAD formation. Decreasing $g_{CaL}$ (or increasing $g_{Kr}/g_{Ks}$) shifted HB points toward prevention of trapping of full system trajectories (EAD formation). Accelerating $I_{Ks}$ activation or inhibition of the transient outward current ($I_{to}$) was also effective in preventing EADs. The ten Tusscher-Panfilov model further reproduced spontaneous SR $Ca^{2+}$ release-mediated EADs via destabilization of intracellular $Ca^{2+}$ concentrations; decreasing SR $Ca^{2+}$ pumping rate was effective in preventing the SR $Ca^{2+}$ release-mediated EAD. This study demonstrates that bifurcation analysis is useful for systematically investigating how EADs can be controlled by modifications of ionic channels and transporters. (COI:No)

## 2P-034

### Analysis of short-term potentiation at the supramammillary nucleus to the dentate granule cell synapses

Eri Tabuchi, Takeshi Sakaba, Yuki Hashimotodani (*Lab Mol Syn Fun, Grad Sch Brain Sci, Doshisha Univ, Japan*)

The supramammillary nucleus (SuM) of the hypothalamus has strong connections with the hippocampus and has been implicated in spatial learning, emotional behavior and sleep-wake state. We recently found that SuM neurons make monosynaptic connections to granule cells (GCs) and interneurons in the dentate gyrus and co-release glutamate and GABA at these synapses. While we previously revealed synaptic connections between the SuM and dentate gyrus, little is known about synaptic plasticity at SuM-GC synapses. Here, we investigated whether SuM-GC synapses can undergo short- or long-term plasticity. To this end, we performed whole-cell patch-clamp recordings from GCs in acute hippocampal slices. To optogenetically activate SuM afferents, we injected AAV carrying channelrhodopsin-2 into the SuM of VGluT2-Cre mice. By light illumination, SuM-GC EPSCs were monitored in the presence of $GABA_A$ receptor antagonist picrotoxin. We found that postsynaptic depolarization induced a robust potentiation of EPSCs, which lasted for 10-15 min. This depolarization-induced potentiation (DIP) is input-specific, as DIP was not observed at perforant-path inputs, main excitatory inputs onto GCs. We further found that postsynaptic loading of the $Ca^{2+}$ chelator BAPTA, or bath application of nifedipine, a L-type $Ca^{2+}$ channel blocker, abolished DIP, indicating that a postsynaptic $Ca^{2+}$ influx by postsynaptic depolarization is required for induction of DIP. To test whether DIP is expressed as presynaptic or postsynaptic mechanism, we monitored pharmacologically isolated NMDA-EPSCs. If DIP is due to an enhancement in glutamate release, NMDA-EPSCs are expected to show a similar potentiation. However, DIP was not observed in NMDA-EPSCs, suggesting that DIP is postsynaptically expressed. Remarkably, DIP was not observed in IPSCs mediated by GABA co-released from SuM terminals, suggesting that DIP changes glutamate/GABA ratio at SuM-GC synapses. Taken together, our results indicate that SuM inputs may have important roles for information processing in the dentate gyrus by temporal changing the excitatory/inhibitory balance. (COI:No)

## 2P-035

### Cholinergic induction of network oscillations in invertebrate olfactory neuron in vitro

Suguru Kobayashi, Yuzuki Murao, Nana Komatsu, Hisayo Sadamoto (*Kagawa Schl Pharmaceut Sci, Tokushima Bunri Univ, Japan*)

Synchronous oscillatory network is vital for cognitive functions of the brain of both vertebrates and invertebrates. In the central nervous system of the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC) and its frequency changes are suggested to encode the olfactory information and memory. We recently found oscillatory activity is generated spontaneously in dispersed cell culture of PC neurons. Application of acetylcholinesterase inhibitor or nicotine increased the number of spontaneous activities and furthermore, induced synchronous oscillatory activity. On the other hand, biogenic amines or neuropeptides often changed the number of spontaneous activities without generation of synchronous oscillation on PC neurons. Previous results suggest that acetylcholine could be function as a driving force on the synchronous oscillatory activity of the PC neuron network via nicotinic acetylcholine receptors activation. In present study, differences between synchronous and asynchronous network were examined in cultured PC neuron (7-21 days). We found a lower excitability and a higher sensibility to cholinergic activation in synchronous PC neurons. PC neurons cultured after 10 days could induce synchronous oscillation, and included two group, (a) acetylcholine sensitive and (b) insensitive/driven by a. (COI:No)

## 2P-036

### The endocannabinoid 2-arachidonoyl glycerol in dentate granule cells suppresses kainate-induced seizures

Yuki Sugaya[1], Kenji Sakimura[2], Masanobu Kano[1] (*[1]Dept. Neurophysiol., Grad. Sch. Med., Univ. of Tokyo, Japan, [2]Dept. Anim. Model Dev., Brain Res. Inst., Niigata Univ., Japan*)

The endocannabinoid 2-arachidonoyl glycerol (2-AG) produced by the activity of diacylglycerol lipase $\alpha$ (DGL $\alpha$) mediates retrograde suppression of synaptic transmission and prevents excessive excitability of neural circuits. In line with this notion, we have demonstrated previously that kainate-induced seizures are substantially more severe in global DGL $\alpha$ knockout mice than in wild-type littermates. However, it remains unclear which cell types are responsible for 2-AG production and ameliorating seizures, although many reports suggest involvement of the hippocampal dentate gyrus in seizures. Therefore, we examined possible roles of 2-AG produced in granule cells of the dentate gyrus in kainate-induced seizures. To obtain conditional knockout mice with DGL $\alpha$ deletion specifically in dentate granule cells, we crossed the pro-opiomelanocortin (POMC)-cre mice with the DGL $\alpha$ floxed mice. Immunofluorescence staining showed that the POMC-cre positive offsprings (gcDGL $\alpha$ -/- mice) lacked DGL $\alpha$ in dentate granule cells but exhibited intact DGL $\alpha$ expression in other hippocampal neurons. In contrast, localizations of $CB_1$ cannabinoid receptors in the dentate gyrus were identical between the gcDGL -/- mice and their cre negative littermates (gcDGL $\alpha$ fl/fl mice). We injected kainate (30 mg/kg, i.p.) to both genotypes and determined the latency to the onset of generalized tonic clonic seizures in gcDGL $\alpha$ -/- and gcDGL $\alpha$ fl/fl mice. We found that the latency was significantly shorter and the mortality was significantly higher in gcDGL $\alpha$ -/- mice than in gcDGL $\alpha$ fl/fl mice. The latency of gcDGL $\alpha$ -/- mice was comparable to that of global DGL $\alpha$ knockout mice. These results suggest that 2-AG produced in dentate granule cells ameliorates seizures and is crucial for survival during hyperexcitability. (COI:No)

## 2P-037

### Postnatal stimuli modulate cortico-hippocampal network dynamics

Yoshiaki Shinohara[1], Shinnosuke Koketsu[2], Takatoshi Ueki[2] (*[1]Dep. Histology, Jichi Medical Univ., [2]Dep Integrative Anatomy, Med, Nagoya City Univ*)

Phase-locked synchronization between the cerebral cortex and hippocampus has been implicated to be important during working memory and spatial navigation. However, how animal experience organizes cortico-hippocampal dynamics is largely unknown. To analyze synchronized activity between the hippocampus and various cortical areas, we used transgenic mice (G7NG817), which expresses the calcium indicator G-CaMP7 in astrocytes and the majority of excitatory neurons in the cortex. The mice allow us to observe the temporal dynamics of cortical calcium in 25-100Hz transcranially. Environmental effects on cortico-hippocampal dynamics were observed by comparing mice reared under two distinct conditions, either in enriched environment (ENR) or isolated condition (ISO). After 4 weeks rearing, we measured cortical calcium dynamics and hippocampal local field potentials simultaneously.

We found that the cortical calcium dynamics was closely correlated to hippocampal EEG status in urethane anaesthetized mice. Regardless of the rearing conditions, calcium activities observed during theta and non-theta states showed distinct spatial and temporal patterns. Cortical calcium level is higher in wide areas of the cortex during the theta state, and the temporal calcium fluctuation is relatively mild. During non-theta states, basal calcium level is lower, but larger cortical calcium elevations that spread over cortical areas co-occurred with hippocampal sharp wave and ripple (SWR) oscillations.

Next, we investigated spatio-temporal dynamics between hippocampal SWR events and cortical calcium using head-restrained un-anaesthetized mice monitoring animal sleep/awake states. In both states, calcium activities preceded hippocampal SWR events in vision-related cortical areas. During awake SWRs, wide areas of cortical calcium elevation co-occurred with hippocampal SWRs, while sleep SWRs showed delayed frontal cortical activation after SWRs. Interestingly, ENR mice exhibited sparser activity patterns. Our data indicate that the cortico-hippocampal dynamics show distinct patterns depending on animal states, but postnatal experience also play a role for modulating the network dynamics. (COI:No)

## 2P-038

### Age-related changes in the M1/M2 polarization of microglia in trigeminal spinal subnucleus caudalis following intra-oral injury

Daisuke Ikutame[1], Masamichi Shinoda[2], Kentaro Urata[1], Shintaro Fujiwara[1], Toshimitsu Iinuma[1], Koichi Iwata[2] (*[1]Dept Complete Denture Prosthodont, Nihon Univ Sch Dent, Japan, [2]Dept Physiol, Nihon Univ Sch Dent, Japan*)

Pain perception in the orofacial region is known to change during aging. It is reported that the microglial activation in the trigeminal spinal subnucleus caudalis (Vc) is involved in orofacial pain hypersensitivity following intra-oral injury in young adult animals, but it is unclear how the aging affects the pain hypersensitivity enhanced by microglial activation following orofacial tissue injury. Here, we examined that aging-related changes in the M1/M2 polarization of microglia in Vc following intra-oral injury in mice.

Senescence-accelerated mouse prone 8 (SAMP8) and senescence-accelerated mouse resistant 1 (SAMR1) as control were used in this study. Palatal mucosal incision (Length: 3mm, Depth: 1 mm) was performed under the deep anesthesia. The mechanical withdrawal threshold (MHWT) in the incised site was measured for 21 days following intra-oral tissue injury. Furthermore, expression pattern of CD11c-immunoreactive (IR) cells (affective microglia (M1)) and CD163-IR cells (protective microglia (M2)), and TNFalpha-IR M1 and M2 in Vc was analyzed immunohistochemically on days 3 and 11 following intra-oral tissue injury.

The decrease of MHWT following intra-oral tissue injury is significantly enhanced in SAMP8. M1 activation was enhanced and the number of TNFalpha-IR M1 and M2 was increased on day 3 following intra-oral tissue injury in SAMP8 compared with SAMR1. On day 11, M1 and M2 activation was observed in both groups, whereas M2 activation was attenuated in SAMP8. Moreover, the increase in the number of TNFalpha-IR M1 was more extensive than that of TNFalpha-IR M2.

The present findings suggest that mechanical allodynia following intra-oral tissue injury was potentiated and sustained in SAMP8 due to the enhancement of TNFalpha signaling following M1 activation and M2 attenuation in Vc. (COI:No)

## 2P-039

### Oxytocin attenuates orofacial mechanical allodynia following infraorbital nerve injury

Masatoshi Ando, Masamichi Shinoda, Koichi Iwata (*Dept Physiol, Sch Dent, Nihon Univ, Japan*)

Oxytocin (OXT) signaling is known to attenuate nociceptive neuronal hyperexcitability which is responsible for pain hypersensitivity. In this study, we examined the effect of oxytocin application into the nerve-injured site on orofacial mechanical hypersensitivity following infraorbital nerve injury (IONI) in rats.

A one-third thickness of the infraorbital nerve was tightly ligated with 6-0 silk under deep anesthesia, MedGel® containing OXT (20 $\mu$l, 50 mg/ml in 0.01M PBS) is placed directly into the ION-injured site. The head-withdrawal threshold to mechanical stimulation (MHWT) of the whisker pad skin was measured using von Frey filament following IONI. Trigeminal ganglion (TG) neurons innervating the whisker pad skin were identified employing retrograde labeling with 3% Fluoro-Gold (FG). FG-labeled OXT receptor-positive, transient receptor potential vanilloid 1 (TRPV1)-positive, and TRPV4-positive TG neurons were examined immunohistochemically on day 5 following IONI.

The MHWT was significantly decreased from day 1 onward following IONI. OXT application into the nerve-injured site attenuated the decrease of the MHWT from day 5 onwards. On day 5 following IONI, OXT receptors were expressed in uninjured ION and FG-labeled TG neurons Furthermore, the number of FG-labeled TRPV1-positive and TRPV4-positive TG neurons was increased on day 5 following IONI, and its increments were significantly depressed by the OXT application into the ION-injured site. In addition, the suppression of the increased number of FG-labeled TRPV1-positive and TRPV4-positive TG neurons was significantly inhibited by concomitant OXT receptor antagonist (Atosiban, 20 $\mu$l, 100 $\mu$M) application.

These findings suggest that OXT application into the ION-injured site inhibits the increases in TRPV1 and TRPV4 expression in TG neurons innervating the whisker pad skin, resulting in the attenuation of orofacial mechanical allodynia following IONI. (COI:No)

## 2P-040

### Orexin receptor activation induces a novel slow afterhyperpolarization that results from the calcium-dependent closure of cation channels in serotonergic dorsal raphe neurons

Masaru Ishibashi[1], Atsuo Fukuda[1], Christopher Leonard S[2] (*[1]Dept of Neurophysiol, Hamamatsu Univ School of Med, Japan, [2]Dept of Physiol, New York Medical College, USA*)

Serotonergic (5-HT) dorsal raphe (DR) neurons regulate numerous brain functions including sleepwake states, circadian phase, reward and mood. Moreover, orexin receptor signaling at 5-HT DR neurons appears critical in the sleep disorder narcolepsy, which emerges following the loss of orexin signaling. We recently reported that in addition to producing a slow depolarization, orexin-A enhances the post-spike afterhyperpolarization (oeAHP), which alters spike encoding by increasing spike frequency adaptation. Mechanistically we found that the oeAHP involved two distinct components that required $Ca^{2+}$ influx. The first was of medium-duration (tau ~ 0.5s) and involved apamin-sensitive SK $Ca^{2+}$-activated $K^+$ channels. The second was of longer duration (tau ~ 5s), was apamin-insensitive (termed the ai-oeAHP) and appeared similar to a slow AHP (sAHP). In this study we used whole-cell patch clamp recordings and $Ca^{2+}$ imaging in mouse brain slices to investigate the mechanisms and function of this ai-oeAHP. we found that the ai-oeAHP was not attenuated by a cesium-based patch solution as expected for a $K^+$ currents, but rather was blocked by substituting NMDG for $Na^+$ in the ACSF or by application of flufenamic acid (FFA), both of which attenuated the orexin-induced inward current. Moreover, we found that the increase in baseline membrane conductance produced by orexin-A was reduced during the ai-oeAHP suggesting that the ai-oeAHP was mediated by a transient, $Ca^{2+}$-dependent closure of the cation channels activated by orexin. These results suggest that ai-oeAHP is a novel type of $Ca^{2+}$-dependent sAHP that is conditionally expressed following orexin-activation of non-selective cation channels. (COI:No)

## 2P-041

### Difference of cholinergic modulation on corticostriatal synaptic plasticity between direct and indirect spiny projection neurons

Atsushi Tamura, Julie Chouinard, Kiyoto Kurima, Yumiko Akamine, Jeffery Wickens R. (*Neurobiology Research Unit, OIST, Japan*)

The cholinergic interneurons (CINs) of the striatum are crucial for behavioral flexibility. CINs of the dorsomedial striatum (DMS) play a role in strategy switching. However, how CINs modulate the neural circuitry underlying strategy switching is unclear. The glutamatergic afferents from the cerebral cortex to the striatum display activity-dependent plasticity in the corticostriatal synapses, and may be involved in certain types of learning. One hypothesis is that strategy switching may be realized by a modulatory effect of CINs on corticostriatal plasticity. Here, we investigated the effect of CINs on activity-dependent plasticity in the corticostriatal synapses. To control tonic firing of CINs, adeno-associated virus (AAV) encoding halorhodopsin (NpHR) was injected into DMS of ChAT-cre mice. AAV injected mice expressed NpHR in CINs and we can optogenetically inactivate CINs firing. We made whole-cell ex vivo slice recordings from spiny projection neurons (SPNs), which are the output neurons of the striatum, and recorded EPSPs induced by electrical stimulation of the corpus callosum. Activity dependent synaptic plasticity was induced by high-frequency stimulation under the Mg-free conditions. This conditioning stimulus combined with optogenetic inactivation of CINs during HFS induced long-term potentiation in some SPNs. However, other group of SPNs showed long-term depression in response to the same conditioning stimuli. To identify the cell type showing opposite plsticity, we observed HFS-induced LTP on either direct or indirect SPNs.These recordings revealed that pause in CINs faculitate LTP on dSPNs and disrupte LTP on iSPNs. This result suggest that pause in CINs work on synaptic plasticity in different manner between dSPNs and iSPNs. (COI:No)

## 2P-042

### Neurological analysis of the GAD67 knockout rats

Dongyu Liu[1], Tomokazu Ohshiro[1], Kazuyuki Fujihara[2], Yuchio Yanagawa [2], Hajime Mushiake[1] (*[1]Dept Physiol, Grad Sch Med, Tohoku Univ, Japan, [2]Dept Gen Behav Neuro, Grad Sch Med, Gunma Univ, Japan*)

An inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) is synthesized by two isozymes, glutamate decarboxylase (GAD) 65 and GAD67. GAD65 is localized in the synapses and responsible for synaptic GABA production. Its deficiency causes spontaneous epilepsy in both mice and rats, indicating indispensable roles of the protein in the central nervous system. On the other hand, GAD67 is a cytoplasmic protein and GAD67 knockout mice are born with severe cleft palate and all of them die at birth, which hampered further detailed analysis. Thus, it remains unknown whether deficiency of GAD67 causes any abnormal brain function in animals.

Recently we generated GAD67 knockout rats by a gene-editing method and found that in contrast to GAD67 knockout mice, GAD67 knockout rats do not exhibit cleft palate and some of them can survive to adulthood. Therefore, these GAD67 knockout rats brought us an opportunity to examine the function of GAD67 *in vivo*. The GAD67 knockout rats exhibited no drastic convolutions or myoclonus as in the case of the GAD65 knockout rats. To examine if the GAD67 knockout rats exhibit any abnormal brain activity, we recorded the EEG from the animals. A hand-made headset with eight wire electrodes was chronically implanted over the epidural space of the homozygous GAD67 knock-out rats. Heterozygous or wild type animals for the gene locus were used as control. Basic time-series analysis of the raw EEG revealed rather normal frequency distributions of the slow sleep waves and the awake fast waves, comparable to those of the control animals. However, a series of short slow waves (3 ~6 seconds) were observed intermittently in the EEG from the GAD67 knockout rats while awake. We are currently investigating whether these paroxysmal waves reflect any petit-mal seizure, by monitoring their behaviors when these paroxysmal waves occur. (COI:No)

## 2P-043

### Two cation current components in GABAergic interneuron precursors in the medial ganglionic eminence of the embryonic brain

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

Proliferation and migration of developing cells are accompanied by cell volume changes, which are achieved by the net ion flux and the resulting passive water movement across the cell membrane. The opening of several types of ion channels and the electrochemical gradients of permeating ions can produce the net ion flux. The precursors of GABAergic interneurons in the cerebral neocortex are born primarily in a region in the ventral telencephalon of the embryonic brain, called the medial ganglionic eminence (MGE), and they migrate toward the neocortex during prenatal and perinatal periods. The types of ion channels involved in the development of interneuron precursors have not yet been identified. By whole-cell patch clamping of acutely isolated interneuron precursors in MGE, we found two cation current components under normal conditions. One component was activated at the membrane voltage level more than −30 mV and exhibited steep outward rectification that was suppressed by the removal of $K^+$ from the patch pipette solution. The tail current generated at −60 mV after depolarizing voltage steps was, however, inward and abolished by external $Na^+$ removal. The reversal potential of the tail current was around −30 mV. Thus, this voltage-gated current component must be generated by an ion channel highly permeable to $K^+$ with a small significant permeability to $Na^+$. Another component was a small $Na^+$ leak current with linear voltage dependence. These two cation current components might cooperatively regulate the development of interneuron precursors. (COI:No)

## 2P-044

### Concentration dependent inhibition of evoked EPSPs produced by ammonium in rat hippocampal CA1 neurons

Naomitsu Kameyama, Eiichiro Tanaka, Yoshinaka Murai, Kiyoshi Kikuchi (*Dept physiol, Sch of Med, Kurume Univ, Japan*)

Hyperammonemia suppresses the central nervous system, e.g., it can induce comas. Why hyperammonemia induces impairments of consciousness has not yet been clarified. On the other hand, a high concentration (1–5 mM) of NH4Cl has been found to inhibit excitatory postsynaptic potentials (EPSPs) in in vivo or in vitro animal model experiments, although the mechanism responsible for the inhibition of EPSPs by NH4Cl is still under discussion. We investigate details of the inhibition of EPEPs by NH4Cl using intracellular and extracellular recordings made from the hippocampal CA1 and CA3 neurons of male Wistar rats. Fast EPSPs were elicited via the focal stimulation of Schaffer collaterals through monopolar electrodes.
The AMPA type fast EPSPs initially increased in amplitude by 5 mM NH4Cl superfusion, then after suppressed and disappeared. Application of various concentration of NH4Cl induced the suppression of the AMPA type fast EPSPs with concentration dependent manner. Application of 5mM glutamate induced a membrane depolarization, which is increased in amplitude in the presence of 5mM NH4Cl. On the other hand, a Na+ spike elicited in a CA3 neuron suppressed by the application of 5mM NH4Cl. A Ca2+-dependent spike was elicited in a CA3 neuron in the presence of 1$\mu$M tetrodotoxin / 20mM tetraethyl ammonium. The Ca2+-dependent spike initially prolonged its duration by application of 5 mM NH4Cl, and then shortened.
These results suggest that sustained suppression of fast EPSP may be due to presynaptic inhibition. Mechanism of the presynaptic inhibition may include, at least, inhibition of the Na+ spike generation or conduction in the excitatory nerve, and of the influx of the Ca2+ into the excitatory nerve terminals. Impairment of consciousness due to hyperammonemia may cause by the reduction of excitatory inputs to the central nervous system due to presynaptic inhibition. (COI:No)

## 2P-045

### The identity of the synaptic vesicle pools underlying evoked and spontaneous synaptic transmission in larval zebrafish neuromuscular junction

Yoshihiro Egashira[1], Akio Ojida[2], Fumihito Ono[1] ([1]*Dept Physiol, Faculty Med, Osaka Medical College, Japan,* [2]*Grad Sch Pharm Sci, Kyusyu Univ, Japan*)

The information transfer at a synapse is mediated by the release of neurotransmitters stored in the synaptic vesicles (SVs). All synapses show both evoked (action potential dependent) and spontaneous (action potential independent) forms of neurotransmitter release. Aside from the mode of neurotransmitter release, neurotransmitter-filled SVs are generally categorized into three distinct pools; a readily releasable pool (RRP), a reserve pool and a resting pool. The RRP and reserve pool together form the recycling pool of SVs. Although the relationship between evoked and spontaneous neurotransmitter release has been investigated using a variety of model synapses, it remains controversial whether the SVs undergoing evoked and spontaneous fusion belong to the same SV pool. Here we addressed this issue in the larval zebrafish neuromuscular junction by generating a novel transgenic fish in which a pH sensitive fluorescent protein (super-ecliptic pHluorin; SEP) and Halo tag were fused in tandem to the luminal side of a SV protein and expressed specifically in the motor neurons. Live imaging of SEP fluorescence allowed us to monitor exo / endocytosis of SVs induced by repeated electrical stimulation, whereas Halo tag-based labeling enabled us to separately tag SVs mobilized by evoked and spontaneous activities. Using this technique, we found that, in zebrafish neuromuscular junction, about 80% of SVs were mobilized by both high frequency electrical stimulation and high K+ depolarization stimulation, and thus constituted the recycling pool. In contrast, SVs fused spontaneously comprised only 12% of total SVs. Sequential labeling of SVs with spontaneous and evoked activities indicated that the two SV populations overlapped completely. Therefore, our data showed that the SVs undergoing spontaneous fusion originated from the identical SV pool responding to action potentials, and the population of the spontaneously-fusing SVs was limited to a small proportion. (COI:No)

## 2P-046

### A crucial role of N-glycosylation of homomeric GluA1 AMPA receptor in LTP induction

Yoshihiko Wakazono[1], Ryosuke Midorikawa[1], Shogo Oka[2], Kogo Takamiya[1] ([1]*Dept Integr Physiol, Fac Med, Univ Miyazaki, Japan,* [2]*Dept Biol Chem, Human Health Sci, Grad Sch Med, Kyoto Univ, Japan*)

The intracellular molecular mechanisms underlying the regulation of the AMPA receptor have been dramatically elucidated in the past few decades. In contrast, the functional regulation of the extracellular domain remains unclear. Here, we focused on N-glycosylation of the AMPA receptor in the extracellular domain and tried to clarify their functions by combining molecular biological and electrophysiological techniques.
We previously reported that 401 asparagine residues (N401), putative N-glycosylation site, in GluA1 subunit might be a responsive site for drastic changes of glutamate responses of AMPA receptor from desensitization to re-sensitization, and that long-term potentiation (LTP) induction of hippocampal slices prepared from N401Q GluA1-expressing lentivirus vector-injected GluA1 KO mice was impossible to maintain its potentiation.
In the present study, we tried to clarify molecular mechanisms underlying the defect of LTP maintenance in N401Q GluA1 mutant. Analysis of miniature EPSC (mEPSC) revealed that the frequency, amplitude and rise times showed no significant change, and that the decay time became significantly faster than that of wild-type GluA1. In addition, when expressed N401Q GluA1 in primary cultured neurons prepared from embryonic GluA1 KO mice brain, the glutamate response did not always show the re-sensitization. These are not consistent with results in N401Q GluA1-expressing HEK cells. To explain this discrepancy, we next examined the involvement of GluA2 subunit, which is possible to form heteromers with GluA1 subunit. Glutamate response in N401Q GluA1-expressing HEK cells reverted the re-sensitization to the desensitization by co-expression with GluA2 subunit. These results suggest that the re-sensitization in N401Q GluA1 homomers might influence to synaptic currents during the LTP induction period, not in the resting state. (COI:No)

## 2P-047

### Long-term survival and proper axonal elongation of grafted hypothalamic Neurons from Mouse ES Cells

Miho Kawata[1], Yu Kodani[1], Yoko Kaneko S.[1], Akira Nakashima[2], Hiroshi Nagasaki[1] ([1]*Dept Physiol, Sch Med, Fujita Health Univ, Japan,* [2]*Dept Physiol chem, Sch Med, Fujita Health Univ, Japan*)

We have established the method to transplant the differentiated hypothalamic neurons from mouse embryonic stem cells (mESC) into the supraoptic nucleus (SON) of the hypothalamus. In this study, we investigated whether grafted neurons could survive for a long time and function in the host brain.
EB5, a mESC cell line, was induced to hypothalamic neurons by floating culture method. The hypothalamic progenitors were purified using the cell-surface antigens. For differentiation, these cells were enzymatically dissociated and cultured on Matrigel-coated cover glass. After day 28, when cells developed to show hypothalamic nature, the cells were infected AAV-CAG-tdTomato for labeling with red fluorescent protein. Then, the cells were dissociated and transplanted to SON of the SCID/NOD mice. To examine the long-term survival and function of the grafted cells, we collected brain samples at 1 month or 3 months after the grafting.
Graft tissue survived up to 3 months. The axons from tdTomato positive neurons were identified widely in hypothalamic area after 1 month. Three months after grafting, the tdTomato positive axons showed beaded-shape, and some of them have reached to the neural lobe of pituitary.
These results suggested the grafted hypothalamic neurons survived in the hypothalamus area of immunnodeficient mice for long-term and followed the pattern of axon guidance as seen in the host hypothalamic neurons. (COI:No)

## 2P-048

### Hyperocclusion suppresses the cognitive activity via the expression of amyloid beta

Munehisa Maeshiba[1,2], Hiroshi Kajiya[1,3], Takashi Tsusumi[2], Takashi Tsuzuki[2], Koji Okabe[3], Jun Ohno[1] ([1]*Res Cent for Regener Med, Fukuoka Dent Coll, Japan,* [2]*Dept Oral Rehabil, Fukuoka Dent Coll, Japan,* [3]*Dept Physiol Sci Mol Biol, Fukuoka Dent Coll, Japan*)

**Background:** In recent years, severe periodontitis has been reported to suppress cognitive ability. However, it is unclear the relationship between malocclusion and cognitive ability. The purpose of this study was to clarify the effects of malocclusion on cognitive ability.
**Materials and Methods:** To create hyperocclusive state in jaw movement stainless wire attached to occlusal surface in upper right molar in 2-month-old and 12-month-old mice. We examined the behaviors analyses such as the 8-arm radial maze test and novel object recognition test on a trial per day. The analyses were measured the total stay duration and error scores in the 8-arm radial maze test and recognitive scores in novel object recognition test. Furthermore, the hippocampus samples were collected on before (0) and after (1 and 4 weeks) hyperocclusion loading in these mice and examined the change in expression of cognition related molecules.
**Results and Discussion:** The long-term cognitive ability and social cognitive ability transiently suppressed on 1 week after hyperocclusion in 2 month-old mice, but not in 12 month-old mice. The expressions of cognition suppressing molecules were also upregulated on 1 week after hyperocclusion using RT-qPCR and Western blotting. The malocclusion suppressed the cognitive ability via the upregulation of suppressive molecules in the cognition, especially amyloid beta, indicating that strict occlusion play an important factor in normal cognitive ability. (COI:No)

## 2P-049

### Optical analysis of nuclear-cytoplasmic $Ca^{2+}$ dynamics in the master circadian clock

Sota Hiro[1,2,3], Yoshiko Yamada[2], Tomomi Nemoto[1,2,3], Ryosuke Enoki[1,2] (*[1]Biophotonics Research Group, Exploratory Research Center on Life and Living Systems, National Institutes of Natural Sciences, Japan, [2]Laboratory of Molecular and Cellular Biophysics, Research Institute for Electronic Science, Hokkaido University, Japan, [3]Graduate School of Information Science and Technology, Hokkaido University, Japan*)

The temporal order of the mammalian physiology and behavior is coordinated by the biological circadian clock that is synchronized with the environmental light-dark cycle. The master clock of circadian rhythm is the suprachiasmatic nucleus (SCN) that is located in the hypothalamus. The SCN is composed of ca. 20,000 neurons, and the individual neurons are thought to keep 24-hour rhythms by the transcriptional and posttranslational feedback loop (TTFL) involving the clock genes and their protein products. The cytoplasmic circadian $Ca^{2+}$ rhythm is thought to regulate the TTFL (Enoki *et al.*, ***Scientific Reports,*** 2017). On the other hand, the SCN neurons exhibited circadian $Ca^{2+}$ rhythms in the cytoplasm but not in the nucleus (Ikeda *et al.*, ***Neuron,*** 2003). It is still a matter of discussion whether the cytoplasmic $Ca^{2+}$ enters into the nucleus or whether the cytoplasmic $Ca^{2+}$ is regulated independently of the nuclear $Ca^{2+}$. In the present study, we simultaneously expressed two genetically-encoded $Ca^{2+}$ probes, GCaMP6s and jRGECO1a: The former expressed specifically in the nucleus of the SCN neurons and the latter in the cytoplasm by combining with nuclear localization signal or nuclear exporting signal, respectively. $Ca^{2+}$ dynamics were monitored at the single-cell level using a Nipkow-disk confocal time-lapse imaging system. We found robust circadian $Ca^{2+}$ rhythms in the nucleus (nuclear circadian $Ca^{2+}$ rhythms). Both nuclear and cytoplasmic circadian $Ca^{2+}$ rhythms had identical topological patterns in the SCN network: the advanced phase in the dorsal region compared with the central and ventral regions. Moreover, nuclear circadian $Ca^{2+}$ rhythms had the same phase as cytoplasmic circadian $Ca^{2+}$ rhythms. These results show that spatiotemporally organized nuclear circadian $Ca^{2+}$ rhythms might play a crucial role in a transcriptional regulatory mechanism in the SCN. (COI:No)

## 2P-050

### Establishment and characterization of neuron-like PC12-derived cell lines with hypersensitivity or hyposensitivity to temperature-regulated repeated thermal stimulation (TRTS)

Tada-aki Kudo[1], Kanako Tominami[1], Satoshi Izumi[1], Yohei Hayashi[2,3], Takuya Noguchi[4], Guang Hong[5] (*[1]Div Oral Physiol, Grad Sch Dent, Tohoku Univ, Japan, [2]CRCBR, IDAC, Tohoku Univ, Japan, [3]Grad Sch Life Sci, Tohoku Univ, Japan, [4]Lab Health Chemist, Grad Sch Pharma Sci, Tohoku Univ, Japan, [5]Liaison Cent Innov Dent, Grad Sch Dent, Tohoku Univ, Japan*)

Neurite formation allows functional formation or restoration of the nervous system. Previously, we developed a novel in vitro method for induction of neuronal cell differentiation in rat neuron-like PC12 cells using temperature-controlled repeated thermal stimulation (TRTS) with a heating plate for temperature regulation of the culture medium. Based on sensitivity to TRTS, the PC12 cells were divided into two types: hypersensitive to TRTS with elongation of the neurites, and hyposensitive to TRTS for unknown reasons. To investigate the mechanism of hyposensitivity, this study aimed to establish two PC12-derived cell lines according to sensitivity to TRTS by a general method of subcloning during the course of neuronal cell differentiation using a 96-well culture plate. To characterize these two models, the cell size, growth rate, and elongation of the neurites were evaluated in the subcloned cells treated with neurite inducers, such as nerve growth factor (NGF) and bone morphogenic protein (BMP), or TRTS. Two types of subcloned cell lines were obtained: PC12-P1F1, TRTS-hypersensitive PC12 cell line and PC12-P1D10, TRTS-hyposensitive PC12 cell line. No significant differences in the cell size and growth rate were observed among the parental PC12 cell line and the two subcloned cell lines. Elongation induction of the neurites by BMP or TRTS was higher in PC12-P1F1 than in the parental PC12 cells, while no induction of neurite elongation was observed in PC12-P1D10. In contrast, NGF-induced neuritogenesis was observed in all the cell lines. These results suggest that PC12 parental cell line shows differences in cell characteristics related to TRTS-induced neuronal differentiation, and BMP signaling pathway is required for TRTS-induced neurite outgrowth in PC12 cells. (COI:No)

## 2P-051

### Cytotoxicity of nanomaterials and neuronal cell death

Yuji Kamikubo, Yoshie Hashimoto, Takashi Sakurai (*Dept Pharmacol, Juntendo Univ Sch Med, Japan*)

With the development of nanoscience, nanomaterials with various sizes, chemical elements, shapes, and surface modifications have been produced for industries and consumers. Although bulk materials have constant features regardless of their size, nanomaterials possess novel size-dependent physical and chemical properties and have been used in a broad range of processes and products including paints, foods, cosmetics, clothes, polishing of fine structures, and pharmaceuticals. Nanomaterials including nanoparticles (NPs) of titanium dioxide, aluminum oxide, zinc oxide, silicon dioxide (silica) are well studied and have been applied in various fields. Because silica nanoparticles are inexpensive, innocuous, and easy to produce and functionalize, they are applied as adsorbents, catalyst carriers, materials for bio-imaging, and drug-delivery systems. Despite these benefits, there are concerns that exposure to nanoparticles can have detrimental effects on certain types of tissues. Because NPs can cross the blood–brain barrier and the blood–placental barrier, they may cause toxic effects such as immune responses, hemolysis, and developmental abnormalities in the brain and developing embryos. Although investigations of the toxicity of NPs to neurons are essential for medicinal use, few studies have assessed the direct effects of NPs on cells derived from the central nervous system. In this study, we showed that treatment with silica and metal oxide NPs caused oxidative stress and neural cell death. Furthermore, we found that these cytotoxicities was reduced by NPs surface functionalization or protein coating, and pretreating cells with an antioxidant, suggesting that contact-induced oxidative stress may be responsible for NPs-induced cell death. These data will be valuable for utilizing NPs in biomedical applications. (COI:No)

## 2P-052

### Characterization of HCN4-immunoreactive neurons that localized at laminae Ⅱ-Ⅲ in mouse spinal cord

Taku Nakagawa[1], Toshiharu Yasaka[2], Noriyuki Nakashima[1], Kensuke Oshita[3], Makoto Takano[1] (*[1]Dept Physiol, Med, Kurume Univ, Japan, [2]Dept Immunol, Grad Sch Med, Kagoshima Univ, Japan, [3]Dept Anesth, Med, Kurume Univ, Japan*)

The hyperpolarization-activated, cyclic nucleotide-gated channels (HCN1～4) are drawing attention as a potential target of pain treatment; in the dorsal root ganglion, it has been reported that HCN2 expressing neurons play the central role in the transmission of inflammatory and neuropathic pain. We have previously reported that HCN4 channels are expressed in the spinal dorsal horn, using transgenic mouse in which the expression locus of HCN4 can be visualized by chemical luminescence by firefly luciferase. However, little is known about the function of HCN4-expressing neurons in the spinal dorsal horn. We therefore aimed to characterize the HCN4 expressing cells using the immunohistochemistry in the transgenic mouse expressing the artificial chromosome in which GFP was knocked into the genetic locus of vesicular GABA transporter (VGAT). We found that the HCN4-immunoreactive cells showed laminar distribution restricted to lamina II inner (IIi) and lamina III, and hardly co-localize with GFP signal derived from VGAT. These findings suggested that most of HCN4-expressing neurons were putatively excitatory interneurons. It has been reported that excitatory interneurons at the border between lamina IIi and III receive the tactile inputs and simultaneously receive the feed-forward-inhibition from the inhibitory interneurons. The failure of such feed-forward-inhibition reportedly underlie the neuropathic pain. Because HCN channels were thought to regulate the synaptic integration by suppressing the fluctuation of membrane potential, HCN4 expressed in the excitatory interneuron might participate in the modulation of such local circuit. (COI:No)

## 2P-053

### The mechanism underlying central sodium sensing for mediating salt-induced hypertension

Kengo Nomura[1], Takeshi Hiyama.Y[1,2], Masaharu Noda[1,2,3] (*[1]Division of Molecular Neurobiology, National Institute for Basic Biology, [2]School of Life Science, SOKENDAI, [3]Research Center for Cell Biology, Institute of Innovative Research, Tokyo Institute of Technology*)

Increases in sodium concentrations ($[Na^+]$) in body fluids elevate blood pressure (BP) by enhancing sympathetic nerve activity (SNA). However, the mechanisms by which information on increased $[Na^+]$ is translated to SNA have not yet been elucidated. We demonstrated that $[Na^+]$ increases in body fluids mediated sympathetic activation leading to BP elevations in wild-type mice, but not in mice which genetically disrupts $Na_x$, an atypical Na channel. We identified $Na_x$ channels expressed in specific glial cells in the organum vasculosum lamina terminalis (OVLT) as the sensors detecting increases in $[Na^+]$ in body fluids, and showed that OVLT neurons projecting to the paraventricular nucleus (PVN) were activated via acid-sensing ion channel 1a (ASIC1a) by $H^+$ ions exported from $Na_x$-positive glial cells. These results provide an insight into the neurogenic mechanisms responsible for salt-induced BP elevations. (COI:No)

## 2P-054

### Response properties to amplitude changes of sound envelope in the neurons of the anterior auditory fields

Sohei Chimoto (*Dept NeuroPhysiol, Div of Med, Univ of Yamanashi, Japan*)

Natural sounds such as human speech and animal species-specific sounds show a broad of time envelope changes in amplitude under acoustic environments. They contain a sound with a quick attack in amplitude (damped sounds) and a sound with a slow increase (ramped sounds). It has been also examined that the temporal amplitude changes influence sound perception and identification in human psychophysical experiments. In the previous studies we found that the higher auditory cortices as well as the primary auditory cortex (A1) include velocity-sensitive cells for the damped- and ramped-sounds and some neurons in the secondary auditory cortex (A2) and posterior auditory fields (PAF) had the sensitivities to direction of the amplitude change. The neurons in anterior auditory fields (AAF) rostral to A1 show tonotopic responses and are also responsive to various kinds of sounds such as noise busts, amplitude modulated sounds, and natural sounds. However, little is known about their response properties during the stimuli with amplitude change. In the present study we recorded single unit activities from AAF of awake animals and examined response properties during ramped and damped sounds. Spike trains responding to each sound were used to construct peristimulus time histograms and the height of PSTH was transformed into the driven rate by subtracting the mean of background firing rates. We found that most of AAF neurons showed sensitivities to the quick change of stimulus envelope and a few neurons showed sensitivities to the slow amplitude changes during both damped- and ramped-sounds. Most AAF neurons also show weak selectivity for damped- or ramped-sounds by comparing between the peak response amplitude during both sounds. These results suggest that the majority of AAF neurons are tuned to the higher velocity of the envelope change but not to the direction of the change. (COI:No)

## 2P-055

### GABAergic inputs to ON and OFF starburst amacrine cells are controlled by different acetylcholine receptors

Mie Gangi, Makoto Kaneda (*Dept Physiol, Nippon Med Sch, Tokyo, Japan*)

Starburst amacrine cells (SACs) are considered to play an important role for the formation of direction selectivity in the retina. They are classified into ON SACs and OFF SACs based on their morphological properties. The ON SACs have cell bodies in the ganglion cell layer and arborize their dendrites in sublamina b of the inner plexiform layer (IPL). The OFF SACs have cell bodies in the inner nuclear layer and arborize their dendrites in sublamina a of the IPL. Because of the easy accessibility to the soma in the whole-mount preparation, the electrophysiological properties of ON SACs were rigorously studied, and the electrophysiological properties of OFF SACs were believed to be same as those of ON SACs. In a series of experiments, we showed that the electrophysiological properties of OFF SAC were not predictable from those of ON SACs. First, P2X2-purinergic signaling pathway is predominantly used in OFF SAC. Second, glycinergic signaling pathway is predominantly used in ON SAC. Further, we recently showed the possibility that the pathways of acetylcholine (ACh) synthesis might be different between ON SAC and OFF SAC.

In the present study, we examined whether such a possible difference of ACh synthesis can produce further difference in cholinergic signaling pathways in the mouse retina. Application of ACh or carbachol (CCh) increased GABAergic IPSCs in both SACs. In ON SACs, a muscarinic agonist increased IPSCs, while in OFF SACs, a nicotinic agonist increased IPSCs. These results indicate that ON and OFF SACs receive GABAergic inhibitory inputs from different amacrine cells expressing different subtypes of ACh receptors. (COI:No)

## 2P-056

### Optimal degree of visual angle for the SSVEP-based brain-computer interface stimuli

Kouji Takano[1], Nana Hayashi-Morita[1], Kenji Kansaku[1, 2, 3] ([1]*Sys Neurosci Sect, Dept of Rehab for Brain Funct, Res Instit of Natl Rehab Cent,* [2]*Dept of Physiol and Biol Information, Dokkyo Medical University School of Medicine,* [3]*Cent for Neurosci and Biomed Eng, The Univ of Electro-Communications*)

**Introduction:** Our group has developed an EEG based steady-state visual evoked potential (SSVEP) brain-computer interface (BCI) system for amyotrophic lateral sclerosis (ALS) patients, including completely locked-in syndrome (CLIS). We reported that a CLIS patient who cannot control the direction of their eyes, was able to control the BCI system (Okahara, 2018). We have experienced that position of visual stimuli (LED flicker) was required to adjust in each patient. In this study, we evaluated optimal degree of visual angle for the SSVEP-based BCI stimuli.

**Methods:** Ten subjects (5 females, age 31.1) participated in this study. The visual stimuli were presented by LED. The LED was placed in the peripheral visual field at 5, 10, 15, 30, and 45 degrees on the opposite side of the dominant eye. Participants were asked to gaze at a central fixation point and attend to or ignore the LED (attention/ignore tasks). EEG signals were measured from Oz, PO7, and PO8. We calculated the power spectrum density (PSD) and imaginary coherence (ImC) of the EEG data as the parameters to discriminant the subjects attend or ignore the LED.

**Results:** In PSD analyses, over 70% accuracies of discrimination were observed at 5, 10, 15 degrees, and in ImC analyses, these were observed at 10, 45 degrees. The highest accuracy was 75% (7 subjects exceeded 70%) at 5 degrees in PSD analyses and 71.9% (6 subjects exceeded 70%) at 45 degrees in ImC analyses.

**Discussion:** The results suggested that optimal degree of visual angle for the SSVEP-based BCI stimuli were dependent on the used analyzing methods (PSD or ImC). (COI:No)

## 2P-057

### Photoreceptor ribbon synapse is a factor regulating light adaptation of the visual information processing in mice

Yuko Sugita[1], Kenichiro Miura[2, 3], Takahisa Furukawa[1] ([1]*Lab. Mol & Dev Biology, Protein Inst, Osaka Univ, Japan,* [2]*Dept. Integ Brain Sci, Grad Sch. Med, Kyoto Univ, Japan,* [3]*National Center of Nerology and Psychiatry, Japan*)

The ribbon synapse is a specialized synaptic structure which connects photoreceptor synapse terminal with bipolar and horizontal cell. An extracellular matrix protein Pikachurin is essential for the proper formation of the ribbon synapse. Several lines of evidence suggest that the Pikachurin deletion specifically influences the ON pathway; the localization of mGluR6 is restricted to the postsynaptic site of ON bipolar cells in the ribbon synapses of the outer plexiform layer.

To understand how influence of ON pathway contributes to visual information processing, we examined optokinetic responses (OKRs) elicited by two-frame animations presented with an inter-stimulus interval (ISI) in Pikachurin null mutant mice that show improper formation of the photoreceptor ribbon synapse.

The wild-type mice showed OKRs to two-frame-motion stimuli in the veridical direction in the absence of an ISI. The OKRs decreased progressively as the ISI got longer and became almost zero when the ISI was 106.7ms. When the ISIs were 106.7 ms or longer, the OKRs were directed in the opposite direction. The Pikachurin$^{-/-}$ mice showed a different dependence on ISIs from that of the wild-type mice. We simulated the dependences on ISIs of individual mice using a computational model of visual motion detection (elaborated Reichardt motion detector) to estimate the characteristics originated from the temporal filters embedded in the visual system of mice.

The frequency characteristics of temporal filters in Pikachurin$^{-/-}$ mice showed no difference in the optimal temporal frequency, but showed stronger adaptation to an exposure to a sustained light signal than the wild-type mice. These results suggest that the ribbon synapse is a factor regulating light adaptation of visual information processing. (COI:No)

## 2P-058

### Electrical synapses of pyramidal cells in developing visual cortex and retinal ganglion cells can enhance excitatory synaptic outputs through synchronous neural spiking between these cells

Soh Hidaka[1], Chiyuki Kaneko[2] ([1]*Dept Physiol, Fujita Health Univ Sch Med,* [2]*Dept Pathol, Faclt Med Tech, Fujita Health Univ Sch Health Sci*)

Electrical synapses are present in many types of visual cells expressing channel subunit, connexins (J. Neurosci., 2004; J. Integra Neurosci., 2008; Brain Res., 2012). Electrical current spread through gap junctions between presynaptic neurons is expected to regulate chemical output synapses from these neurons onto postsynaptic neurons. In our recent studies, physiological properties of electrical synapses between $\alpha$ retinal ganglion cells have been characterized (J. Neurosci., 2004; J. Integra Neurosci., 2016). In the present study, we examined electrical synapses of retinal ganglion cells and pyramidal cells in visual cortex of developing rats and primate common marmosets. First, we investigated the localization of gap junctions between these visual excitatory cells by immunocytochemical studies of connexins. Second, we analyzed physiological properties of electrical synapses between these cells under dual whole-cell patch clamp recordings. We then investigated relationship between electrical synapses and chemical output synapses of these visual cells. Connexin-36 electrical synapses occur between these cells, where depolarizing responses in the cells increased through cells' electrical synapses. Synchronous injection of subthreshold currents (pulses) in two electrically-coupled cells increased (the likelihood of $Na^+$) neural spiking. These electrically-coupled visual cells generated synchronous neural spiking within 4.4msec under patch clamp recordings. These results suggest that visual cells' chemical excitatory synaptic outputs onto postsynaptic cells appear to increase together with synchronous neural spiking through electrical synapses between these gap-junctionally connected visual excitatory cells. (COI:No)

## 2P-059

### Effects on compound action potentials at dorsal root after the injection of QX314 / Flagellin (Q/F) solution to the sciatic nerve in rats

Yoshiyuki Tsuboi (*Dept Physiol, Nihon Univ Sch of Dent, Japan*)

QX-314 is a derivative of lidocaine and block the conduction of C-fibers by binding Na+ channel from the inside after entrance to the inside of nerve fiber through the TRPV1 channel following the capsaicin application. Flagellin is a ligand of the Toll-like receptor 5. Recently, it has been reported that Q/F solution selectively blocked A$\beta$ fibers in mice (Nature 2015). We also found that thresholds for mechanical stimulation to foot were increased in rats with sciatic nerve injury (SNI: Spared Nerve Injury) at 40 min to 3 h after Q/F solution injection to foot skin. Aim of this study is to clarify reduced inputs to dorsal spinal cord qualitatively and quantitatively after injection of Q/F solution to the sciatic nerve trunk. Compound action potentials were recorded with monopolar lead method from the dorsal roots of lumber 4-6 following the electrical stimulation of sciatic nerve at the level of the distal of the mid-thigh under gas anesthesia (2 % isoflurane with 2 l/min $O_2$). We investigated effects of Q+F solution on conductions of Group I to III after an injection of Q+F solution to sciatic nerve trunk at the level of hips. At first, the application of Q+F solution effectively blocked Group II fibers in dose and infusion-velocity dependent manner. But the solution also finally blocked other types of fiber and response did not recover over 3 h. These results suggest that Q+F solution may be required any contrivance for the application. (COI:No)

## 2P-060

### Investigation of the antipruritic mechanism of the kappa opioid receptor agonist in the murine spinal dorsal horn

Kotaro Honda[1], Mitsutoshi Tominaga[1], Fumiya Kusube[1, 2], Fumiyuki Yamakura[3], Hisashi Naito[4], Kenji Takamori[1, 5] ([1]*Inst Env Gend Specif Med, Juntendo Univ, Japan,* [2]*Dep Bio Sci Tec, Fac Ind Sci Tec, Tokyo Univ Sci, Japan,* [3]*Fac Int Lib Art, Juntendo Univ, Japan,* [4]*Inst Health Sports Sci Med, Juntendo Univ, Japan,* [5]*Dep Derm, Juntendo Univ Urayasu Hosp, Japan*)

The kappa opioid receptor (KOR) antagonist is used as a drug for intractable itch, which is resistant to histamine receptor antagonists. However, little is known about specific sites important to the antipruritic effects of the agonist. Because intractable itch is presumed to be abnormal in the neuronal pathway of spinal cord, our research aims to identify the action points of the KOR agonist in the spinal itch pathway.

To identify the anti-pruritic action of the agonist of kappa opioid receptor, in vivo electrophysiology, behavioral tests, and high-sensitive in situ hybridization (ISH) were conducted in murine spinal dorsal horn, using C57BL/6J mice.

Behavioral tests showed that intrathecal injection of the KOR agonist reduced but not eliminated gastrin-releasing peptide (GRP)-evoked scratching behaviors. In vivo electrophysiological recordings revealed that administration of the KOR agonist suppressed chloroquine-responsive dorsal horn neurons in 15.8% (3/19) of mice. Only one of three-suppressed neuron responded to GRP administration in the spinal cord. ISH in three sections of the spinal cord showed that 24.6% (154/777) were double positive for GRP and KOR, and 13.6% (68/567) for GRP receptor (GRPR) and KOR in total KOR+ cells. Most KOR+ cells were negative for GRP and GRPR. Intrathecal injection of dynorphin-saporin did not decrease scratching bouts caused by GRP. Unexpectedly, it tended to increased scratching bouts by the administration of GRP alone and the combination of GRP and the KOR agonist.

In conclusion, the KOR agonist targets both GRP+ KOR+ and GRPR+ KOR + cells which are present in a 2:1 ratio, in the spinal dorsal horn. These findings suggest that GRP+ KOR- and GRPR+ KOR- cells may function as interneurons in the spinal dorsal horn. (COI:No)

## 2P-061

### Contribution of bicarbonate permeability to the reversal potential of GABA responses in bipolar cells of the mouse retina

Chengzhu Yin, Toshiyuki Ishii, Makoto Kaneda (*Dept. Physiol, Nippon Medical School, Tokyo, Japan*)

In the retina, the different polarity of surround responses between ON- and OFF-bipolar cells is thought to be mediated by the differences of the reversal potentials of GABA responses. In retinal bipolar cells, GABA responses are mediated by the $GABA_A$ and $GABA_C$ receptors. In the present study, we examined whether the difference of the bicarbonate ion ($HCO3^-$) permeability to GABA receptors can generate the difference of reversal potentials between the $GABA_A$ and $GABA_C$ receptors using patch clamp technique. We also examined whether the anion permeability of $GABA_A$ and $GABA_C$ receptors reported in heterologous expression system can be true in vivo. The reversal potentials of $GABA_A$ and $GABA_C$ receptors without extracellular $HCO3^-$ did not show significant difference (-4.9 ± 2.4 mV (n = 19) for $GABA_A$ and -5.1 ± 3.6 mV for $GABA_C$ (n = 18)). The reversal potentials of both $GABA_A$ and $GABA_C$ receptors with extracellular $HCO3^-$ (24 mM) did not differ significantly but slightly shifted to the positive side (-2.4 ± 2.6 mV (n = 19) for $GABA_A$ and -0.6 ± 4.1 mV (n = 18) for $GABA_C$). The permeability of anions such as $I^-$, $NO_3^-$, $HPO_4^-$, $CH3COO^-$ to $GABA_A$ and $GABA_C$ receptors of bipolar cells were similar to the permeability reported the previous study in vitro. These results rule out the possibility that the expression level of GABA receptor subtypes with different $HCO3^-$ permeability contributes to form the differences of the reversal potentials of GABA responses between ON- and OFF-bipolar cells, although the permeability of $HCO3^-$ can affect the reversal potential of GABA responses. Our results also show that anion permeability of $GABA_A$ and $GABA_C$ receptors reported in vitro study can be applicable to assess the ionic mechanisms of $GABA_A$ and $GABA_C$ receptors in vivo. (COI:No)

## 2P-062

### Analysis of the peripheral mechanism of muscular mechanical hyperalgesia induced by repeated cold stress in rats

Teruaki Nasu[1], Yoshiko Uchimura[1], Asako Kubo[2], Koutaro Yamada[3], Kazue Mizumura[1,2] ([1]*Coll. Life Health Sci., Chubu Univ, Kasugai, Japan,* [2]*Dept Physiol. Nihon Univ, School of Dentistry,* [3]*ZERIA Pharmaceutical Co. Ltd*)

There are many patients suffering from chronic musculoskeletal pain, however, its mechanism is not well understood so far. Previously we reported that rats exposed to repeated cold stress (RCS) showed long lasting muscular mechanical hyperalgesia (Nasu et al, 2010). Its spinal mechanism has been partly clarified (Nasu et al, 2019), and we also showed RCS changed mechanosensitivity of muscular nociceptors (Wakatsuki et al., 2015). Yet, it is not known what sensitizes peripheral nociceptors in RCS. Purpose of the present study is to clarify factors that sensitize muscular nociceptors.

We examined expression of mRNAs of mediators (NGF, GDNF, family, BDNF, NT-3, IL-1$\beta$, IL - 6, TNF-$\alpha$)by RT- PCR in extensor digitorum longus muscle of the control rats and rats underwent RCS. We also measured pH in tibialis anterior (TA) muscle by a needle type pH electrode under 1.5% isoflurane anesthesia. Consequently, we pharmacologically examined whether vesicular ATPase (V-ATPase) contributes to muscular pH change in RCS, and whether muscular pH change contributes to muscular mechanical hyperalgesia.

RT-PCR revealed that expression of mRNA of any mediators examined were not changed by RCS. We found RCS significantly lowered muscular pH. Subcutaneous injection of bafilomysin (25$\mu$g/Kg), a V-ATPase inhibitor, and APETX2, an ASIC3 antagonist, reversed muscular mechanical hyperalgesia. These results suggest that muscular acidification contributes to persistent muscular mechanical hyperalgesia by RCS.

As we previously reported that chondroitin sulfate (CS) decreased acid-induced facilitation of mechanosensitive currents in dorsal root ganglion neurons (Kubo et al., 2013) and mechanical response of single muscular thin-fibers (Hotta et al, in press), we examined whether CS reversed mechanical hyperalgesia after RCS. As expected CS attenuated the mechanical muscular hyperalgesia after RCS dose dependently. Collectively, these results suggest that RCS induces acidification of the muscles and this acidification sensitizes muscular nociceptors, resulting in mechanical hyperalgesia. (COI:No)

## 2P-063

### The response to whisker stimulation in visual associated area of monocular deprived mice *in vivo*

Akari Hashimoto[1], Akiko Miyamoto[2], Koichiro Haruwaka[1], Daisuke Kato[1], Yoshihisa Tachibana[1], Hiroaki Wake[1] ([1]*Div System Neuroscience, Grad Sch Med, Kobe Univ, Japan,* [2]*Systems Neuroscience Group, AIST, Japan*)

Information processing of sensory inputs in higher-order cortex are crucial to detect the external environment. Blind subjects compensate the disadvantage on it by recruiting and processing the other remaining senses in the visual associated area, which phenomenon is known as "cross-modal plasticity". The previous studies using positron emission tomography (PET) showed that activity of visual cortex occurred in the blind people when reading Braille. Whisker-dependent activation of visual cortex was demonstrated in the eye enucleated mice. However, the functional changes and its mechanism *in vivo* have not been shown yet. In this research, we try to unravel the effect of early monocular deprivation (MD) on the activation of the visual associated area with whisker stimulation. We first visualized the axonal projection from S1 (primary somatosensory cortex) to V2 (extrastriate cortex) by injecting adeno-associated virus coded eGFP with synapsin promotor (AAV1-syn-eGFP). Retrograde labelling of S1-V2 axons by Cholera Toxin Subunit B (Recombinant) Alexa Fluor™ 488 Conjugate also showed the existence of the projection. Then we hypothesized the activation triggered by whisker stimulation in S1 can be transmitted and processed in V2 as well. To verify our hypothesis, we combined *in vivo* two-photon imaging with the injection of AAV coding $Ca^{2+}$ indicators (GCaMP6s/GCaMP6f) and visualized the activity of S1-V2 axons and neurons in V2 during whisker stimulation. We finally assumed that microglia are a key player for the synaptic transferring from V1-V2 connection to S1-V2 axon. To verify the microglial contribution, we assessed morphology and gene expression of microglia, which might be a driving force to modify the neuronal circuit with MD. This study will be an important clue to understand the compensating ability of the cortex for the future therapeutic target. (COI:No)

## 2P-064

### The decreases in taste recognition thresholds in humans by addition of low concentration of salts

Ryusuke Yoshida[1], Keisuke Sanematsu[2], Shingo Takai[2], Shusuke Iwata[2], Noriatsu Shigemura[2] ([1]*Dept Oral Physiol, Grad Sch Med, Dent and Pharma, Okayama Univ, Japan,* [2]*Sect of Oral Neurosci, Grad Sch of Dental Sci, Kyushu Univ, Japan*)

It is well known that sweet taste is enhanced by addition of NaCl, however physiological/molecular mechanisms for sweet enhancement by addition of NaCl is not elucidated. Sweet receptor forms heterodimeric complex of T1R2 and T1R3. Therefore, sweet enhancement by salt may be caused by modulation of T1R2/T1R3 activity by salt. To test this possibility, the effects of addition of salt to tastants on taste recognition thresholds in humans were analyzed. Sweet recognition thresholds for sucrose, glucose and aspartame were significantly lowered by addition of 3 mM NaCl, which was almost tasteless to participants. In contrast, recognition thresholds for bitter (quinine), sour (HCl) and umami (monosodium glutamate + inosine-5'-monophosphate) were not affected by addition of 3 mM NaCl. Other salts such as N-methyl-D-gluconate chloride (NMDG-Cl), KCl and $NaHCO_3$ also lowered sweet recognition threshold for sucrose and glucose but not for aspartame. These results suggest that the addition of salt selectively enhances sweet taste sensitivity in humans and that there are multiple sweet receptor systems in humans. (COI:No)

## 2P-065

### Effect of sleep deprivation on sleep homeostasis in secretin receptor knockout mouse

Aiko Moridera (*Dept Ergo, Grad Sch Med, UOEH, Kitakyushu, Japan*)

Secretin is a peptide hormone released from the duodenum to stimulate the secretion of digestive juice by the pancreas. Secretin also functions as a neuropeptide hormone in the brain. Recently, it is reported that secretin neurotransmission might be related to the pathophysiology of autism. As sleep disturbance is one of the symptoms of autism, we hypothesed that the secretin neutorransmission might play a role in the sleep control. We used secretin receptor knockout (SctR KO) and wild type mice to compare baseline sleep profile and homeostatic response to the sleep deprivation (SD). The mice were surgically implanted a radio transmitter into the peritoneal cavity for measuring ambulatory activity and body temperature, and the electrodes for measuring electroencephalogram and electromyogram under the sevoflurane anesthesia. All animals were put in the home cage for at least one week for recovery and then transferred to the recording chamber. After a 24 hours of baseline recording, SD was performed for four hours from the light onset and rebound responses were recorded for another 24 h. The SctR KO mice showed a tendency to need more time to recover from the SD compared to control group. This result suggests that secretin neurotransmission might have a role in the sleep control especially with sleep homeostasis. (COI:No)

## 2P-066

### Massage-like stroking stimulation increases call rate of 50-kHz ultrasonic vocalizations with various call subtypes in young adult rats

Rie Shimoju[1], Miyo Hori[2], Hideshi Shibata[3], Mieko Kurosawa[1,4] ([1]*Center Med Sci, Int Univ Health & Welfare, Otawara, Japan,* [2]*Fund Advancement of Intl Sci, Tsukuba, Japan,* [3]*Lab Vet Anat Ins Agric, Tokyo Univ Agric & Tech, Tokyo, Japan,* [4]*Dept Pharm Sci, Intl Univ Health & Welfare, Otawara, Japan*)

Rats emit 50-kHz ultrasonic vocalizations (USVs) associated with reward. We have previously shown that massage-like stroking stimulation of the abdomen under vertical holding condition vigorously induced 50-kHz USVs. 50-kHz USVs were categorized according to 14-subtype scheme (Wright et al., 2010), and some of the subtypes are thought to be associated with specific emotional state and emotion-related behaviors. This study sought to classify the subtypes induced by stroking stimulation of the abdomen. In addition, the effects of stroking of other cutaneous areas (back or head) on 50-kHz USVs were examined. The experimenter held tightly the rat's upper back skin with the left hand and stroked gently the skin (abdomen, back or head) with the right hand. USVs were recorded during stroking stimulation. Massage-like stroking stimulation of the back or head significantly increased 50-kHz USVs as stimulation of the abdomen did. The total call numbers of 50-kHz USVs evoked by stroking of the abdomen, back or head during stimulation were not different. On the other hand, stroking stimulation altered 50-kHz call subtype profile in a stimulus site dependent manner. In abdomen-stroked group, "step down" and "composite" calls were more, while "flat" calls were less than back or head-stroked group. Furthermore, we found a new subtype which has not been shown in the 14-scheme by Wright and coworkers. The new subtype was monotonous call in harmonics and we defined it as "harmonic flat". The "harmonic flat" was well observed in response to stroking stimulation of all the skin areas tested. These results demonstrate that stroking stimulation of various skin areas generally evoked 50-kHz USVs; however, emotional state induced by stroking stimulation may be partly different depending on the stimulus site. (COI:No)

## 2P-067

### Constant light exposure leads to the down-regulation of clock genes expression with circadian clock disorganization in pregnant beef cows

Tsuyoshi Otsuka[1], Hiroki Mitsuishi[2], Hiroki Onishi[1], Masato Yayota[2,3] (*[1]Lab. Anim. Pro. Mgmt., Fac. Appl. Biol. Sci., Gifu Univ., Japan, [2]Lab. Anim. feed. Nut., Fac. Appl. Biol. Sci., Gifu Univ., Japan, [3]GeFAH., Gifu Univ., Japan*)

Circadian rhythm is generated by transcriptional translational feedback loop system of clock genes, which are regulated by photoperiod. In mammals, variation in circadian rhythm has an affect on physiological function such as behavior, metabolism, and reproduction. Therefore, understanding the circadian rhythms of clock genes contribute to improvement of production and health in animals. It is well known that hair follicle cells are noninvasive, convenient, and useful biopsy sample for detecting the expression pattern of circadian clock in human. However, whether the expression rhythms of clock genes are able to measure in cows using hair follicle cells remains unclear. Here we demonstrate the expression rhythms of several clock genes (*Bmal1*, *Per1*, and *Cry1*) were shifted by difference of day length and constant light exposure caused a loss of circadian clock in hair follicle cells of non-pregnant beef cows. Interestingly, the expression levels of several clock genes in pregnant beef cows fluctuate by difference of day length. Moreover, pregnant beef cows show the reduction of most clock genes expression levels with disrupted of circadian rhythms by constant light exposure. These findings suggest that it is possible to detect the circadian clock in beef cows using hair follicle cells, and light control may provide a critical impact on circadian rhythm of pregnant beef cows. (COI:No)

## 2P-068

### Spontaneous neuronal activity in the bed nucleus of the stria terminalis across sleep-waking cycles in rats

Kazumi Takahashi, Satoshi Eifuku (*Dept Systems neurosci, Med, Fukushima Med Univ, Japan*)

The bed nucleus of the stria terminalis (BNST), receiving input mainly from the amygdala, is involved in various physiological and behavioral responses to stress, fear and anxiety. It has been also reported that BNST neurons play important roles in the generation of wakefulness through their multiple projections to waking centers in the hypothalamus and brainstem, including the locus coeruleus, the parabrachial nuclei and others. However, the characteristics of the spontaneous activity of BNST neurons across sleep-waking cycles have not yet been fully elucidated. In the present study, we recorded state-dependent changes of neuronal activity in the posterior part of the BNST (BNSTp) through a wire-bundled tetrode along with cortical electroencephalogram and neck muscle activity in freely moving rats. Most neurons recorded in the BNSTp showed tonic and frequent discharge during both wakefulness and paradoxical sleep. During slow-wave sleep, these neurons showed phasic and clustered discharge, which was highly synchronized among the neurons recorded simultaneously, and the timing of the discharge clustering showed a correlation with the phase of cortical slow-wave rhythmicity. These results suggest that a homogeneous neuronal population in the BNSTp may exert strong influences on systems that generate cortical slow-wave activity during slow-wave sleep, in addition to the pathway of cortical activation during wakefulness and paradoxical sleep. (COI:No)

## 2P-069

### The role of PPAR-alpha and ketone bodies in the regulation of arousal during food deprivation

Sachiko Chikahisa, Yoshitsugu Kondo, Tetsuya Shiuchi, Noriyuki Shimizu, Daisuke Tanioka, Hiroyoshi Sei (*Dept Ingeg Physiol, Biomed Sci, Tokushima Univ Grad Sch, Japan*)

Peroxisome proliferator-activated receptor alpha (PPAR $\alpha$) is a ligand-activated transcription factor involved in the regulation of fatty acid metabolism. PPAR $\alpha$ is known to regulate the synthesis of ketone bodies via 3-hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2). Ketone bodies such as acetoacetic acid and $\beta$-hydroxybutyric acid are produced from fatty acids in hepatocytes and brain astrocytes and become the main fuel in many tissues during starvation. We recently found that PPAR $\alpha$ and ketone bodies are involved in the regulation of sleep homeostasis. In this study, we investigated how sleep-wake changes during food deprivation in PPAR $\alpha$ knockout (KO) mice. We evaluated sleep, locomotor activities, body temperature, arterial pressure and heart rate, under ad lib-fed conditions and 24-hour food deprivation. Under ad lib-fed condition, KO mice showed enhanced sleepiness. Under food deprivation, the amount of wakefulness, locomotor activities, arterial pressure and heart rate were markedly decreased in KO mice, compared with that of WT mice. In addition, a lower concentration of plasma ketone bodies and decreased mRNA expression of Hmgcs2 in the liver and brain were observed in KO mice under food deprivation. These results suggest that PPAR $\alpha$ and ketone bodies play an important role in the maintenance of wakefulness during food deprivation in mice. (COI:No)

## 2P-070

### Fibroblast growth factor 5 (FGF5) plays a critical role in central regulation of mouse behavior

Kazuto Matsuda[1], Kai Kimura[1], Kazuaki Nakamura[2], Tsuyoshi Hamada[3,4], Junko Imaki[4], Yasuhiko Kondo[1] (*[1]Dept Ani Sci, Teikyo Univ Sci, Yamanashi, [2]Nat Cent Child Health Develop, Tokyo, [3]Fac Health Med Sport, Teikyo Heisei Univ, Chiba, [4]Dep Develop Anat, Nat Def Med Coll, Saitama*)

Fibroblast Growth factor 5 (FGF5) broadly expresses in the central nervous system. However, our knowledge of the function is limited except for the blood brain barrier, and little has been known on behavioral regulation. In this study, we first demonstrated behavioral characteristic of FGF5 null mutant male mice, compared to that of wild-type (WT) males. When exposing to odors of sexually active males and estrous females, although both WT and mutant males preferred female odor to male odors, mutant males showed significantly shorter investigation for either odors than WT males. In mating tests, mutant males showed lower activities and less ejaculation. Furthermore, performance in Morris water maze was deteriorated in mutant males compared to WT males. Since we found severe dysfunction in a variety of behavior in mutant mice, we reexamined FGF5 mRNA expression in the forebrain, indicating that FGF5 gene expresses broadly such as in the olfactory bulb (OB), the amygdala, the hippocampus, and the hypothalamus. Immunohistochemistry (IHC) and in situ hybridization (ISH) studies detected the signals in common in the hippocampus, the cerebral cortices, and the thalamic reticular nucleus. However, the signals were generally weak and able to confirm the PCR results only partly. Furthermore, the inconsistency was found between IHC and ISH. Intense signals in the granular layer of CA2, CA3 and dentate gyrus, but weak in CA1, of the hippocampus were observed by ISH, while immunoreactive patchy particle (not cellular form) were found in the hippocampus including CA1 by IHC. ISH detected weak signals in the OB, but IHC failed. The current study demonstrates that FGF5 in the central nervous system is involved in neural regulation of various behaviors, suggesting that FGF5 deficiency can be a good model for some psychiatric disease in human. (COI:No)

## 2P-071

### Sexual Activity Correlates Olfactory Sensitivity in Male Rats

Yui Shimomi, Yasuhiko Kondo (*Dept Ani Sci, Teikyo Univ Sci*)

On screening the sexual activity of male rats, researchers became aware of the existence, among naïve rats, of a subpopulation with little or no sexual behavior, even after several mating sessions. The present study investigated whether such sexually sluggish (SS) males show behavioral differences from normal copulatory (NC) males, other than sexual behavior. Sexually naïve Long-Evans male rats were subjected to weekly olfactory preference tests, which measured the time spent nose-poking sexually active males and sexually receptive females for odor exploration in a three-chamber apparatus, each followed by copulation tests with receptive females for 3 weeks. Depending on their sexual behavior performance, the rats were assigned to NC males who showed ejaculation in these copulation tests and SS males who showed very low sexual activity resulting in no ejaculation. The preference tests indicate that although both NC and SS males significantly prefer receptive female odor to male odor, SS males spent significantly less time nose-poking to investigate receptive females than NC males did. Thereafter, the food finding test was performed after overnight fasting. In the test, all the NC males found the buried pellet within 5 min, whereas over 60% of the SS males failed to find it. Furthermore, the males were tested for finding a buried bag containing soiled bedding collected from estrous female cages. The bag was found by 80% of NC males, but only by 20% of SS males. Our current results suggest that the difference between SS and NC male rats is not only in sexual behavior but also extends to other functions such as olfaction to detect foods. (COI:No)

## 2P-072

### How do mice balance the trade-off between exploration and exploitation?

Kuniaki Satori[1], Yu Takarada[1], Masashi Arake[3], Yuji Morimoto[3], Toshiaki Ishizuka[2], Hiroyuki Ohta[2], Tatsuji Takahashi[1] (*[1]Dept Engineering Science, Tokyo Denki Univ, Japan, [2]Dept Pharmacology, NDMC, Japan, [3]Dept Physiology, NDMC, Japan*)

How should an animal choose between exploiting a well-known resource or exploring new options? This problem is widely known as the exploration-exploitation trade-off.
Reinforcement learning models play an important role in understanding the behavior and neural mechanisms of choice action in animals and humans, especially as for the adaptive balancing of exploration and exploitation. Previous studies have shown that in the two- or three-armed bandit problems, the action choice of rats are similar to a reinforcement learning model, the forgetting Q-learning algorithm operated under the softmax action selection policy (Ito and Doya 2009; Cinotti 2019). Our experiments have shown that the action choice of mice shows less exploration when the reward probabilities are high overall.
Thus we suspected that there is a factor that contributes to balancing between exploration and exploitation that is sensitive to the reward probabilities.
Therefore, we proposed models of value-update and policy.
Specifically, a model in which the value-update and the policy parameters are scaled using the estimated mice weight, the food intake within the last 12 hours, and the Q-value.
We also applied satisficing as a policy, which explores until an aspiration level is met, and then the greedy action selection is performed.
As a result of comparing 24 models, a model in which the learning rate of the Q-value is inversely proportional to the magnitude of the Q-value itself best explained the mice behavior. The result indicates that mice put great value on unexpected rewards and preserve highly expected choice values. (COI:No)

## 2P-073

### In vivo analysis of circadian rhythm of the suprachiasmatic nucleus in AVP neuron-specific vesicular GABA transporter knock-out mice

Yusuke Tsuno[1], Takashi Maejima[1], Emi Hasegawa[2], Michihiro Mieda[1]([1]*Dept Physiol, Grad Sch Med, Kanazawa Univ, Japan,* [2]*IIIS, U of Tsukuba*)

The suprachiasmatic nucleus (SCN) is the circadian rhythm center of the mammals. Individual SCN neurons have their intrinsic daily rhythms controlled by transcriptional-translational feedback loops. However, how these individual circadian rhythms can synchronize is largely unknown. GABAergic transmission may be a good candidate, but its contribution remains controversial.

To elucidate the functional meaning of GABAergic transmission within the SCN, we used conditional knock-out mice in which vesicular GABA transporter (VGAT) was specifically deleted in AVP neurons (*AVP-Vgat*$^{-/-}$). We found that *AVP-Vgat*$^{-/-}$ mutant mice showed lengthening and a splitting pattern of the activity time in behavioral circadian rhythm. By recording miniature GABA-mediated postsynaptic potential (mGPSCs), the frequency of small amplitude mGPSCs was decreased in both AVP and non-AVP neurons during the light period, suggesting the decrease of GABA release from AVP neurons. To examine the clock gene expression in the SCN, we made an acute slice to observe the daily rhythm of the clock gene, Per2, expression by using Per2:: Luc mice. We found the peak phase of the clock gene expression was delayed relative to the onset of activity period of behavior in *AVP-Vgat*$^{-/-}$mutant mice, indicating the phase relationship change between SCN molecular clocks and the locomotor activity.

To examine the rhythmicity of the activity of AVP neurons in vivo, we used fiber photometry to visualize calcium rhythm with a calcium indicator (jGCaMP7s) at the right SCN in AVP-Cre (control) and *AVP-Vgat*$^{-/-}$ mutant mice. We found a possible change of phase relationship between circadian calcium rhythm and locomotor activity in *AVP-Vgat*$^{-/-}$ animals. These results suggest that GABAergic transmission of AVP neurons may regulate the phase relationship among the SCN molecular clock, SCN neuronal activity rhythm, and locomotor activity rhythm, and thereby control the timing of SCN output to behavior. (COI:No)

## 2P-074

### Effects of reversible manipulation of estrogen receptor α expression in the ventromedial nucleus of the hypothalamus on aggressive behavior in male mice

Tetsu Hatsukano, Kazuhiro Sano, Sonoko Ogawa(*Faculty of Human Science, University of Tsukuba, Japan*)

Testosterone (T) facilitates male aggressive behavior by acting on estrogen receptor α (ERα) after being aromatized to estradiol in the brain. Using virally mediated RNAi methods for site-specific ERα knockdown, we have found that ERα in the ventromedial nucleus of the hypothalamus (VMH) at the time of behavioral testing in adult is necessary for "activational action" of T to induce aggression (Sano et al., Eur J Neurosci, 2013). However, it is not known whether expression of ERα in the VMN is involved in possible "organizational action" of T during pubertal period. To test this, it is necessary to be able to knockdown ERα expression during pubertal period but not at the time of testing in adult. In the present study, we developed a new method to achieve reversible manipulation of ERα expression by inducing gene knockdown with the tet-on system. We first injected inducible ERα knockdown vectors in adult mice and confirmed that ERα expression in the VMH was suppressed under doxycycline (DOX)+ condition and recovered by terminating DOX treatment. We then knocked down ERα in the VMH during only pubertal period by injecting the inducible ERα knockdown vectors on postnatal day (PND) 21 and giving DOX containing food between PND 21 to 56. When the mice were tested on PND 84 under DOX- condition, they showed equivalent levels of aggressive behavior as mice treated with a control virus during pubertal period. On the other hand, consistent with our previous findings, levels of aggressive behavior in mice injected with the inducible ERα knockdown vectors on PND 70 were reduced under DOX+ condition but restored afterward in DOX- condition. These results suggest that ERα in the VMH is required for the activational action of T in adulthood but not involved in the organization action during pubertal period for male aggression. (COI:No)

## 2P-075

### Memory impairment and changes in brain gene expression on heat-exposed mice

Ryota Kato[1], Kenjiro Sato[2], Ryosuke Hitomi[3], Kazuki Toyoda[3], Nobuhiko Kojima[1,2]
([1]*Grad Sch of Life Sciences, Toyo Univ, Japan,* [2]*Res Ctr for Biomed Eng, Toyo Univ,* [3]*Sch of Life Sciences, Toyo Univ, Japan*)

Global warming increases the risk to health damage including brain dysfunction. It is reported that the serious heat stroke patient shows a symptom of the higher brain dysfunction. However, the details of how heat stress affects cognitive functions are still unknown. In this study we focused on memory function among cognitive functions, and aimed to elucidate the molecular basis underlying heat-induced memory impairment.

To evaluate the memory function of heat-exposed mice, we conducted the novel object recognition test. The heat exposed (1 hour at 45℃) immediately after the object presentation did not affect the retention of the memory tested 24 hours after the heat exposure. By contrast, the second day's test showed a significant memory impairment in the heat-exposed mice. However, the third day's test, the heat-exposed mice showed normal retention of the previous day's memory. Thus, memory impairment was only detected first day after heat exposure, suggesting that heat-induced memory impairment seems to be transient.

To understand the molecular mechanism that caused this transient memory impairment, we examined the effect of heat exposure on the expression of genes in the brain. PCR array analysis showed that heat exposure caused changes in expression of various synaptic plasticity-related genes in the brain. Thus, it is considered that the increased expression of these genes by heat stress causes functional and structural changes in synapses and remodeling and reconstruction of neural circuits.

We also found that the changes in expression of apoptosis-related genes and the inflammatory marker were observed 24 hours after heat exposure. Such pathological events may impact on subsequent memory acquisition.

These molecular changes after heat stress may be involved in not only transient impairment of memory, but also recovery of memory processes. Our studies may contribute to better understanding of molecular basis for heat-induced cognitive deficit. (COI:No)

## 2P-076

### Is drebrin required for metabotropic glutamate receptor-mediated changes in anxiety-related behavior and spine morphology?

Mai Sawabe[1], Yohei Yamashita[1], kenji Hanamura[2], Hiroyuki Yamazaki[2], Tomoaki Shirao[2], Nobuhiko Kojima[1]([1]*Dept Life Science, Grad Sch of Life Science, Toyo univ, Japan,* [2]*Dept of Neurobio and Behav, Grad Sch of Med, Gunma Univ, Japan*)

Dendritic spine is dynamic structure and its morphology is related not only to synaptic plasticity of individual neurons, but also to higher functions of the brain. Actin cytoskeleton supports the structure of spines and regulates dendritic spine remodeling in response to neuronal activity. Recent lines of evidence indicate that the group I metabotropic glutamate receptor (mGluR) is involved in structural plasticity of dendritic spines. It is known that mGluR activation triggers long-term depression in synapses and dysregulation of mGluR signaling causes aberrant spine morphology. These findings suggest that mGluR activity may link to actin cytoskeleton. However, the intracellular molecular cascade that relays mGluR activity to actin cytoskeletal dynamics is largely unknown. We have demonstrated that the actin-binding protein drebrin plays a critical role in spine morphogenesis and plasticity. Drebrin possesses a binding motif of Homer that is known as a scaffold protein of mGluR. So, we make the hypothesis that drebrin mediates the interplay between mGluR and F-actin through the Homer-binding. In this study, we examined the effects of the lack of drebrin on animal behavior, spine morphology and protein interaction in dendritic spine using drebrin knockout (DXKO) mice. We found that these KO mice showed increased anxiety in the open field test. Histological investigation with Golgi-Cox staining revealed that a significant decrease in spine density in hippocampal CA1 regions of DXKO mice. Among the types of spines, stubby, thin and mushroom-type spines were decreased, and filopodia-type spines were conversely increased. These observations suggest that the lack of drebrin affect the maturation of dendritic spines. We have now examined whether the lack of drebrin cancels out mGluR-mediated changes in animal behavior and spine morphology. This study will uncover the molecular basis for the roles of mGluR on anxiety-related disorders and structural plasticity. (COI:No)

## 2P-077

### The motor recovery and synaptic plasticity was affected by the exercise in the hemorrhage model rat

Chihiro Sato[1], Kunikazu Tanji[2], Mana Kishimoto[1], Shun Mori[1], Shuhei Koeda[1], Misaki Mikami[1], Junko Yamada[1]([1]*Dept of Occcupi Ther, Grad Sch Health Sci, Hirosaki Univ, Japan,* [2]*Dept of Neuropathology, Grad Sch Med, Hirosaki Univ, Japan*)

Previously, we reported that the exercise promotes the motor recovery and the recovery of voluntary exercise (V-Ex) was earlier than forced exercise (F-Ex) in the intracerebral hemorrhage (ICH) rats. However, the factors to promote the motor recovery were unknown. The aim of this study is to clarify the mechanisms of recovery by the rehabilitation. We focused on the early phase when there was significantly difference between the trained groups.

To clarify the synaptic function of the injury side, DiI was injected into the right striatum. Whole cell patch clamp recordings were performed from DiI labeled cells in the layer V in motor cortex (M1) using voltage -clamp mode (V hold = -70 mV). The amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) were recorded in the presence of TTX at 32 ℃. The amplitude and frequency of mEPSCs were increased in both V-Ex and F-Ex groups than the non-exercise group.

To assess the difference between the V-Ex and F-Ex group, the motivation and stress levels with exercise were compared among three groups. The immunoreactivity of ΔFosB in nucleus accumbens was used as a marker of activated neuron on the reword system, and corticosterone concentrations were analyzed to assess the stress level. The expression of ΔFosB was increased in the V-Ex group than the N-Ex group. The plasma corticosterone was higher in the F-Ex group than the other groups.

These data suggested that the motor recovery was due to the synaptic plasticity in M1 that project to striatum, and the synaptic functions might be affected by the motivation or stress.

It is known that the KCC2 level is decreased after brain injury in mature neuron. There might be alterations in KCC2 expression in brain on the recovery. We need to determine the effect of the KCC2 for the neural recovery. (COI:No)

## 2P-078

### Possible relationship between estrogen-dependent hypothalamic oxytocin synthesis and body fat accumulation in female rats

Kazuaki Nishimura[1,2], Kazuhiko Baba[1], Kenya Sanada[1], Haruki Nishimura[1], Kentaro Tanaka[1], Satomi Sonoda[1], Mitsuhiro Yoshimura[1,3], Takashi Maruyama[1], Kiyoshi Yoshino[2], Yoichi Ueta[1]([1]*Dept Physiol, UOEH, Japan,* [2]*Dept ObGyn, UOEH, Japan,* [3]*Dept Translational Health Science, Sch Med, Univ Bristol, UK*)

Oxytocin (OXT) is produced in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus and is secreted into the systemic circulation from the posterior pituitary gland (PP). Although OXT regulates labor and lactation, OXT is also related to feeding as an anti-obesity peptide. However, the relationships between estrogen, hypothalamic OXT synthesis and body weight, in particular fat mass are unclear in women. Our aim in the present study was to evaluate whether estrogen regulates hypothalamic OXT synthesis and body fat accumulation in female rats that express the OXT-monomeric red fluorescent protein 1 (mRFP1) fusion gene.

The synthesis of OXT in the hypothalamus was evaluated by the intensity of the mRFP1 fluorescence in the SON, PVN and PP. The distribution of body fat accumulation was measured by microcomputed tomography (micro-CT). The fluorescence intensity of OXT-mRFP1 in the SON, PVN and PP were most strongly observed during the estrus stage of female rats and decreased significantly in ovarectomized (OVX) rats. The subcutaneous fat mass was significantly increased in OVX rats in comparison with sham-operated rats. Estrogen replacement in OVX rats caused significant increases of the fluorescent intensities in the SON, PVN and PP in a dose-dependent manner. Depending on the dose of estrogen, subcutaneous fat mass was reduced at low doses of estrogen replacement and visceral fat mass as well as subcutaneous fat mass was reduced at high doses of estrogen replacement. These data suggest that hypothalamic OXT synthesis and accumulation of body fat mass may be regulated by estrogen level. (COI:No)

## 2P-079

### Cardiovascular response evoked by social defeat stress and projections to the rostroventral medulla from the midbrain in rats

Mio Matsuyama[1], Ena Yamamoto[1], Yuliang Hu[2], Jouji Horiuchi[1,2] (*[1]Department of Biomedical Engineering, Toyo University, Japan, [2]Dept Biomedical Engineering, Toyo Univ, Japan*)

Acute psychological stressor evokes typical cardiovascular responses, such as increases in blood pressure (BP) and heart rate (HR) accompanied by a physical reaction. It has been shown that the sympathetic vasomotor pathway of acute psychological stress is mediated via neurons in the rostroventral medulla (RVM) indirectly from the dorsomedial hypothalamus which is a possible autonomic center for the stress response. However, it is still unknown detailed neural projections from the hypothalamus to the RVM. In the present study, we investigated that direct projections to the RVM and distribution of c-Fos expressed neurons during social defeat stress (SDS) in conscious rats. In order to verify neural projections to the RVM, we injected a retrograde tracer, FluoroGold (FG) into the unilateral RVM in the rat. The FG injected rat was allowed to recover for a week, and then was exposed to the SDS challenge. After the SDS exposure, neuro-excitatory marker, c-Fos and FG were stained and observed throughout the brain. We have already shown that the SDS challenge evoked pressor response and tachycardia in our previous study. The FG labeled neurons were observed from the pons to the hypothalamus. The double-labeled (c-Fos and FG) neurons were distributed in the lateral/ventrolateral periaqueductal grey matter (PAG) in the midbrain. Therefore, we microinjected GABA antagonist, Bicuculline (Bic) to excite neurons distributed in the lateral/ventrolateral PAG in some anesthetized rat preparations (urethane;1.3g/kg, iv). The Bic injection into the PAG caused profound increases in BP and renal sympathetic activity. Taken together with all our results, it is suggested that neurons in the lateral/ventrolateral PAG contribute to the pressor response evoked by acute psychological stress, like the SDS, via sympatho-excitatory vasomotor pathway. (COI:No)

## 2P-080

### Distribution of c-Fos expressed neurons and the cardiovascular reaction evoked by social defeat stress in serotonin-deficient rats

Akari Hori[1], Yuka Ichinotsuka[2], Tatsuya Kumaki[2], Ena Yamamoto[1], Mio Matsuyama[1], Jouji Horiuchi[1,2] (*[1]Department of Biomedical Engineering, Toyo University, [2]Dept Biomedical Engineering, Toyo Univ, Japan*)

Social defeat stress (SDS) that mimics stress caused by interpersonal relationship trouble, is a kind of psychological stress and causes us to have various autonomic responses such as hypertension and tachycardia. In our previous study, central activation of 5-hydroxytryptamine $_{1A}$ receptors suppressed the stress-induced cardiovascular response seen when stimulating the dorsomedial hypothalamic area (DMH) in anesthetized rats. Also, we found that the SDS increased blood pressure (BP) and heart rate (HR) and increased the neural excitability in the DMH in conscious rats. Thus, it is suggested that central serotonin system plays an important role in the stress-induced cardiovascular response, but the detailed mechanism is not clear. In this study, we investigated the effect of the SDS on the cardiovascular response and the excitability of the DMH neurons in serotonin-deficient model rat (FH/Ham rat). Experimental rats were implanted a telemetry probe to measure BP and HR during the SDS and then the rats' brains were fixed and stained a neuro-excitatory marker, c-Fos in the DMH. The initial change in BP caused by SDS in FH/Ham rats was the same as those from Wistar rats (control). However, the pressor response in FH/Ham rats soon returned to pre-SDS levels, whereas the pressor response persisted during the SDS exposure in the control group. The immediate increase in HR after the SDS in FH/Ham rats was significantly suppressed, and the tachycardia did not sustain during the SDS period. The number of c-Fos immunoreactive neurons in the DMH significantly increased in FH/Ham rats compare to the control. Therefore, the neural excitability of the DMH, which is one of stress centers, augmented in FH/Ham rats. These results suggest that there is a discrepancy between DMH neural excitability and the cardiovascular responses during SDS in serotonin-deficient rats. (COI:No)

## 2P-081

### Oxytocinergic Transmission from Paraventricular Hypothalamic Nucleus to Rostral Medullary Raphe Stimulates Brown Adipose Tissue Thermogenesis

Akihiro Fukushima, Kazuhiro Nakamura (*Dept Integrative Physiol, Nagoya Univ Grad Sch Med, Japan*)

Oxytocin (OXY), a neuropeptide synthesized in the paraventricular hypothalamic nucleus (PVH) contributes to many brain functions including maternal, social and sexual behaviors and stress responses, which all involve autonomic responses. To investigate the roles of PVH oxytocinergic neurons in the central regulation of autonomic functions, in this study, we first performed neural tract tracing in rats from oxytocinergic PVH neurons to the rostral medullary raphe region (rMR), which contains sympathetic premotor neurons controlling brown adipose tissue (BAT) thermogenesis. A recombinant adeno-associated viral vector (OXY-Tet-palGFP) was injected into the PVH to express palGFP under the oxytocin promoter. Confocal imaging showed OXY-immunoreactive puncta in PVH-derived, palGFP-expressing fibers distributed in the rMR. palGFP-expressing fibers in the rMR were also closely associated with neurons expressing vesicular glutamate transporter 3 (VGLUT3), a marker for BAT sympathetic premotor neurons. Next, we co-injected another viral vector (TRE-ChIEF-mCherry) with the OXY-Tet-palGFP vector into the PVH to express the engineered channelrhodopsin variant, ChIEF, in OXY neurons. In whole-cell slice recordings of PVH neurons, GFP(+)/mCherry(+) double-labeled neurons faithfully exhibited action potentials in response to 10-Hz blue-light stimuli. In *in vivo* BAT sympathetic nerve recording, optogenetic stimulation of PVH-derived OXY terminals in the rMR increased BAT sympathetic nerve activity and temperature, expired CO2, and heart rate, eliciting thermogenic and cardiac sympathetic responses. Moreover, prior nanoinjection of OXY into the rMR significantly potentiated BAT sympathetic nerve activity and other sympathetic responses evoked by NMDA nanoinjection into the rMR. These findings indicate that OXY transmission from the PVH to the rMR stimulates thermogenic and cardiac sympathetic outflows to BAT and the heart. This OXY pathway may be involved in emotion-related metabolic, thermal and cardiac responses. (COI:No)

## 2P-082

### Does physical activity in daily life cause differences in circulatory dynamics and autonomic nervous system activity between men and women?

Xinru Sun, Junko Hoshi, Hiromasa Tanno, Emi Kanno, Ryoko Maruyama (*Health Science, Grad Sch Med, Tohoku Univ, Japan*)

Physical activity (PA) provides health benefits: the PA profile (type, intensity, amount) is associated with enhanced health and QOL. We have used non-intervention methods to observe the association between PA in daily living, circulatory dynamics, and autonomic nervous system (ANS) activity in healthy young adults. We aimed to investigate how PA in daily life affects ANS activity and circulatory dynamics in men and women.

Forty-five participants aged 20–30 years were recruited, and we measured the PA of daily living over 1-week using pedometer. Accordingly, the high PA group (≥28 METs/week) comprised 7 men and 16 women and the low PA group (<28 METs/week) comprised 8 men and 14 women. Height, weight, BMI, muscle mass, visceral fat rating, and basal metabolic rate were measured using a body composition analyzer. We also measured blood pressure (BP) and heart rate variability (HRV) at rest and during the postural change from the supine to the sitting position. Spectral analysis of HRV was performed to evaluate low-frequency (LF) power, high-frequency (HF) power, and LF/HF ratio to observe the changes in cardiac sympathetic and parasympathetic nerve activities.

Significant differences were observed between the men groups that the high PA group in terms of body muscle mass is higher. The women in the high PA group had significantly higher leg muscle mass than those in the low PA group. During the postural change from all positions, women groups of ANS activities were more sensitive than that in men groups. Thus, we observed greater PA of daily living affects body muscle composition, and the impact on ANS activities exhibited gender differences. (COI:No)

## 2P-083

### Does listening to music affect HR, BP and autonomic nervous activity in men and women differently?

Junko Hoshi, Xinru Sun, Hiromasa Tanno, Emi Kanno, Ryoko Maruyama (*Dept Nursing, Grad Sch Med, Tohoku Univ, Japan*)

It has been reported that listening to music has beneficial effects on physical and mental health. Moreover, it has been reported that autonomic cardiac control and auditory perception differs between men and women. Despite these widespread claims, previous studies have shown inconsistent results regarding physiological responses to music. We aimed to evaluate the gender differences in heart rate (HR), blood pressure (BP) and autonomic nervous system (ANS) activity in healthy adults in response to listening to music. Twenty-five volunteers participated in 3 tasks consisting of 3 experimental conditions. Including 10 min of rest and test-to-load on sympathetic nervous activity, the conditions were listening to Mozart's Piano Sonata for Two Hands, D-major (K448) or silence as control for 8 min in randomized order. Electrocardiography (ECG) was continuously performed from start to end of each data collection point. There were no significant differences between men and women in terms of changes in HR and BP. However, significant decrease after listening to K448 in the ratio of low frequency (LF)/ high frequency (HF) as an index of sympathetic nervous activity was observed only young women. Conversely, natural logarithmized HF (logHF) as an index of parasympathetic nervous activity in men significantly decreased after listening to K448. Our finding suggests that ANS activity in response to music varies between men and women whereas this gender difference was not noted in terms of HR and BP. (COI:No)

## 2P-084

### Analysis of the effect of intraspinal administration of alpha-melanocyte-stimulating hormone on colorectal motility

Hiromi Ueda[1], Mitsuya Shiraishi[1], Hiroyuki Nakamori[2], Takahiko Shiina[3], Yasutake Shimizu[3], Kiyotada Naitou[1] (*[1]Dept. Basic Vet. Sci., Fac. Vet. Sci., Kagoshima Univ., Kagoshima, Japan, [2]Dept. Cell Physiol., Nagoya City Univ. Grad. Sch. Med. Sci., Nagoya, Japn, [3]Dept. Basic Vet. Sci., Lab. Physiol., Unit. Grad. Sch. Vet. Sci., Gifu Univ., Gifu, Japan*)

Colorectal motility is regulated by two defecation centers, one located in the brain and one in the spinal cord. However, the precise mechanisms of central regulation of colorectal motility are still unclear. An orexigenic peptide, ghrelin, reportedly affects the spinal defecation center and enhances colorectal motility. Previously, we showed that somatostatin, which has the opposite effect to ghrelin in the hypothalamus, enhanced colorectal motility through acting on the spinal defecation center. In this study, we focused on alpha-melanocyte-stimulating hormone (alpha-MSH), another neuropeptide with an opposite effect to ghrelin in the hypothalamus. We aimed to elucidate the action of alpha-MSH on the spinal defecation center and the consequent effect on colorectal motility in anesthetized rats. Colorectal motility was enhanced by intraspinal administration of alpha-MSH at the L6-S1 level (the location of the spinal defecation center), but not by intravenous injection. Intraspinal alpha-MSH still enhanced colorectal motility when the thoracic cord was transected at the T4 level. Prevention of parasympathetic outflow from the spinal cord by cutting the pelvic nerves, but not prevention of sympathetic outflow by cutting the colonic nerves, abolished the effect of alpha-MSH. Our results demonstrate that intraspinal alpha-MSH activates the parasympathetic outflow to the colorectum by acting on the spinal defecation center and enhances colorectal motility. The findings provide novel insight into the central regulation of colorectal motility. Considering the role of alpha-MSH in hypothalamic appetite regulation, we suggest that this neuropeptide may link appetite modulation to the central regulation system for defecation. (COI:No)

## 2P-085

### Generation and analysis of scn4aa and scn4ab double knock-out zebrafish

Chifumi Terai, Souhei Sakata, Fumihito Ono (*Dept Physiol, faculty Med, Osaka Med Col, Japan*)

Muscle excitation starts by activation of the acetylcholine receptor (AChR), followed by the action potential generation, opening of the dihydropyridine receptor (DHRP) and the elevation of the intracellular calcium level. In contrast to this classical model, muscle contractions without the action potential have been reported in the slow-twitch fibers of the zebrafish trunk muscle (Buckingham et al.) and the trunk muscle of juvenile ascidians (*Ciona intestinalis*) (Nishino et al.). In these muscle fibers, the depolarization directly contracts the muscle and enables animal locomotion. To re-examine the significance of action potentials, we knocked out voltage-gated sodium channels (Nav, scn) in zebrafish fast-twitch fibers. If the muscle fibers lacking the action potential contract, the fish will swim normally. Alternatively, if the fibers lose their activity, the fish will show abnormal swimming pattern as reported in Zempo et al. (In this meeting).
Initially, we performed RNA sequencing and found that the abundant read counts of *scn4aa* and *scn4ab* in the zebrafish trunk muscle. In addition, much smaller counts of *scn5lab* (*scn12ab*) were also observed. The PCR experiment confirmed the gene expression of *scn4aa* and *scn4ab*, while *scn5lab* was absent. Depending on these data, we generated a double-knock out zebrafish line - lacking *scn4aa* and *scn4ab* - using the CRISPR-Cas9 system. Unexpectedly, the double knock-out fish exhibited swimming pattern similar to wild-type fish. This may imply the dispensability of the action potential. We are recording the sodium current from the knock-out zebrafish. At the meeting, we will discuss the role of scn genes and the action potential in swimming activity of zebrafish. (COI:No)

## 2P-086

### Detection of muscle hardness after cramping

Akihiro Kaizu, Yoshiyuki Tsuboi (*Dept Of Physiol, Sch Dent, Nihon Univ, Japan*)

**Background:** Muscle clamps are painful involuntary contraction. Muscle clamps have a good prognosis, but many people complain of pain and hardness even after muscle clamping. Stretch is commonly used for pain relief. However, little is known about the cause of remaining pain or hardness after clamping.
**Purpose:** In this study, we aimed to measure muscle properties after cramping.
**Methods:** Seven-week-old SD rats (average 239 g) were used. A wire electrode was connected and attached to the rat tibial nerve under general anesthesia (2% isoflurane). Tetanic contraction was caused by applying electrical stimulation. The rat lower leg muscle clamping model was obtained by performing electrical stimulation for 5 minutes at an intensity (frequency 50 Hz, duration 100 $\mu$s, 10 times the threshold) that causes complete tautness. Muscle properties such as Tone (Hz), Stiffness (N/m) and Elasticity was measured by using Myotone Pro®. In addition, in order to examine the effect of stretching, the lower leg muscles after cramping were stretched for one minute, and same parameters were evaluated.
**Results:** Muscle Tone (Hz) and Stiffness(N/m) were increased significantly ($P<0.05$). Although there was no significant difference, Elasticity was also increased after muscle cramping. These indicators were improved by stretching treatment.
**Discussion:** This study suggests that remaining hardness after cramping may be caused by muscle morphological changes. Because Muscle hardness was detected even in the absence of electrical stimulation. To consider the details, cytohistological study is necessary. (COI:No)

## 2P-087

### 1-Fluoro-2, 4dinitorobenzene delays the relaxation process of the skinned carotid artery

Sachiko Ootsuka (*Dept Frontier Health Science, Grad Human Health Sciences, Tokyo Metropolitan Univ, Japan*)

**Purpose:** Phosphocreatine (PC) is known to accelerate $Ca^{2+}$ removal induced relaxation process in smooth muscle. To investigate whether the acceleration of the relaxation is due to ATP regeneration through Lohmann reaction, we examined the effects of 1−Fluoro−2, 4dinitorobenzene (FDNB), a potent inhibitor of creatine kinase, on the relaxation process using beta escin skinned (cell membrane permeabilized) taenia cecum and carotid artery from guinea−pig.
**Materials and Methods:** A small intact strip of carotid artery or taenia cecum from guinea−pig was firstly skinned with beta escin and Ca ionophore A23187, then contracted with 10 $\mu$M $Ca^{2+}$. When the active force reached to a steady level, $Ca^{2+}$ was quickly removed by application of ethylene glycol-bis(2-aminoethlylether)-*N, N, N', N'*−tetraacetic acid (EGTA) in the presence or absence of FDNB.
**Results and Discussion:** FDNB significantly slowed the relaxation process both in skinned carotid artery and taenia cecum at 30 $\mu$M and higher. Regression analysis of the relaxation process suggested that FDNB accelerated reattachment and/or slowed subsequent detachment and/or slowed subsequent detachment of myosin with actin rather than the initial fast cross-bridge dissociation. Since the slowing effects of FDNB on the relaxation process was observed even in the absence of PC in the intracellular solution, FDNB might affect intrinsic PC regenerating mechanisms and/or other Lohmann reaction independent mechanisms of the skinned smooth muscle. (COI:No)

## 2P-088

### Withdraw

## 2P-089

### Role of cyclic AMP pathway on expression of MyHC Ⅱ and IL-6 mRNAs in mouse myocytes

Junko Yamaji[1], Reiko Hiroshima[2], Yoshiaki Mori[2] ([1]*Deptf Nutr. Sci., Kansai Univ of Welf. Sci., Japan,* [2]*Dept Rehab, Kansai Univ of Welf. Sci., Japan*)

CGRP is a neuropeptide secreted from motor neuron at neuromuscular junctions and also activates cyclic AMP (cAMP) pathway. However, the role of the cAMP pathway in expression of MyHCII mRNA in skeletal muscle remains unknown. Recently, we reported that IL-6 induced by calcineurin activation might increase MyHCIIb mRNA, but that MyHCIIb mRNA level was decreased by medium supplemented with CGRP in C2C12 cells. In this study, we investigated that the role of cAMP pathway and CGRP on expression of MyHCIIb and IL-6 mRNAs in C2C12 myocytes.
C2C12 cells were cultured by medium containing with or without agents at the beginning of differentiation. The levels of mRNA expression were measured by quantitative RT-PCR method using Taqman probes.
Then our results are as follow:
(1) The mRNA expression level of MyHCIIb was significantly upregulated by IL-6 induced by calcineurin activators and was significantly attenuated by calcineurin inhibitor.
(2) The mRNA expression level of MyHCIIb was not affected by medium supplemented with forskolin, with 8-bromo cAMP and with clenbuterol.
(3) The IL-6 mRNA level was not affected by medium supplemented with CGRP and with forskolin.
These results suggested that calcineurin-mediated IL-6 production increases MyHCIIb mRNA but that cAMP pathway is not essential for expression of IL-6 and MyHCIIb mRNAs in C2C12 cells. (COI:No)

## 2P-090

### Activation CGRP-cAMP-dependent signal transduction pathways upregulate MyHC I mRNA in C2C12 cells

Yoshiaki Mori[1], Junko Yamaji[2], Reiko Hiroshima[1], Manabu Miyamoto[1] ([1]*Dept of Rehabil Sci, Kansai Univ of Welf Sci, Kashiwara, Japan,* [2]*Dept of Nutr Sci, Kansai Univ of Welf Sci, Kashiwara, Japan*)

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide which belongs to a family of related peptides including calcitonin, amylin, and adrenomedullin. CGRP peptides are mainly localized in sensory and central neurons and have been implicated in a variety of physiological processes. CGRP has also been identified in spinal motoneurons of several species and in the nerve terminals of the rodent neuromuscular junction. Our previous study using differentiated C2C12 cells indicated that myosin heavy chain type I (MyHC I) mRNA expression level was significantly increased by the application of CGRP, but myosin heavy chain type IIb (MyHC IIb) mRNA expression level was not increased by the application of it. C2C12 cell line appears to express CGRP receptors coupled to adenylyl cyclase activity. Therefore, we examined the contribution of cAMP-dependent pathways on the upregulation of MyHC I mRNA levels in C2C12 cells. C2C12 cells were induced to differentiate to myotubes by medium exchange to D-MEM containing 2%FBS. The cells were incubated in D-MEM containing 2%FBS with chemical compounds at the beginning of differentiation and removed after 24hr, and were maintained in differentiation medium for 3 days. MyHC I and MyHC IIb mRNA expression levels were measured by the real-time PCR method. MyHC I mRNA levels were significantly increased by the administration of CGRP, although MyHC IIb mRNA levels were not affected by it. Additionally, the effects of forskolin or 8-Br-cAMP on these mRNA levels were identical to that of CGRP. However, MyHC I mRNA expression levels were not affected by the application of cAMP response element binding protein (CREB) inhibitor, 665-15. These results suggest that the upregulation of MyHC I mRNA level by the activation of CGRP-cAMP-dependent signal transduction pathways is not involved in cAMP/CREB-mediated transcriptional regulation in C2C12 cells. (COI:No)

## 2P-091

### Elucidation of the pathogenic mechanisms in HSPB8 myopathy

Yukika Kawabata-Sakata, Genri Kawahara, Yukiko Hayashi K.（*Dept Pathophysiol, Tokyo Medical Univ, Japan*）

Heat shock protein B8 (HSPB8), a member of the small heat shock protein family, prevents protein denaturation and aggregation, and plays a role in degrading abnormal proteins via the chaperone-mediated selective autophagy (CASA) pathway in skeletal muscle. Previous studies reported that mutations in *HSPB8* cause hereditary motor neuropathy and myopathy. Recently, two novel candidate mutations of *HSPB8* were identified in families with protein-aggregated myopathy. However, the pathogenic mechanisms of HSPB8 myopathy remains to be clarified. In this study, to elucidate the pathological mechanisms of HSPB8 myopathy, we analyzed phenotypes and intracellular aggregates using zebrafish and cultured cells with novel *HSPB8* mutations. We carried out microinjection of wild-type or mutant human *HSPB8* mRNA in zebrafish embryos. Then their phenotypes at 5 days post-fertilization were analyzed. Overexpression of mutant *HSPB8* mRNA resulted in morphological abnormalities at higher rate compared to expressing wild-type *HSPB8* mRNA-injected and uninjected fish. Furthermore, it revealed that these abnormal fish had severe muscle degeneration and protein aggregation. Aggregate formation was also observed in cultured cells. In addition, the aggregates contained CASA-related molecules along with mutant HSPB8. Our data suggest that failure of the protein quality control system with HSPB8 may cause myopathy with protein aggregation. (COI:No)

## 2P-092

### $Ca^{2+}$-dependent and –independent contractile responses to 1-fluoro-2, 4-dinitrobenzene (FDNB) in the taenia caecum isolated from the guinea pig

Yukisato Ishida[1, 2], Richard Paul[2], Satoko Mihashi[1], Sachiko Ohtsuka[1], Naoya Nakahara[3], Masaru Watanabe[1]（*[1]Grad Sch Human Health Sci, Tokyo Metropolitan Univ, Tokyo, Japan, [2]Coll Med, Univ Cincinnati, Cincinnati, Ohio, USA, [3]Dept Mol Physiol, Jikei Univ Sch Med, Tokyo, Japan*）

In the guinea pig taenia caecum, both cytosolic and mitochondrial cratine kinases are present (Ishida *et al*., 1991), and phosphocreatine (PCr) production is dependent on the presence of oxygen (Ishida and Paul, 1990), indicating the compartmentation of PCr and ATP metabolism in a smooth muscle cell (Ishida *et al*., 1994). Since FDNB inhibits creatine kinases, we here investigate effects of FDNB on contractile responses and phosphagen contents of the taenia. The presence of 0.4 mM FDNB gradually elicited a contractile response of the intact taenia for more than 60 min with a peak tension approximately 15 min after the onset of contraction. The depletion of $Ca^{2+}$ from the medium with 0.2 mM EGTA inhibited the FDNB-induced peak tension by 60% of control, but did not inhibit the late phase of contraction. On the other hand, pre- or post-treatment with 10 $\mu$M verapamil did not affect the contractile response to FDNB. In the skinned taenia with $\beta$-escin and A23187, 0.1 mM FDNB elicited a contractile response in the medium contained with CTP as a nucleotide, but not with ATP and PCr. In the intact taenia, the presence of 0.4 mM FDNB gradually reduced tissue ATP (by half at 15 min and nearly depleted at 60 min), but not PCr, and increased ADP and AMP contents, measured by isotachophoresis, indicating that FDNB inhibits creatine kinases in the intact taenia. These results suggest that FDNB elicits two components of contraction in the intact taenia, where the early phase of $Ca^{2+}$ channel-insensitive contraction is dependent on the presence of external $Ca^{2+}$, but the late phase is independent. The early phase seems to be an active contraction, but the late phase be a rigor- or latch-like contraction of the taenia due to the elevation of ADP in the presence of FDNB. (COI:No)

## 2P-093

### Amiloride and spirantol sensitivity for NaCl responses in the taste-sensitive neurons in the rostral nucleus of the solitary tract in rats

Tatsuko Yokota, Katsunari Hiraba（*Dept Physiol, Sch Dent, Aich-Gakuin Univ, Japan*）

In the present study, we investigated whether amiloride (epithelial $Na^+$ channel blocker) and spirantol (salt-taste enhancer, a pungent extract from an aster family, Spilanthes acmella) affected low-threshold (LT) and/or high-threshold (HT) neurons for NaCl in the first-order taste relay, the rostral nucleus of the solitary tract (rNST) in the medulla. Here, we recorded extracellular single unit activities in the rNST neurons using multi-barrel glass micropipettes while under urethane anesthesia. Taste solutions were applied to the tongue and the oral cavity, and rinsed by distilled water (water-condition), amiloride and spirantol. Net responses during taste stimulations were calculated by subtractions of averaged spikes during water applications. Amiloride significantly reduced the net responses for 0.1 ~ 0.8 M NaCl in 33% (10/30 neurons), and all of the amiloride-sensitive neurons were the LT neurons. Spirantol increased the net responses for NaCl in 50% (6/12 neurons) by spirantol. The spike activities for the LT neurons were frequently increased by spirantol solution for itself. The HT neurons which did not respond to 0.1 and/or 0.2 M NaCl during water-condition responded to their low concentrations during spirantol administration. The enhancement of spirantol may be prominent in the HT neurons with amiloride-insensitive. These results suggest that enhancement of sodium taste by spirantol result from not only increased response magnitudes but also recruitment of neurons. (COI:No)

## 2P-094

### Withdraw

## 2P-095

### Oral discrimination of viscosity in sweet flavor in humans and rats

Yuichi Miyamura[1, 2]（*[1]Dept Physiol, Grad Sch Dent Med, Kyushu Dent Univ, Japan, [2]Dept Oral Radiol, Grad Sch Dent Med, Kyushu Dent Univ, Japan*）

The unpalatability of the texture and/or flavor of an additive cause some methodological problems in studies of texture perception. Here, we examined oral viscosity discrimination in humans and rats in sweet flavor to improve unpalatability and additive flavor. In nine healthy volunteers, the palatability and viscosity perception of xanthan gum (0-3%)-contained fluids were evaluated by visual analogue scales. Apple sweet flavor greatly improved the palatability of fluids without impairment of viscosity perception. In animal experiments, carboxymethyl cellulose (CMC: 0-3%)-contained saccharin fluids were fed to food-deprived rats. The intake of viscous fluids was same as non-viscous fluid until 1% and slightly decreased in 3%. Next, we performed conditioned avoidance test by viscous fluid without saccharin and LiCl or saline (vehicle) to evaluate viscosity discrimination in rats. Relative intake of the viscous saccharin-containing fluid against vehicle treatment was significantly lower than that of the non-viscous saccharin-containing fluid in 1% CMC conditioning, but not in 0.3% conditioning. These results suggest that viscosity can be discriminated in the oral cavity under improvements of the unpalatability of texture and/or flavors of an additive by adding sweet flavors. (COI:No)

## 2P-096

### Nutrient sensing mechanisms in gustatory ganglion cells of rats

Takeshi Suwabe, Toshiaki Yasuo, Noritaka Sako（*Dept Oral Physiol, Sch Dent, Asahi Univ, Japan*）

**Purpose:** Sensory function is influenced by nutritional balance. However, little is known about mechanism for integration of sensory and nutritional informations. In this study, we investigated the existence of taste receptors for nutrient sensing in the rat geniculate ganglion to determine possible mechanism for integration of nutrition and gustatory informations in the peripheral nervous system.
**Methods:** Subjects were adult male (7-8 weeks old) rats. Reverse transcribed total RNA extracts of geniculate ganglia from rats were tested by qRT-PCR for the presence of the renin-angiotensin system (angiotensinogen, renin and angiotensin-converting enzyme) and angiotensin II receptor (type 1a, type 1b and type 2 receptors) mRNAs. 10-$\mu$m-thick free-floating cryosections of geniculate ganglia were prepared and incubated with anti-angiotensin II antibody, stained with Alexa Fluor 568-conjugated secondary antibody and Alexa Fluor 488-conjugated anti-NeuN (a neuronal cell maker) antibody.
**Results:** A salt taste receptor aENaC, a sweet taste receptor component T1R3, a taste-specific G protein subunit a-gustducin and the downstream element Trpm5 mRNAs were detected in geniculate ganglia of rats by qRT-PCR. Angiotensinogen, renin and angiotensin-converting enzyme mRNAs were detected in the geniculate ganglia by qRT-PCR. Angiotensin-II-immunoreactivity was detected in the cytoplasm of NeuN (a general neuronal marker)-immunopositive cells of the geniculate ganglia by immunohistochemistry. Angiotensin II type 1a, type 1b and type 2 receptor mRNAs were detected in the geniculate ganglia by qRT-PCR.
**Conclusions:** A salt taste receptor and a sweet taste receptor component mRNAs were expressed in the geniculate ganglion suggesting the ability to sense fluctuations of sodium and glucose concentrations in body fluid. The renin-angiotensin system and angiotensin II receptor mRNAs were co-expressed in the geniculate ganglion suggesting cellular communication via angiotensin II signal within the ganglion. (COI:No)

## 2P-097

### Mechano-sensitive ion channel of rat squamous cell carcinoma

Motoki Ishizaki[1], Haruna Toda[1], Sadao Ohyama[1], Wataru Ohfusa[1], Asuka Higashikawa[1], Maki Kimura[1], Yoshiyuki Shibukawa[1], Tatsuya Ichinohe[2] (*[1]Dept Physiol, Tokyo Deantal Univ, Japan, [2]Dept Dental Anesthesiology, Tokyo Deantal Univ, Japan*)

Some patients with oral cancer in early stage do not complain any pain sensation while pain is one of the important symptoms of oral cancer. Recent study has suggested that cancer cells are capable to release pain-inhibiting substances to the surrounding tissue to suppress pain caused by increasing tissue pressure during tumor growth. In this study, we aimed to investigate the mechano-sensitivity of rat squamous cell carcinoma cells, and whether cancer cells are capable to release diffusible pain inhibitors into surrounding environment or not.
Rat squamous cell carcinoma cells (SCC-158) were used. Intracellular free Ca2+ concentration ([Ca2+]i) was measured by fura-2/AM. Direct mechanical stimulation to single SCC-158 cells was performed, and [Ca2+]i changes were recorded from mechanically stimulated cells and their neighboring cells, to investigate the intercellular signal communication among cancer cells. We utilized Gd3+ (non selective mechano-sensitive cation channel inhibitor) and GsMTX4 (Piezo1 channel inhibitor), as mechanosentive channel antagonists.
During direct mechanical stimulation to SCC-158, we could observe transient [Ca2+]i increase. Repeated mechanical stimuli to the SCC-158 induced repeated [Ca2+]i increases, but the increases did not show desensitizing effects. Transient [Ca2+]i increases were also observed in neighboring cells to the stimulated SCC-158 cells. Application of Gd3+ inhibited mechanical stimulation-induced Ca2+ increase in the 35% of stimulated SCC-158 cells (5 cells/14 cells). Application of GsMTX4 inhibited mechanical stimulation-induced Ca2+ increase in the 33% of stimulated SCC-158 cells (5 cells/15 cells).
We suggest that rat squamous cell carcinoma cells express mechano-sensitive cation channels, and their activation may result in releasing intercellular transmitters from the cells. (COI:No)

## 2P-098

### Withdraw

## 2P-099

### Sexually dimorphic neural projections of calbindin-D28K neurons in the medial preoptic area of mice

Masahiro Morishita[1], Kota Ono[1], Yousuke Tsuneoka[2], Shuhei Horio[3], Shigeki Kato[4], Kazuto Kobayashi[4], Shinji Tsukahara[1] (*[1]Div Life Sci, Grad Sch Sci and Engr, Saitama Univ, Japan, [2]Dept Med, Toho Univ, Japan, [3]Div Endocrinol and Metab, Natl Inst for Physiol Sci, Japan, [4]Dept Mol Genet, Inst Biomed Sci, Fukushima Med Univ, Japan*)

The medial preoptic area (MPA) of mice contains a sexually dimorphic nucleus composed of neurons expressing calbindin-D28K (Calb) [thereafter termed the calbindin-sexually dimorphic nucleus (CALB-SDN)]. The CALB-SDN exhibits a male-biased sex difference in the number of Calb neurons, although the projection site of Calb neurons and its sex difference remain unclear. To determine the projection site of Calb neurons and its sex difference, we conducted an anterograde neurotracing study using an adeno-associated virus (AAV) vector that enables Cre recombinase-expressing cells to express green fluorescent protein (GFP) and wheat germ agglutinin (WGA). We injected the AAV vector into the MPA of transgenic mice that expresses Cre recombinase in Calb-expressing cells (Calb-Cre mice) and observed the brain sections of male and female Calb-Cre mice to find GFP-expressing fibers and WGA-expressing cell bodies. As a result, we found both GFP-expressing fibers and WGA-expressing cell bodies in the ventral tegmental area (VTA), suggesting that the VTA is a projection site of Calb neurons. Next, we performed a retrograde neurotracing study using an AAV vector that infects neurons at nerve terminals and expresses tdTomato after Cre recombination. We injected the AAV vector into the VTA of male and female Calb-Cre mice and counted the number of tdTomato-expressing Calb neurons in the CALB-SDN of Calb-immunostained brain sections. As the results, many tdTomato-expressing Calb neurons were found in the male CALB-SDN, but few tdTomato-expressing Calb neurons in the female CALB-SDN. Calb neurons that did not express tdTomato were also observed in the CALB-SDN of both sexes, but no sex difference was found in such neurons. These results suggest that the CALB-SDN contains two types of Calb neurons, which are neurons serving as interneurons within the CALB-SDN and neurons projecting to the VTA. The latter may be a significant component for forming a sexually dimorphic neural circuit. (COI:No)

## 2P-100

### Mechanical hypersensitivity induced by adult-onset hypothyroidism due to peripheral nerve hyperexcitability based on voltage-gated potassium channel downregulation in mice

Machiko Suda[1], Yusuke Takatsuru[2], Izuki Amano[1], Shogo Haraguchi[3], Noriyuki Koibuchi[1] (*[1]Dept of Integrative Physiol, Grad Sch Med, Gunma Univ, Japan, [2]Dept of Psychiatry, Johmoh Hospital, [3]Dept of Biochem, Showa Univ Sch of Med*)

Thyroid hormone (TH) is crucially involved in the function of both the central and peripheral nervous system. Approximately half of adult-onset hypothyroid patients suffer from sensory symptoms including pain, which may be caused by the peripheral neuropathy. However, the mechanisms causing the pain in hypothyroidism have not yet been clarified. We generated adult-onset hypothyroid mouse model by administrating 50 ppm propylthiouracil (PTU) for 5 weeks. The mechanical hypersensitivity, examined by von Frey test, was observed during PTU exposure and recovered after the termination of PTU treatment. Compound action potential of the sciatic nerve was also analyzed. Threshold, conduction velocity and amplitude of A $\delta$ - and C-fibers were measured by single pulse stimulation. The latency delay and amplitude decrease during train pulse stimulation were also measured. No significant change in thresholds, conduction velocities, or amplitudes by single pulse stimulation was observed in PTU administered group. However, the latency delay and amplitude decrease in A $\delta$ -fiber components by train pulse stimulation were less in PTU administered group 4 week after the PTU exposure, indicating relative hyperexcitability in nociceptive fibers. In the C-fiber component, less conduction delay by the train pulse stimulations was observed in PTU administered group. Voltage-gated potassium channel 1.1 and 1.2 protein levels decreased significantly in the sciatic nerve of PTU administered group simultaneously. These results indicate that adult-onset hypothyroidism in mice causes mechanical hypersensitivity due to hyperexcitability of peripheral nerves, and reduction of Kv1.1 and Kv1.2 may be involved in such alteration. (COI:No)

## 2P-101

### Bromocriptine administration to pregnant mice during late pregnancy induces impaired expression of maternal behavior in offspring

Kwan Ee Oh[1], Seika Sato[1], Shinnosuke Masuda[1], James Taku Sairenji[1], Mitsue Miyazaki[2], Noriaki Shimokawa[1, 2], Noriyuki Koibuchi[1] (*[1]Dept of Integrative Physiology, Gunma University Graduate School of Medicine, Japan, [2]Dept of Nutrition, Takasaki University of Heatlh and Welfare, Gunma, Japan*)

Although maternal behavior is one of the indispensable behaviors for the preservation of species, our understanding towards the development of maternal behavior is still limited. Using genetically modified mice, we previously reported the possibility that maternal prolactin (PRL) during prenatal period could be the critical factor for the development of maternal behavior in the subsequent generation. However, it is yet unclear whether the change in maternal PRL itself influences the development of maternal behavior. Here, using wild-type mice administered with bromocriptine, a D2-dopamine agonist, we show that PRL signaling during late pregnancy is important in the development of maternal behavior in offspring. We first measured the plasma PRL concentration during late pregnancy. Antepartum PRL surge was identified from gestational day 19 and lasted until parturition. Interestingly, we also identified a small nocturnal surge of PRL occurring daily at late pregnancy. By inhibiting secretion of PRL from the pituitary gland using bromocriptine, we managed to keep the plasma PRL concentration at low level throughout late pregnancy. For the evaluation of maternal behavior in the next generation, we measured the survival rate of pups. Mice born to mother administered with bromocriptine showed a lower survival rate of pups, indicating impaired expression of maternal behavior. In contrast, when PRL was administered together with bromocriptine, survival rate of pups showed no significant difference compared to the controls. Collectively, these findings suggest that PRL signaling during late pregnancy contributes to the normal development of maternal behavior in offspring. (COI:No)

## 2P-102

### Effects of chronic iodine excess on brain function

Izuki Amano[1], Yusuke Takatsuru[2], Shogo Haraguchi[3], Noriyuki Koibuchi[1] (*[1]Dept of Integrative Physiol Gunma Univ Grad Sch of Med, Gunma, Japan, [2]Department of Medicine, Johmoh Hospital, Gunma, Japan, [3]Department of Biochemistry, Showa University School of Medicine, Tokyo, Japan*)

Iodine is one of the essential micronutrient which is required for the synthesis of thyroid hormones. Thus, iodine deficiency may result in the hypothyroidism. Iodine deficiency is one of the most common cause of preventable mental retardation and brain damage in the world. On the other hand, Japanese iodine intake exceeds that of most other countries, due to the significant seaweed consumption such as kelp. Iodine tolerance among individual humans varies greatly, and the excess iodine can cause both hyper- and hypo- thyroidism. Furthermore, the effect of thyroid dysfunction due to iodine excess on brain function has not been clarified. In this study, we generated a mouse models for chronic iodine excess and evaluated its effect on brain development. C57BL/6 mice were treated with $KIO_3$ 37.4mg/l and 374mg/l through drinking water. The visual discrimination test and the three-chambered social test were conducted at 10 weeks old. After the experiment was completed, thyroid hormones were measured and tissues were collected. Excess iodine intake caused hypertrophy of thyroid follicles regardless of the administered dose. Female mice administered highest dose showed a slight decrease in thyroid hormone levels, whereas male mice showed a slight increase. In the behavioral analysis, female mice showed an increase in learning ability. In summary, the high overdose of iodine affects thyroid hormone levels, cognitive learning function, and social behavior. The gender difference in the consequence was also observed. From the above, it was clarified that the high dose exerts various changes, although the body is tolerated with excess iodine. (COI:No)

## 2P-103

### Elucidation of the relationship between TNF receptor subtypes and the development of insulin resistance

Yoko Kaneko S.[1], Yu Kodani[1], Miho Kawata[1], Akira Nakashima[2], Hiroshi Nagasaki[1]
([1]*Dept Physiol, Sch Med, Fujita Health Univ, Japan,* [2]*Dept Physiol Chem, Sch Med, Fujita Health Univ, Japan*)

Type2 diabetes is a multifactorial disease and develops by overlapping multiple genetic predispositions and environmental factors. Among them, obesity by overeating, high-fat diet (HFD) intake and insufficient exercise are related to it. Recently, it has become clear adipocytes secrete various physiologically active substances called adipokines. TNF-$\alpha$, one of them, is involved in the development of insulin resistance.
There are two receptor subtypes of TNF-$\alpha$. In this study, mice lacking type1 TNF receptor (R1-KO), mice lacking type2 receptor (R2-KO), mice lacking the both (D-KO) and C57BL/6 mice (WT) were reared with normal chow or HFD. The amounts of ingested calories and body weight were measured at every three days up to eight weeks. The concentration of triglyceride, glucose and insulin in blood were measured with assay kit.
The weight-gain rate of R1-KO, R2-KO, D-KO and WT reared with HFD was 57.7%, 75.3%, 73.4% and 73.4%, respectively. R2-KO and D-KO gained significant weight even with normal chow and it was 13.2% and 19.6%, respectively. The blood glucose level increased about 1.5 times and the concentration of insulin increased more than 5 times in R2-KO, D-KO and WT by HFD feeding. The responses to HFD of R1-KO were not significant in the experiments. HOMA-IR index is calculated from insulin and glucose levels in fasting blood, and it is used as an indicator of insulin resistance. The value was 35.2 in R2-KO, 40.0 in D-KO and 21.0 in WT reared with HFD, indicating they showed severe insulin resistance. The index of D-KO with normal chow was 4.3, indicating they were in wild insulin resistant state. Those data suggested there was a difference in the development of insulin resistance depending on the TNF receptor subtypes that transduce the different signals into cells. (COI:No)

## 2P-104

### Analysis of Cell Behaviors in Pancreatic Islet during Pancreatic $\beta$ cell regeneration in larval zebrafish

Hiroki Matsuda[1], Fumihiro Kato[1], Yukihiko Kubota[2] ([1]*Deprt Biomed Sc, Coll Life Sc, Ritsumeikan Univ, Japan,* [2]*Deprt Bioinformatics, Coll Life Sc, Ritsumeikan Univ, Japan*)

Pancreatic $\beta$ cells produce Insulin and play a central role for glucose homeostasis. Regenerative capacity of mammalian $\beta$ cells is limited, so that loss and dysfunction of $\beta$ cells causes type 1 and type 2 diabetes, respectively. On the other hand, zebrafish have high regenerative capacity of pancreatic islets, including $\beta$ cell, throughout their entire life. Thus, zebrafish is an attractive model for the study of $\beta$ cell regeneration.
Previously several groups have reported about molecular mechanisms underlying pancreatic $\beta$ cell regeneration. However, it is still unclear from what cells regenerating $\beta$ cell arise and when $\beta$ cell regeneration is completed. Therefore, we analyzed behaviors of pancreatic $\beta$ cells, $\alpha$ cells and neurod1 expressing endocrine progenitor cells in the regenerating islet using several transgenic lines, to address these two questions. Our results revealed 1) that pancreatic $\beta$ cell arose from neurod1 expressing cells during $\beta$ cell regeneration and 2) that pancreatic $\beta$ cell completely regenerated within 13 days after $\beta$ cell ablation. (COI:No)

## 2P-105

### Change in polyunsaturated fatty acid metabolite profiles in plasma upon progression of chronic renal failure

Takeshi Kato (*Nutr Phyol, Grad Phar Sci, Josai Univ, Japan*)

It is very important to prevent the progression of renal failure in order to reduce medical expenses for a super-aging society in Japan. Among the polyunsaturated fatty acids (PUFAs), arachidonic acid (ARA) is important for kidney development and function, while docosahexaenoic acid (DHA) is important for anti-inflammatory action. In this study, chronic renal failure model rats were fed different PUFAs, and the effects on the progression of renal failure and changes in PUFA metabolites during that progression were examined. Sprague Dawley male rats were randomly divided into 4 groups (Control, ARA, DHA, and ARA+DHA) and were fed diets containing different compositions of PUFAs for each group, ad libitum. One month later, 5/6 of the kidney was excised from each rat. After 4, 8, 12, and 16 weeks, blood and urine samples were collected. Creatinine levels in plasma and urine were quantified, and creatinine clearance (Ccr) and urinary albumin (U-ALB) excretion were determined. Further, PUFA metabolites in plasma were quantified using Liquid chromatography tandem-mass spectrometry. Ccr decreased after renal failure treatment but was not affected by PUFA intake. U-ALB increased after renal failure treatment. The U-ALB levels in the DHA+ARA group significantly decreased compared to the Control group. DHA metabolites, ProtectinD1, and ResolvinD1, increased in the DHA and DHA + ARA diet groups. On the other hand, ARA metabolites increased in the ARA group. The correlation between PUFAs and U-ALB was examined, and a negative correlation was found between DHA and U-ALB in the renal failure model rats after 4 weeks. These results suggest that the effects of the combined use of DHA and ARA may slow the progression of renal failure. (COI:No)

## 2P-106

### Ezrin regulates multiple solutes reabsorption via the regulation of several membrane protein localization in the proximal tubules

Ryo Hatano[1], Mikiko Takayama[2], Kotoku Kawaguchi[2], Toshiyuki Fukutomi[3], Toru Kimura[3], Hiroyuki Sakurai[3], Eunyoung Lee[1], Takashi Miki[1], Shinji Asano[2] ([1]*Dept Med Physiol, Grad Sch Med, Chiba Univ, Japan,* [2]*Dept Mol Physiol, Col Pharm Sci, Ritsumeikan Univ, Japan,* [3]*Dept Pharmacol Toxicol, Kyorin Univ Grad Sch Med, Japan*)

Ezrin is a member of ERM (ezrin-radixin-myosin) proteins, which commonly possess a binding domain for membrane proteins and phosphoinositide, which is located at amino-terminal and a binding domain for actin cytoskeleton, which is located at carboxyl-terminal. In kidneys, intense expression of ezrin is observed in proximal tubules, and it is postulated that ezrin plays important roles in tubular solute reabsorption via the regulation of apical membrane localization of several transporters. We previously reported that ezrin knockdown (*Vil2*$^{kd/kd}$) mice show hypophosphatemia due to misvocalization of Sodium dependent phosphate transporters and its scaffold protein, NHERF1. However, we haven't investigated the influence of loss of ezrin on the membrane localizations of other transporters and membrane proteins. Thus, we performed comprehensive proteomic analysis of renal brush border membrane vesicles (BBMVs) of proximal tubules from Wild type (WT) and *Vil2*$^{kd/kd}$ mice in this study. We prepared BBMVs by $MgCl_2$ precipitation method from renal cortex. We identified totally 1,412 proteins from six mice. Scaffold proteins including NHERF1 and PDZK1 were significantly decreased in *Vil2*$^{kd/kd}$ mice. Several transporters including Slc5a1, Slc5a11, Slc22a4 and Slc22a5 showed marginally significant reduction. We also found that BBMV localizations of several other solute transporters associated with the reabsorptions of amino acids, glucose, and organic anions were totally decreased in *Vil2*$^{kd/kd}$ mice, suggesting that *Vil2*$^{kd/kd}$ mice exhibit the phenotype of Fanconi syndrome, which is accompanied with massive urinary solute waste. *Vil2*$^{kd/kd}$ mice showed a defective endocytosis of FITC-labeled beta2-microglobulin similar with Fanconi syndrome model mice, suggesting that ezrin plays important roles in the regulation of several membrane transporter localizations in the proximal tubules. (COI:No)

## 2P-107

### Visualizing expression of hypothalamic arginine vasopressin change during peritoneal dialysis by using transgenic rats

Kenya Sanada[1, 2], Hiromichi Ueno[1, 2], Kazuhiko Baba[1], Kentaro Tanaka[1], Haruki Nishimura[1], Kazuaki Nishimura[1], Satomi Sonoda[1], Mitsuhiro Yoshimura[1, 3], Takashi Maruyama[1], Tetsu Miyamoto[2], Yutaka Otsuji[2], Yoichi Ueta[1] ([1]*Dept Physiol, UOEH, Japan,* [2]*Department of Cardiovascular medicine and Nephrology,* [3]*Translational Health Sciences, School of Medicine, University of Bristol, UK*)

**Background:** Peritoneal dialysis (PD) is a renal replacement therapy that stores hypertonic substances in the abdominal cavity to drain excess water and waste products. In PD patients, volume excess is a major clinical problem. Arginine vasopressin (AVP), which is synthesized in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus, increases water reabsorption in the collecting duct. AVP synthesis and release in the hypothalamus are mainly controlled by plasma osmolality and blood volume. We hypothesized that intraperitoneal administration of hypertonic substances like PD solutions could affect the synthesis of AVP by altering osmolality and blood volume.
**Methods:** First, after administration of 3% hypertonic saline (HTN) as a dialysis solution for a short-time dwell or polyethylene glycol (PEG) as a dialysis solution for a long-time dwell, we evaluated the AVP-enhanced green fluorescent protein (eGFP) fluorescent intensity in the hypothalamus. The eGFP intensity is a quantitative indicator of AVP synthesis in transgenic rats. Second, we then quantified the Fos-like immunoreactive (IR) cells in several brain regions that are known to be involved in maintaining fluid homeostasis by control of AVP synthesis and/or which have interactions with the hypothalamus. Next, we evaluated the gene expressions of AVP, GFP and CRH *in situ* hybridization histochemistry.
**Results:** eGFP fluorescent intensities were significantly increased in the hypothalamus after administration of HTN and PEG. Immunohistochemistry for Fos revealed that several brain areas were activated after the administration of HTN and PEG. The gene expressions of AVP, GFP and CRH were revealed by *in situ* hybridization.
**Conclusion:** We visualized and semiquantitatively evaluated upregulation of AVP-eGFP synthesis and neuronal activations during PD. Upregulation of the hypothalamic AVP might be involved in the mechanism of fluid retention in PD patients. (COI:No)

## 2P-108

### Effect of transcranial direct current stimulation to M1 on subcortical neural activity

Ryota Tamura[1, 2], Nobuo Kunori[1], Ichiro Takashima[2, 3] ([1]*Neuro Rehab, HIRI, AIST, Japan,* [2]*Grad Sch Comprehensive Human Sci, Tsukuba Univ, Japan,* [3]*HIRI, AIST, Japan*)

Transcranial Direct Current Stimulation (tDCS) is a non-invasive neuromodulation technique that delivers a low direct electric current via electrodes placed on the skin of head. It has recently attracted attention as one of the therapeutic techniques expected to be effective for various brain disorders. Previous several studies on animals and humans have reported that it has an effect of changing cortical excitability directly under the stimulation electrode (Nitsche and Paulus, 2000). However, it has not fully understood how tDCS affects neuronal activity of distant brain regions that is anatomically connected to the stimulation site. In the present study, in order to verify the effect of tDCS in the brain region away from the stimulation site, first, we injected retrograde adeno-associated virus (AAV) vector genetically encoded a calcium indicator jGCaMP7f (pGP-AAV-syn-FLEX-jGCaMP7f-WPRE) into the primary motor cortex (M1) of rats and identified the brain areas where GCaMP expressed by immunohistological analysis. Furthermore, we chronically recorded the neuronal activity in these GCaMP-expressing brain regions of behaving rat by using fiber photometry calcium imaging and analyzed the changes in activity associated with DC stimulation of the M1. Preliminary results showed transient enhancement of calcium signaling associated with DC stimulation in multiple brain regions, such as motor thalamus and M2. This result suggests that DC stimulation applied to the cerebral cortex may affect neural activity of surrounding cortex and subcortical areas which has connected with injection site. (COI:No)

## 2P-109

### Protein Kinase Cγ regulates the 'gain' of excitatory signal during propagation from Climbing Fiber-Purkinje Cell synapses to the cell body

Masashi Watanave, Nobutaka Takahashi, Ayumu Konno, Hirokazu Hirai (*Dept Neurophysiol, Grad Sch Med, Gunma Univ, Japan*)

Protein kinase C γ (PKC γ) is expressed exclusively in neurons of the brain. In the cerebellar cortex, only Purkinje cells (PCs) expressed PKC γ. PKC γ-deficient PCs show normal cerebellar long-term depression, but persistent innervation by multiple climbing fibers (CFs) and impaired motor coordination. We found that viral vector-mediated re-expression of PKC γ in PCs resulted in significant rescue of behavioral defects seen in PKC γ-deficient mice, suggesting that PKC γ had a pivotal role in adult PCs and thereby, regulating the cerebellar function. To clarify a role of PKC γ in mature PCs, we compared the electrophysiological properties of PKC γ-null PCs with those of wild-type (WT) PCs. We found no difference in synaptic transmission from PF to PC and from interneuron to PC between WT and KO mice. In contrast, we found significant decrease in CF-EPSC amplitudes and imput resistances (Ri) in KO PCs, which was elicited in the presence of EGTA in the internal solution at holding current (Hc) of -10mV, but not at Hc of -70mV, suggesting the voltage dependency of CF-PC EPSC and Ri modulation by PKC γ.
Intriguingly, CF-EPSC amplitudes and Ri were comparable between genotypes when using the internal solution containing 10mM BAPTA, instead of EGTA, or the extracellular solution containing 500μM TEA, suggesting that modulation of CF-EPSC amplitude and Ri by PKC γ was Ca2+ and K+ dependent. Based on these results, we hypothesized that the modulation on CF-EPSC was mediated through activation of large conductance potassium (BK) channels, which was activated by voltage-gated calcium channels. Namely, we assumed that BK channel function was augmented in KO mouse PCs. Enhanced BK current in KO PCs could cause shunting effect and attenuate the excitatory signal during the propagation from CF-PC synapses to cell-body. To test this, we are planning to measure the BK current and show you the results. (COI:No)

## 2P-110

### Isolation of spinal motoneurons of a defined pool: diversity in gene expressions of motoneurons

Noriko Isoo[1], Naoyuki Murabe[1], Hiroshi Kameda[1], Satoshi Fukuda[1], Miki Ikeda[1], Sayaka Teramoto[2,3], Toshihiro Hayashi[1], Shin Kwak[2,3], Masaki Sakurai[1] (*[1]Dept Physiol, Sch Med, Teikyo Univ, Japan, [2]Grad Sch Med, Tokyo Univ, Japan, [3]Dept Mol Neuropathogen, Tokyo Med Univ, Japan*)

Motoneurons control various types of skeletal muscles for voluntary movement. Muscles exhibit considerable diversity, e.g. distal forearm muscles producing fine movements and axial muscles mainly involved in posture control. Motoneurons innervating a single muscle constitute a motoneuron pool. Motoneuron pools are organized in functional ensembles in the spinal cord, which could predict diversity of motoneurons in each pool. The molecular signaling pathways underlying the diversity remain poorly understood. Here we attempted to develop a method for isolation of motoneurons in a defined pool to investigate differences in gene expression. To determine the locations of the forearm and paraspinal pools, motoneurons innervating forearm or paraspinal muscles were retrogradely labeled by cholera toxin subunit B-conjugated Alexa Fluor 594. According to the distribution and morphological characteristics, motoneurons in both pools stained with toluidine blue were isolated by laser captured microdissection. Cells identified as motoneurons exhibited immunoreactivity to ChAT. They expressed substantial amounts of ChAT mRNA, but negligible amounts of Olig2 and GFAP mRNA. These findings confirmed the collected cells as motoneurons. Whole genome microarray analysis of the total 26,597 gene expression changes was performed to identify the gene expression profiling of the collected cells. The number of upregulated genes (ratio ≧ 2.0-fold, p-value <0.05) and downregulated genes (ratio ≦ 0.5, p-value <0.05) were 799 and 1,641, respectively in motoneurons in the forearm pools compared with those in the paraspinal pools. Gene set enrichment analysis revealed that expression amounts of gene set related to axonal transport were reduced. Using our method, as many as 2,440 genes demonstrated significant differences in expression between two pools, suggesting comparable diversity in motoneuron pools. This method developed to isolate motoneurons of a defined pool would be expected to contribute further to clarification of molecular mechanisms underlying diversity in motoneurons.(COI:No)

## 2P-111

### Administration of dopamine receptor antagonists to the striatum attenuates the swallowing reflex in the rat

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

Since dysphagia is frequently caused by hemorrhage of the striatum putamen and the Parkinson's disease, there is no doubt that disorders of the basal ganglia are related to dysphagia. However, the mechanism is not completely understood, and it is not known how the basal ganglia and neural pathways are related to swallowing function. In this study, we investigated the effect of microinjection of dopamine receptor antagonists into the striatum on swallowing reflex. These experiments were performed on rats anesthetized by urethane (1.3 g/kg, i.p.). Electromyograms were recorded from the mylohyoid and thyrohyoid muscles to identify swallowing event. The swallowing reflex was evoked by repetitive electrical stimulation (0.2 ms duration, 30 Hz) of the right superior laryngeal nerve (SLN). The dopamine receptor antagonists were microinjected (2 μl, 90 seconds) into the central part of bilateral striatum. D1 receptor antagonist Sch-23390 (5 μg/μl) alone, or Sch-23390 (2.5 μg/μl) together with D2 receptor antagonist Domperidone (2.5 μg/μl), or D2 receptor antagonist Sulpiride (20 μg/μl) alone were used. Co-administration of Sch-23390 and domperidone to the striatum suppressed SLN-evoked swallows. The onset latency of the first swallow was not significantly difference between before and after administration of dopamine receptor antagonist. These results suggested that the direct or indirect pathways of the basal ganglia may be involved in induction of the swallowing reflex. (COI:No)

## 2P-112

### Abnormal basal ganglia activity of L-dopa-induced dyskinesia model mice

Indriani Dwi Wahyu (*Div. System of Neurophysiology, NIPS, Japan*)

Parkinson's disease (PD) is caused by progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta in the basal ganglia (BG) and characterized by akinesia, rigidity, tremor, and postural instability. Administration of dopamine precursor, L-dopa, can compensate for DA reduction and ameliorate PD symptoms. However, as the disease progresses with long-term use of L-dopa, PD patients may develop uncontrollable movements known as L-dopa-induced dyskinesia (LID). This study tried to elucidate the mechanism underlying LID by recording neuronal activity in mice model of LID under awake state. LID mice were generated by treating 6-hydroxydopamine-lesioned PD mice chronically with an intraperitoneal injection of L-dopa. The extracellular recording was performed in the external segment of the globus pallidus (GPe), a relay station of BG, and substantia nigra pars reticulata (SNr), an output nucleus of the BG. Spontaneous recording of GPe and SNr activity showed increase of burst firings during PD, dyskinesia-off (24 hours after L-dopa treatment), and dyskinesia-on (after L-dopa treatment, when mice developed dyskinesia) in comparison to the control mice. In normal mice, cortical stimulation induces a triphasic response both in the GPe and SNr, consisting of early excitation, inhibition, and late excitation. PD state increased cortically evoked late excitation in GPe neurons, which later decreased during the dyskinesia-on state. In the SNr, cortically evoked inhibition was decreased during PD state. During dyskinesia-on state, cortically evoked inhibition was prolonged, while late excitation was reduced. Cortically evoked inhibition and late excitation in the SNr are mediated by the direct and indirect pathways of the BG that contribute to releasing and stopping movements, respectively. Therefore, the present result suggests that increased facilitatory signal through the direct pathway and decreased stop signal through the indirect pathway for movements lead to the emergence of LID symptoms. (COI:No)

## 2P-113

### Motor training strengthens the GABAergic synapses onto corticospinal tract neurons in the primary motor cortex

Hiroyuki Kida, Koh Sakai, Hiroyuki Ohtaku, Fumimaro Tsukamoto, Zin Han Thiri, Dai Mitsushima (*Dept Physiol, Grad Sch Med, Yamaguchi Univ, Japan*)

Nerve fibers in the corticospinal tract originate from pyramidal cells in layer V of the motor cortex. To analyze motor learning-induced plasticity at layer V synapses in the primary motor cortex (M1), we trained rats with a rotor rod test (10 sessions per day). The motor performance was significantly improved at the final session of the 1st day of training. Then, rats were further trained up to 2 days. First, we examined the role of local glutamatergic transmission in the behavioral study. Compared with vehicle injected controls, bilateral pretreatment with either CNQX or APV significantly impaired motor performance. Second, we took layer V specific sample from the M1 to quantify the protein level of AMPA receptor GluA1 subunit. In the synaptosome fraction, 2-days trained rats significantly increased the GluA1 subunit level, suggesting long-term increase in the training-induced AMPA receptor delivery into the excitatory synapses. Third, we made acute brain slices of the M1 to evaluate synaptic plasticity. Vertical fibers from layer II/III neurons to layer V neurons were stimulated to evaluate the ratio of AMPA receptor-mediated current vs NMDA receptor-mediated current (AMPA/NMDA ratio). The ratio in 2-days trained rats increased the ratio up to 177 % of untrained rats. Finally, we recorded miniature EPSC and IPSC to evaluate synaptic plasticity in layer V neurons by retrograde tracer in C4 cervical spinal cord. Compared to untrained controls, trained rats showed significantly higher miniature IPSC amplitude and frequency, suggesting the training-induced strengthen at $GABA_A$ receptor mediated inhibitory synapses.
Thus, the motor-training induced dynamic change in the plasticity at layers V synapses in M1. Together with the plasticity at layers II/III synapses (Kida et al, 2016), the plasticity at $GABA_A$ receptor mediated synapses in the M1 circuit may contribute to reorganize the control system of pyramidal tract after the motor training. (COI:No)

## 2P-114

### Modulation of indirect corticospinal excitation prior to visually-guided online adjustment of target reaching movement

Shinya Suzuki[1,2], Tsuyoshi Nakajima[2], Shun Irie[2], Ryohei Ariyasu[2], Hiroyuki Ohtsuka[2], Tomoyoshi Komiyama[3], Yukari Ohki[2] (*[1]Dept Phys Ther, Sch Rehabil, Hlth Sci Univ Hokkaido, Japan, [2]Dept Integr Physiol, Sch Med, Kyorin Univ, Japan, [3]Div Hlth Phys Educ, Fac Educ, Chiba Univ, Japan*)

Cervical interneurons have been suggested to receive the motor commands for visually-guided reaching (VGR) adjustment of the forelimb in cats. However, their roles in humans have not been elucidated. We investigated modulations of indirect corticospinal excitation while human subjects executed VGR adjustment of the arm.
Healthy subjects, who all gave informed consents, were seated with recording of surface electromyograms (EMG) from the right biceps brachii (BB) muscle. Subjects were asked to reach with the right arm toward the illuminated target in front of the subjects. The target position was sometimes changed to the right or left by 10 cm just after movement initiation to evoke VGR adjustment. Transcranial magnetic or electrical stimulation (TMS/TES) over the contralateral motor cortex was applied at various timing to induce motor-evoked potentials (MEPs) in BB. In a spatial facilitation test, TMS and electrical stimulation of the ipsilateral ulnar nerve (NERVE) were delivered separately or in combination. Inter-stimulus interval for the combined stimulation (CS) was set at 10 ms (NERVE ahead) to ensure simultaneous convergence of both inputs onto the cervical interneurons.
In BB, background EMG activities were enhanced in the left jump trials to make adjustments to the new target. The MEP was also significantly enhanced from 70 ms after target jump in the left jump trials, which started ~20 ms earlier than the onset of the background EMG modulation. In contrast, the MEP was suppressed from 100 ms after right jump of the target. The spatial facilitation by CS was observed in any trial, but it was stronger in the left jump compared to the no jump trial. The present findings suggest that the corticospinal excitation in the cervical interneurons is enhanced just prior to VGR adjustment, which may result in increased descending commands to BB motoneurons. (COI:No)

## 2P-115

### Reactive oxygen species (ROS) are necessary for capacitation but are independent of cAMP/PKA phosphorylation pathways

Gen Takei[1,2], Darya Tourzani[2], Bidur Paudel[2], Pablo Visconti[2] (*[1]Dept Reg Physiol, Dokkyo Med Univ, Japan, [2]VASCI, UMass, Amherst*)

Mammalian sperm have to undergo capacitation, a collective name of biochemical and physiological changes that occurs within female reproductive tract, to achieve fertilization. Several studies have reported that appropriate amount of ROS has a positive role on capacitation. It has been reported that human sperm activate ROS generation during capacitation. However, molecular identity of ROS-generating enzyme and signaling pathways involved upstream of capacitation-activated ROS generation is still controversial. In addition, it has not been shown whether ROS is necessary for fertilization.

In the present study, we investigated the association of known capacitation-associated pathways with ROS generation using mice sperm as a model.

First, we confirmed that ROS generation was up-regulated during capacitation. ROS generation was monitored by chemiluminescence using luminol. Bicarbonate ion and bovine serum albumin in capacitating media act synergistically to up-regulate ROS generation. When nicotinamide adenine dinucleotide phosphate (NADPH) was extracellularly added, mouse sperm greatly enhanced ROS generation. Apocynin, an inhibitor of NADPH oxidase (Nox), significantly inhibited ROS generation by mouse sperm. These results suggest that ROS are generated by Nox.

Although it has been reported that ROS activates cAMP synthesis and tyrosine phosphorylation in human and bovine sperm, removal of ROS by catalase and superoxide dismutase had no effect on phosphorylation of PKA substrates and tyrosine residues. On the other hand, inhibitors or activators of adenylate cyclase and PKA did not affect ROS generation. These results indicate that ROS generation and capacitation-associated cAMP/PKA and tyrosine phosphorylation pathways are regulated independently.

Finally, we investigated the effect of ROS on fertilization. The result showed that in vitro fertilization was completely blocked when sperm were preincubated with catalase to remove ROS. Taken together, these results suggest that ROS are necessary for capacitation/fertilization, and that its generation is regulated independent of cAMP/PKA/ and tyrosine phosphorylation signaling pathways. (COI:No)

## 2P-116

### Suppressive action of dicalcin on female fertility via interaction with mouse cumulus cell-oocyte complex

Hiromasa Satoh[1], Mayu Hanaue[1], Ken Takamatsu[2], Naofumi Miwa[1] (*[1]dept Physiol, Saitama Med Univ, Japan, [2]Dept Physiol, Toho Univ, Japan*)

Fertilization is a well-coordinated and sequential process, beginning with taxon-specific sperm-binding to egg-coating structures. Although the principal cellular events at fertilization have been eludiated, the molecular mechanisms whereby the fertilization is controlled remain largely unknown. We discovered that *Xenopus* dicalcin, present in the frog egg envelope, remarkably suppresses fertilization *in vitro*. This action was brought about by its binding to envelope-constituent glycoproteins, and the dramatic changes in the orientation pattern of the envelope filaments and the viscoelasticity of the entire envelope. We have also found that mouse dicalcin is present in the ciliary oviductal cells and the cumulus cells among cumulus cell-oocyte complex prepared from the oviduct lumen following ovulation. In this study, we investigated the potential role of dicalcin on mouse *in vitro* and *in vivo* fertilization. Pretreatment of the cumulus cells-oocyte complex with mouse dicalcin substantially reduced the efficiency of *in vitro* fertilization as judged by the formation of two pronuclei, whereas pretreatment with anti-dicalcin antibody increased the efficiency. Next, we examined the efficiency of *in vitro* fertilization using the cumulus cells-oocyte complex prepared from dicalcin knock-out mice, and found that the cumulus cell-oocyte complex of dicalcin (-/-) female mice showed a higher efficiency than those of dicalcin (+/-) and wild type. In addition, the number of born pups from dicalcin (-/-) female mice was significantly greater than those of dicalcin (+/-) mice and wildtype female, following the natural mating with wild type male. These results suggested that mouse dicalcin inhibits fertilization efficiency by hampering sperm-egg interaction via its direct action on cumulus cells among cumulus cell-oocyte complex. There are no conflicts of interest to declare. (COI:No)

## 2P-117

### Effects of serotonin on sperm hyperactivation and IVF in mice

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

Capacitation is an essential process for mammalian sperm in order to be fertilized. Capacitated sperm exhibits a specialized flagellar movement that refers to "HYPERACTIVATION". In addition, it is showed that ability of sperm hyperactivation is associated with success of IVF. Previously, I reported that hyperactivation was enhanced by serotonin and was suppressed by GABA in hamster sperm (Fujinoki (2011) Reproduction, 142, 255-266; Fujinoki and Takei (2017) Journal of Reproduction and Development, 63, 67-74). However, I was not able to examine effects of serotonin on in vitro fertilization (IVF) because it is very difficult to perform hamster IVF. In the present study, I show that mouse sperm hyperactivation is regulated by serotonin. Moreover, I also show effects of serotonin on IVF.

At first, I examined effects of serotonin on mouse sperm hyperactivation. Serotonin increased hyperactivation through 5-$HT_2$, 5-$HT_3$, 5-$HT_4$ and 5-$HT_7$ receptors. In the next step, I examined effects of serotonin on IVF. Serotonin, 5-$HT_2$ receptor agonist, 5-$HT_4$ receptor agonist and 5-$HT_7$ receptor agonist increased the success of IVF although 5-$HT_3$ receptor agonist did not affect it.

These findings show serotonin regulates fertilization through inducing of hyperactivation in mice. In mouse, serotonin is released from cumulus cells and matures an oocyte. Therefore, it is likely that serotonin regulates an interaction between sperm and the oocyte in mice. (COI:No)

## 2P-118

### Oocyte selection by membrane potential measurement after freeze-thaw cycle

Masao Miyake[1], Susumu Yoshie[1], Satoru Kaneko[2], Akihiro Hazama[1] (*[1]Dept Cellular and Integrative Physiol, Fukushima Med Univ Sch Med, Japan, [2]Ichikawa General Hospital, Tokyo Dental Coll, Ichikawa, Japan*)

Morphological inspection is the most commonly used technique to pick quality oocytes and embryos for artificial fertilization. To raise reproductive ratio, a new selection method from new point of view is needed, however, there are few techniques which is able to distinguish good oocytes. The membrane potential reflects expression of ion channels and completeness of cell membrane, it may evaluate oocyte quality. We previously showed that there was a wide dispersion of membrane potential among eggs without morphological difference, using MII and 4-cell stage eggs. It implied this technique could be applied for quality selection.

In this study, we analyzed the relationships between embryogenic outcome and membrane potential of mouse oocytes after freeze-thaw cycle. Oocytes were collected from hyperovulated 4-week old female mice. After freeze-thaw cycle, oocytes were used to measure membrane potential. And we measured membrane potential with voltage-sensitive fluorescent dye after direct recording using single electrode, following insemination. Some of oocytes performed good morphological characteristics, could reach blastocysts.But most of oocytes which performed near zero voltage stopped development. The near zero voltage oocytes are possible to be scratched during conventional protocol. This method may be applicable to ignore damaged oocytes. And the effect of mechanical damage on membrane potential will be discussed. All animal experiments were planned toward institutional guidelines and reviewed by institutional animal care and use committee. (COI:No)

## 2P-119

### Class II PI3Ks a and b Are Required for Rho-Dependent Uterine Smooth Muscle Contraction and Parturition in Mice

Sho Aki[1], Md Azadul Kabir Sarker[1], Kazuaki Yoshioka[1], Koji Kuno[2], Yasuo Okamoto[1], Kazuhiro Ishimaru[1], Noriko Takuwa[3], Yoh Takuwa[1] (*[1]Department of Physiology Kanazawa University School of Medicine, Japan, [2]Cancer Research Institute, Kanazawa University, Japan, [3]Department of Health Science, Ishikawa Prefectural University, Japan*)

Class II phosphoinositide3-kinases (PI3Ks), PI3K-C2$\alpha$ andPI3K-C2$\beta$, are highly homologous and distinct from class I and class III PI3Ks in catalytic products and domain structures. In contrast to class I and class III PI3Ks, physiological roles ofPI3K-C2$\alpha$ and PI3K-C2$\beta$ are not fully understood. Because we previously demonstrated that PI3K-C2$\alpha$ is involved in vascular smooth muscle contraction, we studied the phenotypes of smooth muscle–specific knockout (KO) mice of PI3K-C2$\alpha$ and PI3K-C2$\beta$. The pup numbers born from single PI3K- C2$\alpha$–KO and single PI3K-C2$\beta$–KO mothers were similar to those of control mothers, but those from double KO(DKO)mothers were smaller compared with control mice. However, the number of intrauterine fetuses in pregnant DKO mothers was similar to that in control mice. Both spontaneous and oxytocin-induced contraction of isolated uterine smooth muscle (USM) strips was diminished in DKO mice but not in either of the single KO mice, compared with control mice. Furthermore, contraction of USM of DKO mice was less sensitive to a Rho kinase inhibitor. Mechanistically, the extent of oxytocin-induced myosin light chain phosphorylation was greatly reduced in USM from DKO mice compared with control mice. The oxytocin- induced rise in the intracellular $Ca^{2+}$ concentration in USM was similar in DKO and control mice. However, Rho activation in the intracellular compartment was substantially attenuated in DKO mice compared with control mice, as evaluated by fluorescence resonance energy transfer imaging technique. These data indicate that both PI3K-C2$\alpha$ and PI3K-C2$\beta$ are required for normal USM contraction and parturition mainly through their involvement in Rho activation. (COI:No)

## 2P-120

### The role of cerebral vessels in the healthy life expectancy on Alzheimer's disease

Yu Hasegawa[1,2], Shokei Kim-Mitsuyama[2] (*[1]International University of Health and Welfare, School of Health Sciences at Fukuoka, Fukuoka, Japan, [2]Department of Pharmacology and Molecular Therapeutics Kumamoto University Graduate School of Medical Sciences*)

**Objective:** Increasing evidences support the notion that vascular factors play a pivotal role in the progression of Alzheimer's disease (AD). We herein evaluated the role of vascular factors in the pathogenesis of AD using 5XFAD mouse.

**Methods and Results:** 1) Continuous intracerebroventricular injection with angiotensin II (AngII) was performed to 5XFAD and wild-type mice through 4 weeks. Although both mice showed similar increased blood pressure, AngII significantly augmented accumulation of amyloid-$\beta$ (A$\beta$) on cortical arteries, cognitive dysfunction, and sarcopenia in 5XFAD. 2) Continuous intracerebroventricular injection with angiotensin (1-7), which suppresses AngII-induced vascular injuries, was performed to 5XFAD through 4 weeks. Angiotensin (1-7) improved cognitive dysfunction and the effect was associated with increased cerebral vasoreactivity and decreased accumulation of A$\beta$ on cortical arteries. 3) Brain A$\beta$ was significantly reduced in wild-type mice with deletion of apoptosis signal-regulating kinase 1 (ASK1), a member of the MAPK kinase kinase kinase. In addition, increased cerebral vasoreactivity was observed in the mice. On the other hand, there was no significant difference on cognitive function, vasureactivity, or cytotoxic changes in aged 5XFAD with deletion of ASK1.

**Conculsion:** Those results suggest that additive vasotoxic effects on cerebral amyloid angiopathy deteriorate healthy life expectancy. However, healthy cerebral arteries might inhibit the accumulation of A$\beta$ and cognitive impairment in AD. (COI:No)

## 2P-121

### The developmental change of gap junction in starburst amacrine cells of the mouse retina

Takuma Maruyama[1], Toshiyuki Ishii[2], Makoto Kaneda[1] (*[1]Dept Physiol, Nippon Med Sch, Tokyo, Japan, [2]Dept physiol, Nippon Med Sch, Tokyo, Japan*)

In the retina, electrical synapses formed by gap junctions are used in two different ways. Gap junctions between the homologous neurons, which are common electrical coupling in the adult retina, are used for the summation of signals and the cancellation of background noise, while gap junctions between heterologous neurons are used for the conservation of synaptic inputs. In the previous study, we have shown that chemical synaptic inputs onto starburst amacrine cells (SAC), an only cholinergic neuron in retina, are different between ON-type SAC (ON-SAC) and OFF-type SAC (OFF-SAC). In this study, we examined how the gap junctions are used for signal processing in ON- and OFF-SAC in the mouse retina using patch clamp technique. In the whole mount preparation, membrane capacitance (Cm) of ON-SACs with intact dendrites decreased after an application of meclofenamic acid (MFA), a gap junction blocker, at P10, suggesting the presence of electrical coupling. Because of the difficulty to access OFF-SACs in whole mount preparation, systematic comparison of Cm between ON- and OFF-SACs during development was carried out in slice preparation, which might give a possible damage to dendrites of SACs. In both ON- and OFF-SAC, Cm was relatively high until P10 and started to decrease at P15. Reduction of Cm continued until P28. Finally, to evaluate the cell type connected with SACs through gap junction, we injected gap junction permeant tracer, neurobiotin, to ON-SACs in whole-mount preparation. The filled ON-SACs coupled with other types of neurons but not with neighboring ON-SACs at P10. These results suggest that both ON- and OFF-SACs do not summate homologous signals after eye opening. Gap junctions in SACs and heterologous neurons in the early developmental stage might be used for the signal transfer pathway of the retinal waves, which are important for neural circuit formation in the retina. (COI:No)

## 2P-122

### Postnatal development of the temporomandibular joint of the Sprague Dawley rat measured by MR imaging

Keitaro Satoh[1,2], Kaori Satoh[2], Yoshie Imaizumi-Ohashi[2], Mika Yokoi-Hayakawa[2], Toshiyuki Fukada[3,4,5], Yoshiteru Seo[2] (*[1]Dept Pharmacol, Sch Dent, Asahi Univ, Japan, [2]Dept Physiol, Sch Med, Dokkyo Med Univ, Japan, [3]Lab Mol Cell Physiol, Fac Pharma Sci, Tokushima Bunri Univ, Japan, [4]Div Pathol, Sch Dent, Showa Univ, Japan, [5]Lab Integr Genom, IMS, RIKEN, Japan*)

Temporomandibular joint (TMJ) growth studies previously focused on the condyle and its cartilage, and largely paid less attention to the articular disc and temporal bone. In this study, we employed magnetic resonance (MR) images to observe the temporal bone, mandibular condyle, and articular disc growth as a single unit. The changes in the size and relative position of each component were observed using 7-tesla MR images during the postnatal growth. Growth of the mandibular condyle chondrocytes was assessed by immunohistochemistry using the expression of the zinc transporter ZIP13. We obtained 3-dimensional $T_1$-weighted ($T_{1w}$) MR images of the TMJ of Sprague Dawley rats at 4 – 78 days-old (P4 – P78) with a voxel resolution of 65 $\mu$m. Two-dimensional $T_{1w}$ MR images were obtained after a subcutaneous injection of the contrast reagent gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA). Sections of the TMJ were incubated with an anti-ZIP13 antibody. The $T_{1w}$ MR images showed that the mandibular condyle was located posterior to the temporal bone until P20, but afterwards, it changed to a location underneath the temporal bone. In the Gd-DTPA enhanced images, the articular disc could be identified as a region with a lower signal intensity. The number of ZIP13-positive chondrocytes at P6 was larger than that at P24. These observations suggest that the mandibular condyle with cartilage and disc grows in the posterior side of the mandibular fossa until P20. After the growth of the articular disc, joint cavity, and condyle cartilage, the condyle fits to the fossa, and completes the functional unit of the jaw joint. (COI:No)

## 2P-123

### *In vitro* modeling of hypothalamic tanycyte development using mouse embryonic stem cells

Yu Kodani[1], Yoko Kaneko S.[1], Miho Kawata[1], Akira Nakashima[2], Hiroshi Nagasaki[1] (*[1]Dept Physiol, Fujita Health Univ Sch Med, Japan, [2]Dept Physiol Chem, Fujita Health Univ Sch Med, Japan*)

Tanycytes are specialized ependymal cells lining the floor and ventro-lateral walls of the third ventricle and an important modulator of hypothalamic functions. Recent studies have identified tanycytes as an adult neural stem cell (NSC) population that supplies new neurons to the hypothalamus and that releases exosomes showing anti-aging effects. Tanycytes are considered to be remnants of radial glial cells (RGCs), a major embryonic NSC population, but the molecular events underlying functional maturation remain unknown. We previously reported that mouse embryonic stem cell (mESC)-derived hypothalamic tissues generate tanycyte-like NSCs. Here, we examined whether this mESC culture system can recapitulate the developmental transition from RGCs to tanycytes. To visualize both cell types, we used a Rax::GFP knock-in mESC line (Rax is a transcription factor specific to RGCs and tanycytes in the hypothalamus). Rax::GFP$^+$ cells comprised around 50% of total cells during the early phase of neurogenesis from mESCs (~3 weeks) and then decreased to less than 10% during the late phase (~5 weeks). The proliferation marker Ki-67 was expressed in 67.5% of Rax::GFP$^+$ cells on day 14 but only in 1.6% on day 35, indicating that residual Rax$^+$ cells after neurogenesis are mostly in a quiescent state like mature tanycytes. We newly identified a cell surface antigen for native tanycytes and it was expressed on Rax::GFP$^+$ cells at rates of 28.2% and 94.9% after 14 and 35 days of mESC differentiation, respectively. Along with the surface antigen expression, the morphology of Rax::GFP$^+$ cells was shifted from RGC-like to tanycyte-like one. Our results suggest that in the mESC-derived hypothalamic tissue, Rax$^+$ cells reproduce the RGC-to-tanycyte transition process that occurs in the developing hypothalamus. Thus, this culture system provides a useful *in vitro* model for understanding the regulation of tanycyte development, maintenance, and function. (COI:No)

## 2P-124

### Appropriate combinations of herbal components of Japanese Kampo medicines exert laxative actions on colonic epithelium cells through activation of K and Cl channels

Tomohiro Numata[1], Kaori Sato-Numata[2], Yasunobu Okada[3,4], Ryuji Inoue[1] (*[1]Dept Physiol, Grad Sch Med, Fukuoka Univ, Japan, [2]JSPS, [3]Dept Physiol, Kyoto Pref Univ of Med, Japan, [4]NIPS, Japan*)

Japanese Kampo medicines Junchoto and Mashiningan are mixtures of numerous herbal plant extracts and empirically known to exert laxative actions by stimulating fluid secretion from the colonic epithelium, thereby softening stools and promoting intestinal movement. However, it is unknown which and how the herbal components of these crude Kampo drugs are effective to stimulate ion effluxes causing colonic fluid secretion. Here, we selected four herbal components of Junchoto and Mashiningan, Mashinin (MSN), Kyonin (KYN), Tonin (TON), and Daio (DIO), which are putatively laxatives, and examined their effects on the ion channel activity of human colonic epithelial Caco-2 cells.

Patch-clamp analyses revealed that in both Caco-2 and CFTR-expressing HEK293T cells, MSN activated whole-cell current characteristic of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel, which is sensitive to an adenylyl cyclase inhibitor and a CFTR blocker whereas KYN, TON, and DIO activated whole-cell potassium currents, which is sensitive to a $K^+$ channel blocker. $K^+$ currents activated by DIO, but not KYN and TON, were eliminated by intracellular $Ca^{2+}$ chelation with 5 mM BAPTA. In addition, single-channel currents activated in a cell-attached patch by KYN, TON or DIO exhibited a unitary conductance of around 200 pS.

The coulter counter for electronic cell size measurement showed that MSN induced the secretory volume decrease (SVD) sensitive to a CFTR blocker, whereas TON, KYN, and DIO induced SVD sensitive to a $K^+$ channel blocker.

In conclusion, MSN and TON, KYN, or DIO promote fluid secretion from colonic epithelial cells by activating CFTR chloride channels and the large-conductance and voltage-activated $K^+$(BK) channels, respectively. Thus, Japanese Kampo medicines, Junchoto and Mashiningan, exert anti-constipation actions by inducing KCl efflux in colonic cells through the combined actions of CFTR- and BK-stimulating herbal components. (COI:No)

## 2P-125

### Water intake-mediated secretion of ATP from myofibroblast cells in rat small intestine

Moyuru Hayashi[1,2], Tomomi Watanabe-Asaka[1,2], Daisuke Maejima[2], Yoshiko Kawai[1,2], Toshio Ohhashi[2] (*[1]Dept Physiol, Med, TMPU, Japan, [2]Dept Medical-Health, Med, Shinshu Unv, Japan*)

We demonstrated currently water intake increased mesenteric lymph flow and the total flux of albumin, long-chain fatty acids, and ILC-3-dependent IL-22 in rats (Nagashio S et al., Am J Physiol 316: G155-G165, 2019). In the present with animal, cells, and molecular experiments, we attempted to examine the effects of shear stress stimulation produced by water intake on the myofibroblast cells in rat small intestine walls and then evaluate pivotal roles of the shear stress stimulation in innate immunological and physiological meanings. Thus, the shear stress stimulation around 1 dynes/cm$^2$ on cultured myofibroblast cells isolated from rat small intestines produced significantly release of ATP. In agreement with the finding, the immunohistochemical expression of cell surface $F_1/F_0$ ATP synthase was marked observed on the cultured myofibroblast cells. In rat in vivo experiments, an intragastric administration of distilled water caused significant increases of mesenteric lymph flow and the total flux of albumin and ILC-3-mediated IL-22, but no or little total flux of ATP in the mesenteric lymph. In concomitant with the in vivo experiments, the immunohistochemical expression of a specific ATP nucleotidase was confirmed on the submucosal cells including central lacteal canals in the small intestine. ATP also induced specifically significant immunohistochemical expression of podoplanin in the bottom region of submucosal interstitial tissues in the small intestine. In addition, ATP produced a significant increase of IL-22 mRNA expression in the isolated ILC-3 from the submucosal tissues. In conclusion, water intake-mediated shear stress stimulation produced a significant release of ATP from myofibroblast cells in rat small intestine, which may play key roles in the IL-22-dependent innate immunology in the body. (COI:No)

## 2P-126

### Regulation of esophageal striated muscle motility via ATP-dependent potassium channels

Takahiko Shiina[1], Kazuhiro Horii[1], Shouichiro Saito[2], Sawa Onouchi[2], Yasutake Shimizu[1,3] (*[1]Dept Basic Vet Sci, Lab Physiol, Unit Grad Sch Vet Sci, Gifu Univ, Gifu, Japan, [2]Dept Basic Vet Sci, Lab Anat, Unit Grad Sch Vet Sci, Gifu Univ, Gifu, Japan, [3]G-CHAIN, Gifu Univ, Gifu, Japan*)

The external muscle layer of the mammalian esophagus consists of striated muscle fibers and smooth muscle fibers. In the present study, we clarified roles of ATP-dependent potassium channels ($K_{ATP}$ channels) in motility of the striated muscle portion in the esophagus. A segment of the esophagus was isolated from the rat and was placed in an organ bath. Mechanical responses of the esophagus were recorded using a force transducer. Electrical stimulation of the vagus nerve trunk with single pulses evoked monophasic (twitch-like) contractile responses in the rat esophagus. The contractile responses were abolished by treatment with d-tubocurarine, a blocker of nicotinic acetylcholine receptors. Application of glibenclamide, an antagonist of $K_{ATP}$ channels, increased amplitude of vagally mediated twitch contractions of the rat esophagus. On the other hand, minoxidil, an agonist of $K_{ATP}$ channels, decreased amplitude of twitch contractions. RT-PCR revealed the expression of subunits of $K_{ATP}$ channels in esophageal tissue. In addition, immunopositivity for subunits of $K_{ATP}$ channels was observed in the striated muscle cells of the esophageal muscle layer. These findings indicate that $K_{ATP}$ channels might be involved in motor regulation of striated muscle in the rat esophagus. (COI:No)

## 2P-127

### Inhibition of glucose absorption by acetic acid in isolated small intestine of mice – Effects of $Na^+$ concentration

Tomoo Homma[1, 2], Saemi Sugiyama[1], Kazuya Terashima[2], Satoru Ishihara[3], Shin-Ichiro Karaki[4] (*[1]Dept Biotech, Fac Tech, Maebashi Inst Tech, Japan, [2]Div Biotech, Grad Sch Tech, Maebashi Inst Tech, Japan, [3]Gunma Agric Tech Center, Japan, [4]Lab Physiol, Dept Env Life Sci, Univ Shizuoka, Japan*)

Authors reported that cabbage vinegar (CV) inhibited glucose (Glu) absorption in isolated small intestine of mice and the main factor of this inhibition by CV was acetic acid (AA). In order to clarify inhibitory mechanism of Glu absorption by CV, instead of CV, we have been studying inhibitory mechanism of Glu absorption by AA. The present study investigated effects of $Na^+$ concentration on Glu and AA absorptions by using everted sac specimen of isolated small intestine of mice, because it was known that both Glu and AA were absorbed by $Na^+$-dependent transporters. Absorbed quantities of Glu and AA were measured after 20 min and 40 min since Glu and/or AA applications. In the presence of AA, Glu absorption was inhibited, but AA was absorbed. On the other hand, under the condition that Glu was continuously absorbed, Glu absorption was inhibited in the middle after AA was applied. Absorbed quantity of AA was larger than that of Glu under the low $Na^+$ concentration (10 mM). At then, absorbed $Na^+$ seems to be preferentially used for AA absorption, and $Na^+$ for Glu absorption might become short. For further analysis, stoichiometry and affinity of substrates & $Na^+$ of transporters for Glu and AA should be considered. (COI:No)

## 2P-128

### N-terminal region of apoptosis-inducing factor facilitates dimer formation by stabilizing the charge transfer complex

Tetsuo Yamashita[1], Takeshi Hashimoto[1], Junsuke Igarashi[1, 2], Hiroaki Kosaka[1], Katsuya Hirano[1] (*[1]Dept Cardiovasc Physiol, Fac of Med, Kagawa Univ, Japan, [2]Dept Med Eng. Fac of Heal Sci, Morinomiya Univ of Med Sci, Japan*)

Apoptosis-inducing factor resides in mitochondria as $AIF_{mit}$, which lacks a mitochondrial targeting signal (1-53), and its release from the membrane is a prerequisite for induction of apoptosis. $AIF_{mit}$ has been considered to be anchored to the membrane by an N-terminal region. However, we have shown that both $AIF_{mit}$ and $AIF_{sol}$, which further lacks a region of 54-101 in $AIF_{mit}$, bind to the membrane via ionic bond and preferably in a dimer form. NADH facilitates the membrane binding of $AIF_{mit}$ to a greater extent than that seen for $AIF_{sol}$. NADH-bound AIF is known to favor the dimer form. It is therefore hypothesized that the N-terminal region stabilizes NADH binding and facilitate dimer formation of $AIF_{mit}$. The present study addressed this hypothesis. $AIF_{mit}$ and $AIF_{sol}$ were expressed as recombinant proteins tagged with $(His)_6$ in *E. coli*. Gel filtration assay revealed that both $AIF_{mit}$ and $AIF_{sol}$ exist as monomers in the absence of NADH, and as homodimer in the presence of NADH. Under aerobic conditions, the addition of NADH to both $AIF_{mit}$ and $AIF_{sol}$ decreased the absorbance at 451 nm, which reflect the reduced state of FAD, and increased the absorbance at 750 nm, which reflect the formation of charge transfer $FADH_2$-$NAD^+$ complex. The dissociation constant of NADH for $AIF_{sol}$ was three times higher than seen with $AIF_{mit}$, whereas the NADH oxidase activity of $AIF_{sol}$ was significantly higher than that of $AIF_{mit}$. These findings indicate that the charge transfer complex in $AIF_{sol}$ is unstable. The N-terminal region of $AIF_{mit}$ is suggested to prevent electron transfer from FAD to oxygen and stabilize the charge transfer complex, thereby facilitating dimer formation and membrane binding. (COI:No)

## 2P-129

### The cellular mechanisms of skin irritation through TRP channels activation

Masayuki Takaishi[1], Maori Kono[1], Hidefumi Ikeda[1], Makoto Tominaga[2] (*[1]mandom corp, Osaka, Japan, [2]Exploratory Research Center on Life and Living Systems, National Institutes of Natural Sciences*)

TRPA1 or/and TRPV1 is known to be one of the factors to mediate inflammatory pain. Skin preparation such as cosmetics cause continuous slight skin stimulation without injury to lead skin irritation. It is not clear how these TRP channels act as molecular sensors of skin irritation through signaling pathway activated by intracellular calcium increases or thereby increasing the membrane potential. Therefore, we investigated TRPs-mediated signal transduction pathways and transcription factors that lead to skin irritation.

In this study, we focus on secreted proteins through intercellular signaling system activated by TRPA1 and TRPV1. We established HEK293T cell lines stably expressing high levels of human TRPA1 (hTRPA1), human TRPV1 (hTRPV1) or MOCK-transfected cells (MOCK). We selected HEK293T cells since these cells do not express endogenous TRPs including TRPA1 and TRPV1, and do not have crosstalk between TRPs, meaning that HEK293T cell line is considered to be appropriate for detecting only TRP channel-dependent reactions at high sensitivity. At first, we conducted micro array analysis to determine the relative expression levels of genes among hTRPA1, hTRPV1 and MOCK treated with or without AITC (a TRPA1 agonist) or capsaicin (a TRPV1 agonist) and the relative signaling pathway. We picked TRPA1 or TRPV1-dependent signaling pathways and downstream releasing factors via activation of the specific pathway. We especially focused on secreted protein as chemokines and cytokines because some of them play an important role in the pathogenesis of skin irritation. We identified that some common chemokines and cytokines were involved in the activation of both TRPA1 and TRPV1, and some of them were uniquely associated with the activation of either TRPA1 or TRPV1.

In the future, we will investigate production of chemokines and cytokines in various time-points following various agonist-exposure to clarify which is the most important factor for skin irritation though TRP channel activation. (COI:No)

## 2P-130

### Activation of airway ciliary beating by an $[Ca^{2+}]_i$ increased via nifedipine-sensitive $Ca^{2+}$ channels stimulated by amboxol

Daichi Saito[1], Kotoku Kawaguchi[1], Shinji Asano[1], Yoshinori Marunaka[2], Takashi Nakahari[2] (*[1]Dept. Mol. Physiol., Coll. Pherm. Sci., Ritsumei Univ, Japan, [2]Res. Unit for Epithelial Physiol., Res. Org. of Sci. and Tech., Ritsumeikan Univ., Japan*)

The effects of a mucolytic agent, ambroxol (ABX), on ciliary beat frequency (CBF) and ciliary bend distance (CBD, an index of amplitude) were examined in lung airway ciliary cells of mice. Airway ciliary cells were isolated by an elastase treatment from lungs of mouse. Ciliary beatings of airway ciliary cells were observed by the videomicroscopy equipped with high speed camera (500 fps) and CBD and CBD were measured by a image analysis program using a recorded images. ABX(10 μM) increased CBF and CBD by 30 %. ABX-stimulated increases in CBF and CBD were partially inhibited by $Ca^{2+}$-free solution and nifedipine. However, addition of BAPTA-AM (10 μM) completely inhibited increases in CBF and CBD stimulated by ABX. Measurement of $[Ca^{2+}]_i$ revealed that ABX stimulated a transient increase in $[Ca^{2+}]_i$ in a $Ca^{2+}$-free solution, suggesting ABX stimulates $Ca^{2+}$-release from internal stores followed by $Ca^{2+}$ influx. The KCl solution, in which NaCl is replaced with KCl, alone stimulated increases in CBF and CBD, which were inhibited by nifedipine, and ABX still increased CBF and CBD in the KCl solution. These indicate that nifedipine-sensitive $Ca^{2+}$ channels exist in airway ciliary cells and were activated by ABX. Moreover, we are going to identify voltage-activated $Ca^{2+}$ channels in airway ciliary cells of mice. The role of nifedipine-sensitive $Ca^{2+}$ channels on airway ciliary cells still remains uncertain, however, ABX appears to stimulate the nifedipine-sensitive voltage activated $Ca^{2+}$ channels leading to increase CBF and CBD in airway ciliary cells. (COI:No)

## 2P-131

### Cytoskeletal actin fiber elongation was perturbed by cesium application in NIH/3T3 cells

Daisuke Kobayashi, Ziasmin Khatun, Natsumi Nishimura, Akihiro Hazama (*Dept Cell Integrat Physiol, Grad Sch Med, Fukushima Med Univ, Japan*)

Our laboratory previously showed that cesium inhibited the growth of the human cancer cells by inhibition of the glycolytic pathway. We also investigated the effects of cesium on murine fibroblast cells (NIH/3T3) proliferation and migration. Regarding microscopic observation of migration assay, a shape of migrating cells seemed to be different between in the absence and in the presence of cesium. We assumed that the morphologic difference was caused by cytoskeleton difference. In this study, a structure of cytoskeletal actin fiber was visualized with AlexaFluor568-conjugated phalloidin. The NIH/3T3 cells treated with 10 mM cesium chloride have a tendency to round shape, and its actin fibers were condensed at peripheral membrane ruffle compared with control. Moreover, there were structures look like slender fingers of membrane, as well as filopodia in cesium chloride application cells. These results indicated that perturbation of cytoskeletal actin fiber elongation was caused by cesium. (COI:No)

## 2P-132

### Perfusion culture improves functions of hiPSC-liver tissue with large blood vessel

Kazuki Nanjo (*Dept of Regen Med, Grad Sch of Med, Yokohama City Univ, Japan*)

We previously developed a technology to generate the vascularized liver bud composed of human induce pluripotent stem cell (hiPSC)-derived hepatic endoderm, endothelial cells and mesenchymal cells and proposed the novel cell therapeutics for acute liver failure (Takebe T et al., Nature, 2013). To apply this technology to end stage liver failure such as liver cirrhosis, it is necessary to reconstitute large liver tissue. As low oxygen and less nutrients inside of the large tissue cause apoptosis, it is necessary to generate large tissue with blood vessel to supply oxygen and nutrients by perfusion through large blood vessel.

In this study, we generated hiPSC-liver tissue with large blood vessel (0.5-1 mm diameter) and examined the effect of perfusion on liver tissue functions. At first, we tried to fuse hiPSC-liver tissue and mouse aorta punctured by needle. hiPSC-liver tissue successfully fuses with aorta and mouse endothelial cells were observed in human liver tissue under the stimulation with VEGF. Next, large vessels from different origins (femoral artery and vein from mouse and rat, and mouse aorta) were fused with hiPSC-liver tissue and compared their albumin secretion. Among these samples, hiPSC-liver tissue with rat femoral artery showed the highest albumin secretion. Finally, perfusion culture of hiPSC-liver tissue with large blood vessel was conducted at a flow rate of 30 ul/min. As a result, hepatic clusters and albumin secretion were increased in hiPSC-liver tissue with perfusion culture.

Taken together, novel culture technology to generate hiPSC-liver tissue with large blood vessel was developed and perfusion culture through large blood vessel improved hiPSC-liver tissue functions such as increase of hepatic clusters and albumin secretion. This technology could be a substitute for organ transplantation in the future and contribute to solve donor shortage of the organ transplantation. (COI:No)

## 2P-133

### Building-up the primary culture system for ependymal ciliary cells from newborn mice

Yurie Namoto[1], Yuki Ishii[1], Tomonori Okazaki[1], Shinji Asano[1], Yoshinori Marunaka[2], Takashi Nakahari[2] (*[1]Dept Mol Phisiol, Coll of Pharm Sci, Ritsumeikan Univ, Japan, [2]Res Uni for Epithelial Phisiolgy, Res Org of Sci Tech, Ritsumeikan Univ, Japan*)

The brain ventricle wall is covered with beating cilia. The ependymal ciliary beating is thought to drive cerebrospinal fluid flow, because its impairment induces a hydrocephalus. To study ependymal ciliary beating, the stable ependymal ciliary cells in primary culture are required. In the present study, we are exploring the culture condition to obtain the stable sheet of ependymal ciliary cells in primary culture.

We tested two culture conditions for ependymal ciliary cells in primary culture. The whole brain from a newborn mouse was cut into small pieces and then, they were digested by a trypsin treatment. The isolated cells were cultured with DMEM containing 10% FBS in the flask for a week. After the first culture, cells were seeded on the transwell filter and cultured further 2 days. In the first culture method (culture A), cells were cultured with MEM without FBS in apical and basolateral sides. In the second culture method (culture B), cells were cultured with MEM without FBS in the apical side and with DMEM containing 10% FBS in the basolateral side. Beating cilia of ependymal ciliary cells on the transwell filter were observed by a video microscope equipped with a high-speed camera (500 fps). The number of ependymal cells growing on the transwell filter was much larger in culture B than culture A. The ependymal ciliary beating is activated by an application of $CO_2/HCO_3^-$-free solution in both cultures, suggesting they are stimulated by an elevation of $pH_i$.

In conclusion, ependymal ciliary beating was observed in the primary culture systems A and B. The culture B seems to be better compared with the culture A to get ependymal ciliary cells primary culture. Furthermore, the ciliary beating of ependymal ciliary cells in primary culture may be stimulated by an elevation of $pH_i$. (COI:No)

## 2P-134

### The molecular mechanism of intracellular $Cl^-$ in tumor progression by regulating JAK-STAT signaling cascades

Hiroaki Miyazaki (*Dept Life Sci, Fac Sci Eng, Setsunan Univ, Japan*)

A malignant tumor is a serious health problem because cancer cells can spread to distant parts of the body, so-called metastasis. For example, gastrointestinal cancer cells arising in the gastrointestinal tract enter the bloodstream and spread to distant organs such as the lung. Since metastasis is the most common cause of death from cancer, this process is an important therapeutic target. However, the molecular mechanisms of the metastasis are not fully understood. We previously clarified that the reduction of intracellular $Cl^-$ concentrations ($[Cl^-]_i$) inhibits the cell proliferation of gastric cancer MKN28 cells by diminishing the transition rate from G1 to S cell cycle phase. If it is clarified that the intracellular chloride acts as a signal to regulate function of cancer cells, it may lead us to development of novel and unique therapeutic approaches. From this viewpoint mentioned above, in the present study, we investigated whether the intracellular $Cl^-$ regulates cancer cell proliferation, cell migration and cell invasion abilities in human prostate and esophageal cancer cell lines. Our study indicates that the intracellular $Cl^-$ is a key factor regulating JAK-STAT signaling cascades involved in tumor proliferation, migration and invasion. These results strongly suggest that changes of [Cl-] i would play important roles in cancer migrations and invasion. (COI:No)

## 2P-135

### Differential regulation of cortical actin cytoskeleton by intracellular calcium in mouse eggs

Hideki Shirakawa, Shunta Arakawa, Takashi Yoshida (*Dept Eng Sci, Grad Sch Info Eng, Univ Electro-Comm, Japan*)

In mouse eggs, actin filaments (F-actin) are localized in the cortex beneath the plasma membrane, and play various roles in the cellular events at fertilization, such as sperm incorporation, cortical granule exocytosis, and polar body emission. In the present study, we investigated the spatiotemporal changes in F-actin organization, in relation to repetitive rises in intracellular $Ca^{2+}$ concentration, or $Ca^{2+}$ oscillations, induced by sperm-borne egg-activating protein PLC $\zeta$ . The actin cap (AC), which is a thick cortical layer of F-actin near the chromosomes in mature eggs arrested at the metaphase of the second meiosis, rapidly degraded after a few $Ca^{2+}$ transients. Calpain, a $Ca^{2+}$-dependent protease, may participate in this process, since the treatment with calpain inhibitor delayed the onset of the AC. Interestingly, the timing of the AC degradation coincided with the beginning of chromosome separation, suggesting that it is regulated coordinately with the metaphase-to-anaphase transition in meiotic cell cycle. In contrast to the AC, the F-actin forming the bundles in and beneath microvilli distributed over the cortical region other than the AC, increased transiently upon $Ca^{2+}$ rises. The increase was suppressed by inhibitors for actin polymerization, but not by CK-666 or SMIFH2, inhibitors for actin nucleation mediated by Arp2/3 or formins, respectively. By examining the effects of several inhibitors for kinases, it was suggested that calmodulin-dependent kinase II and Src family tyrosine kinases are involved in the regulation of $Ca^{2+}$-dependent increase in cortical F-actin. In the presentation, the relationship between the $Ca^{2+}$-dependent changes in the cortical F-actin and those in $PIP_2$ will also be discussed. (COI:No)

## 2P-136

### Functional expression of ascorbate peroxidase derived from *Cyanidioschyzon merolae* in mammalian cells

Suzuro Hitomi[1], Shoichiro Kokabu[2], Kenichiro Matsumoto[3], Izumi Ujihara[1], Kentaro Ono[1] (*[1]Div Physiology, Kyushu Dental Univ, Kitakyushu, Japan, [2]Div Mol Signal Biochem, Kyushu Dental Univ, Kitakyushu, Japan, [3]NIRS, Chiba, Japan*)

Ascorbate peroxidase (APX) is an anti-oxidative enzyme limited to plants and photosynthetic protists. The primitive red alga *Cyanidioschyzon merolae* (*C. merolae*) has high adaptive ability to live in high-temperature and acidic environment. Probably, *C.merolae*-derived APX (cAPX) has greater anti-oxidative effect than other plants living in normal environment. In the present study, we evaluated the possibility of increasing anti-oxidative ability in cAPX-transfected mammalian cells. The cAPX gene was introduced into the mouse embryo fibroblast cell line 10T1/2 cells by lipofection. Cell proliferation was not changed by cAPX transfection. cAPX-expressed cells were more tolerant to oxidative stress induced by application of $H_2O_2$ than mock cells. Cell viabilities of the cAPX-expressed cells cultured in low pH (pH 3) was increased compared with those of mock cells. Our present study demonstrated that APX gene from *C. merolae* could be functionally expressed in mammalian cells. The cAPX-expressed cells will be available for cytotherapeutic approaches, such as cell transplantation for tissue regeneration. (COI:No)

## 2P-137

### $Cl^-$ channel regulates epithelial to mesenchymal transition in oral/head and neck squamous cell carcinoma

Akihiro Hazama, Kei Kakinouchi, Susumu Yoshie (*Dept Cell Integrative Physiol. Sch Med, Fukushima Med Univ, Japan*)

**Background:** Squamous cell carcinomas in the oral/head and neck region are particularly aggressive due to high incidence of recurrence and distant metastasis. However, the mechanism of epithelial to mesenchymal transition (EMT) and distant metastasis in the oral/head and neck region has not been fully elucidated. It has been known that $Cl^-$ channel controls cell volume and several signaling pathways. Thus, $Cl^-$ channel may be important trigger for morphological change such as EMT. The aim of this study is to investigate the role of $Cl^-$ channel on EMT in OSC 20 cell line, which is a squamous cell carcinoma line of the oral/head and neck region.

**Methods:** OSC-20 cells were cultured with low serum medium containing TGF $\beta$ 1, which is a potent inducer of EMT, or $Cl^-$ channel blocker (NPPB, DIDS, and 9AC) for 5 days. The morphological change, gene expression, migration, and signaling pathway of OSC-20 cells treated with TGF $\beta$ 1 or $Cl^-$ channel blocker were evaluated.

**Results:** The morphology of OSC-20 cells treated with TGF $\beta$ 1 was not so changed. On the other hand, OSC-20 cells treated with $Cl^-$ channel blocker showed typical morphology of mesenchymal cells. Furthermore, the expression levels of mesenchymal markers such as vimentin, ZEB1, and snail in these cells were higher than that of untreated cells and TGF $\beta$ 1-treated cells. A large number of vimentin-positive cells were appeared in OSC-20 cells treated with $Cl^-$ channel blocker. Additionally, these cells showed migration. Interestingly, $Cl^-$ channel blocker did not activate smad2 signaling pathway, which is a signaling pathway that causes EMT.

**Conclusion:** OSC-20 cells treated with $Cl^-$ channel blocker showed mesenchymal phenotype via non-smad2 signaling pathway. These results suggest that $Cl^-$ channel is a key regulator of EMT in the oral/head and neck region. (COI:No)

## 2P-138

### Toxicity evaluation of Turritopsis sp. secretory component

Masahiro Kondo[1], Tomoyasu Hirano[1], Kazunao Hobo[2], Shin Kubota[3], Yuri Mukai[1,2] (*[1]Dept Electr Bioinform, Sch Sci Tech, Meiji Univ, Japan, [2]Dept Electr, Grad Sch Sci Tech, Meiji Univ, Japan, [3]Turritopsis Res Inst Reg Biol, Shirahama, Japan*)

In a wide range of the fields centered on drug discovery and regenerative medicine, it is strongly expected that the control of cell death and cell differentiation will be useful for treatment of intractable diseases. Therefore, to find the protein which is as an inducer of cell death or cell differentiation of human cells is urgent. On the other hand, the loss of biodiversity due to the uniform use of agricultural and fishery resources and the deterioration of the agricultural and fishery resources production base have become major problems in Japan in recent years, and the sustainable production infrastructure is also required for pharmaceutical and medical development in the next-generation. Thus, Turritopsis sp. is focused on as a completely metamorphic and sustainable organism that develops large-scale cell death and cell differentiation including the reversal of the life cycle in this study.

Aiming at the construction of an artificial production base for Turritopsis sp., the laboratory culture was tried in this study. As a result, it was found that mucus is secreted from vicinity of tentacles of medusa and polyps. It was also found that this mucus is toxic to the adult Artemia, which is a feed for the Turritopsis sp., and plays a major role in preying by limiting the activity of Artemia. Furthermore, from the viewpoint of discovering medicinal and medical seeds, the effects of Turritopsis sp. body fluids on HeLa cells were examined. The relationship with the secretory components obtained was clarified in this study. (COI:No)

## 2P-139

### Identifying the heterogeneity of ground state pluripotency in mouse embryonic stem cells and elucidating its regulatory mechanism

Kyoji Horie, Junko Yoshida (*Dept Physiol II, Nara Med Univ, Japan*)

Mouse embryonic stem cells (ESCs) maintain their pluripotent states in serum-free medium supplemented with Mek, Gsk3 inhibitors and LIF (2i/LIF), which is called a ground state culture condition. Core pluripotency transcription factors (TFs) known to fluctuate under serum/LIF such as Nanog are homogeneously expressed under 2i/LIF, implicating this ground state pluripotency is static in nature. However, recent reports of single-cell transcriptome analyses revealed the existence of heterogeneously expressed gene modules in the ground state, with the significance of this remains elusive. Here we report heterogeneity of ground state ESCs correlating with distinct differentiation potencies. A gene trap vector using Venus as a reporter was randomly inserted genome-wide and clones showing heterogeneous Venus expression were isolated. By sorting Venus-positive and -negative ESCs in each clone and inducing differentiation, we identified a clone showing different differentiation potencies between Venus-positive and -negative ESCs. In this clone, a noncoding gene with no known function was trapped. When we generated chimeric mice, we observed higher chimerism in Venus-negative ESCs compared to Venus-positive, indicating heterogeneity of ESC pluripotency in the ground state. To elucidate the mechanism regulating this heterogeneity, we conducted single-cell ATAC-seq and identified TF-binding motifs differentially accessible between Venus-positive and -negative ESCs. We disrupted these TFs by CRISPR/Cas9 and identified TFs regulating this heterogeneity. Our results show that the ground state pluripotency is not static in nature but is dynamically fluctuating. (COI:No)

## 2P-140

### Glutamatergic and GABAergic populations of prostaglandin EP3 receptor-expressing preoptic neurons are heat-responsive

Yoshiko Nakamura (*Dept Integrative Physiol, Nagoya Univ Grad Sch Med, Japan*)

The thermoregulatory center in the preoptic area (POA) receives thermosensory inputs from peripheral thermosensors to control body temperature. The POA also contains neurons expressing the EP3 subtype of prostaglandin E receptors, which receives the pyrogenic mediator, prostaglandin $E_2$ to trigger fever. However it is unknown whether EP3 receptor-expressing neurons in the POA contribute to basal body temperature regulation. In this study, we examined the responsiveness of EP3 receptor-expressing POA neurons to environmental temperatures and the neurotransmitters contained by these neurons by performing histological analyses combining fluorescent immunohistochemistry and in situ hybridization in rats exposed to 36, 24 or 4℃ for 2 hours. Analyzing confocal images showed that EP3 receptor-immunoreactive POA neurons consisted of a large population expressing the glutamatergic marker, VGLUT2 mRNA and a smaller population expressing the GABAergic marker, GAD67 mRNA. Heat exposure (36℃) significantly increased expression of c-Fos, a marker for neuronal activation, in both glutamatergic and GABAergic populations of EP3 receptor-expressing POA neurons, compared with control exposure (24℃). In contrast, cold exposure (4℃) did not increase c-Fos expression in EP3 receptor-expressing POA neurons. These observations suggest that EP3 receptor-expressing POA neurons contribute to heat-defensive control of body temperature under hot environments. Further studies are required to elucidate the roles of the glutamatergic and GABAergic populations of EP3 receptor-expressing POA neurons in the central circuit mechanisms for thermoregulation and fever. (COI:No)

## 2P-141

### Effects of environmental enrichment on fecal corticosterone levels and reinforcement learning in mice

Mitsugu Yoneda[1], Yui Kikuchi[1], Tsukika Noda[1], Kyoko Nishikawa[1], Honoka Hasegawa[1], Tomoaki Fujisaku[1], Hayate Tanigami[1], Masataka Nakagawa[1], Takashi Yoneda[2], Takako Ohno-Shosaku[1] ([1]*Rehab, Health Sci, Kanazawa Univ, Japan,* [2]*Inst Liberal Arts & Sci, Kanazawa Univ, Japan*)

Repeated exposure to stress induces depression-like behavior in mice. Environmental enrichment has been reported to increase BDNF (brain-derived neurotrophic factor) levels in the cortex and hippocampus, and reduce the effect of stress. Previously, we have reported that orally-administered theobromine can increase BDNF levels and improve motor learning in three-lever operant task in mice. In this study, we examined effects of environmental enrichment (EE) on fecal corticosterone levels and the lever-pressing behavior in mice. We used the operant box containing three levers. The mice were trained to press one of active levers for a food reward (1-lever task). The number of active levers was initially set to three, then decreased to two, and finally to one, depending on the performance. In the next step, the mice were trained to press three levers in a given sequence (3-lever task), and then in a reversed order (reverse 3-lever task). Before starting operant task, mice were housed in large cages (25,000 cm3) supplemented with running wheels, tunnels, huts, and mazes (EE mice) or small cages (2,200 cm3) (control mice) for 4 weeks. For corticosterone measurements, feces were collected 1, 10, 20, and 26 days after mice arrived at our laboratory, and once a week after starting operant task. Compared with control mice, EE mice showed a slightly lower fecal corticosterone level, smaller number of sessions required for completion of one-lever task, and some difference in win-stay and lose-shift behavior in the 1-lever task. Our data suggest that housing conditions (EE or control) can influence corticosterone levels and reinforcement learning in mice. (COI:No)

## 2P-142

### Sleep duration and melatonin secretion in preschool children in different regions in Japan

Maki Sato[1,2], Dominika Kanikowska[3], Toshiaki Shiomi[4] ([1]*Dept Physiol, Sch Med, Aichi Med Univ, Japan,* [2]*Institutional Research, Sch Med, Aichi Med Univ, Japan,* [3]*Pathophysiology, Sch Med, Poznan Med Univ, Poland,* [4]*Sleep Disorders Center, Aichi Med Univ, Japan*)

Adequate sleep duration is an essential factor for general health, including cardiovascular, metabolic and mental health, immunologic function, developmental health, and human performance. Amount of sleep for children is decreasing in past decades. According to recommendations of the Academy of Sleep Medicine (2016) for the pediatric population (children 3 to 5 years of age), the amount of sleep should be 10 to 13 hours. Shortage of sleep durations induces the consequences of the children growth problems due to inadequate hormonal secretion. Recently, the use of smartphone and tablet has increased among children, and in childcare. Blue light from smartphone and tablet has a disruptive effect for melatonin secretion and sleep quality at night.

We investigated the melatonin secretion in preschool children in different regions in Japan. Total number of twenty-six children of age 3-6 years old from two regions of urban and rural areas participated in this study. Saliva samples for melatonin were collected three times a day (at 20:00 h, 7:00 h and 12:00 h). The smartphone usage and sleep duration for 2 weeks were asked by questionnaire and sleep daily. In the results, smartphone usage were 58.3% of children in urban and 35.7% of children in rural areas in this study. The sleep duration of children in age 5-6 years old was shorter in urban than in rural area. However, melatonin concentration was not different between the two areas. These data suggested that children in urban area might be exposed to blue light more than that in rural area, indicating the LED blue light at night should be limited duration particular in young children. (COI:No)

## 2P-143

### Emotional behaviors of next-generation offspring mice would be affected by paternal stress just before mating

Noriyuki Shimizu, Sachiko Chikahisa, Tetsuya Shiuchi, Daisuke Tanioka, Hiroyoshi Sei (*Dept Integ Physiol, Inst of Biomed Sci, Grad Sch Univ of Tokushima, Japan*)

Emotional behaviors including anxiety, depression and fear are influenced by psychological stress. However, these mechanisms are still unclear. In recent study, we found a possibility that paternal psychological stress affects emotional behaviors in next generation. On the basis of this result, we hypothesized that paternal stress just before mating would affect a formation of offspring's emotional behaviors.

To make a paternal stress, we used a dexamethasone (glucocorticoid receptor agonist). Male C57/BL6 mice (aged 8-9 weeks) were subjected to i.p. injection of dexamethasone (200 $\mu$g/kg). Administration of dexamethasone was continued for 2 weeks, and then each male mouse was mated with same aged virgin female for 2 days. Pregnancy was confirmed in vaginal plug formation. When the offspring have reached 10 weeks of age, we performed a behavioral analysis to evaluate emotional behaviors.

Prenatally dexamethasone-treated offspring mice showed enhancement of anxiety, depression-like behavior and a strong fear response to electrical stimulation compared with vehicle-treated control mice. Furthermore, when paternal mice had recovery period for 2 weeks after last administration of dexamethasone before mating, their offspring mice showed normal emotional behavior, which was similar to vehicle-treated control mice.

Collectively, these results suggest a possibility that enhancement of paternal stress before mating would affect a function of offspring's emotional behaviors. (COI:No)

## 2P-144

### Recall of fear memories activates hypothalamic paraventricular neuronal and sympathetic nerve activity in conscious rats

Shizuka Ikegame, Kenju Miki, Misa Yoshimoto (*Dept Physiol, Nara Women's Univ, Japan*)

Recall of fear memories activates the sympathetic nervous system and exerts adverse effects on cardiovascular function. The hypothalamic paraventricular nucleus (PNV) is involved in the regulation of sympathetic nerve activity (SNA) and cardiovascular function, suggesting that the PVN may be involved in SNA and cardiovascular responses when recalling fear memories. However, the potential contribution of PVN neuronal activity (PVNNA) to the SNA responses and cardiovascular function when recalling fear memories remains unclear. The aim of the present study was to investigate functional relationships among PVNNA, SNA, and cardiovascular function in response to recalling fear memories in rats. Male Wistar rats were chronically instrumented with multiple electrodes (100-μm stainless steel wires) for measurement of PVNNA, renal SNA (RSNA), lumber SNA (LSNA), and electroencephalogram, electromyogram, and electrocardiogram data, and a catheter was used to measure arterial pressure (AP). In the fear conditioning trials, a tone (conditioned stimulus) paired with a brief electrical shock (0.1 mA, 1 s) was administered to rats twice per day over 2 days. During the course of fear conditioning, rats showed freezing behavior in response to only the tone without foot shock. Upon presentation of the tone, PVNNA, RSNA, LSNA, and AP immediately and simultaneously increased, while the heart rate increased gradually. These data suggest that recalling fear memory evoked by a conditional stimulus could activate PVNNA, resulting in simultaneous increases in RSNA, LSNA, and AP, while PVNNA may not be directly involved in the heart rate response in conscious rats. (COI:No)

## 2P-145

### Effects of social defeat stress on hippocampal neuronal and sympathetic nerve activity in conscious rats

Kana Yaguchi, Yuzuka Masuda, Kenju Miki, Misa Yoshimoto (*Dept autonomic, Nara women's Univ, Japan*)

The hippocampus has been implicated in depression symptoms. Chronic social defeat stress is associated with depression, an increase in anxiety-like behavior, and disorders of autonomic function in rodents. It remains unclear how chronic social defeat stress influences hippocampal and autonomic function. In the present study, we attempted to measure the time course of changes in hippocampal CA1 neuronal activity (CA1NA), renal sympathetic nerve activity (RSNA), and lumber sympathetic nerve activity (LSNA) during social fighting and social defeat in conscious rats. Wistar rats were chronically implanted with a combined probe consisting of multiple electrodes for measurement of CA1NA, RSNA, LSNA, and electroencephalogram, cervical electromyogram, and diaphragm electromyogram data, and a catheter was used to measure arterial pressure (AP). Social defeat was induced by introduction of a Long-Evans rat, which is typically more aggressive and heavier than a Wistar rat, into the Wistar rat's home cage for 30 minutes. The intrusion of the Long-Evans rat sometimes resulted in fighting between the Ling-Evans and Wistar rats. Wistar rats that were defeated displayed a submissive posture and then exhibited immobilization for serval minutes. Immediately after fighting, CA1NA was suppressed abruptly and in a sustained manner during the immobilized behavior. However, the AP, heart rate, RSNA, and LSNA were not suppressed during the immobilized behavior. These findings showed that defeat stress suppressed hippocampal function in a tonic manner, and RSNA, LSNA, AP, and heart rate responses were dissociated from the CA1NA response, suggesting that these peripheral parameters are not an accurate index of hippocampal function during defeat stress. (COI:No)

## 2P-146

### Antidepressant-like peptide derived from Rubisco, a major protein from green leaves

Hikaru Fujii, Takashi Nishida, Saeko Kimura, Shigenobu Matsumura, Kazuo Inoue, Kousaku Ohinata (*Div Food Sci and Biotech, Grad Sch Agr, Kyoto Univ, Japan*)

Currently, many bioactive peptides have been identified from natural food proteins. We previously reported that bioactive peptides can act on the nervous system and alter emotional behavior. Rubisco, the key enzyme for carbon dioxide fixation and photorespiration, is known as the most abundant protein in the entire biosphere. We identified Rubisco anxiolytic-like peptides (rALPs) from the digest of rubisco based on comprehensive peptide analysis and structure-activity relationships [1]. In this study, we investigated the antidepressant-like effects of rALPs.

Antidepressant-like effects were evaluated by the tail suspension test, which can be used to screen antidepressants, using male mice. We also chemosynthesized and purified peptides by reversed-phase HPLC. We found that a heptapeptide, named rALP-1(1-7), released from spinach Rubisco gastrointestinal proteases reduced the immobility time after oral administration (0.03 mg/kg). Thus, rALP-1(1-7) exhibits potent antidepressant-like effects that are comparable or better than those of general known antidepressants after oral administration.

Next, we investigated the mechanism of the antidepressant-like effects of rALP-1(1-7). We used an antagonist of the serotonin 5-HT1A receptor, which is associated with antidepressant-like behavior. The antidepressant-like activity of rALP-1(1-7) was inhibited by WAY100135, a 5-HT1A antagonist. The anxiolytic-like effects of rALP-1(1-7) were inhibited by the same antagonist, suggesting that 5-HT1A mediated both the antidepressant-like and anxiolytic-like effects of rALP-1(1-7). At present, we are investigating whether other mediators function in the antidepressant-like effects.

In conclusion, a green leaf protein-derived heptapeptide, rALP-1(1-7), exhibits antidepressant-like effects after oral administration by activating the 5-HT1A receptor.

[1] Kimura S et al., Biochem Biophys Res Commun. 2018;505(4):1050-1056 (COI:No)

## 2P-147

### Evaluation of antioxidant property, cytotoxicity and toxicity of Cydonia Oblonga fruit extract

Fatma Zahra Sakhri[1, 2], Naoki Adachi[1], Zahia Kabouche[2], Sakina Zerizer[2], Masataka Sunagawa[1] (*[1]Dept Physiol, Sch Med, Showa Univ, Japan, [2]Univ des freres Mentouri, Constantine [1], Lab d'obtention de substances therapeutiques, Constantine-Algeria*)

The present investigation was carried out to evaluate the antioxidant activity of *Cydonia Oblonga* fruit (CO), and its toxicity. For this purpose, hydroalcoholic extract of this fruit was prepared ($EtOH/H_2O$; 80/20) and the antioxidant activity was determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. In the acute toxicity test, female Wistar rats received oral administration of CO as 2,000 mg and 6,000 mg/kg Bwt. Mortality, signs of toxicity, body weights were assessed for 14 days. Blood samples were collected for hematological assessments as well as organs were weighed. To more fully evaluate the toxicity potential of CO, *in vitro* cytotoxicity was determined in primary culture of rat cortical cells.

The CO extract had an inhibitory concentration $IC_{50}$ value of (480.46 ± 3.46) $\mu$g/ml for the DPPH scavenging activity. Neither mortality nor treatment related changes in their behaviour and external appearance were observed, indicating that the LD50 value of CO is higher than 6,000 mg/kg Bwt. No significant differences were noticed in body and organ weights and in hematological parameters between the control and treated groups. The CO extract did not show significant cytotoxic effect in the range from 0.1 to 5.0 mg/ml.

The present research allows to conclude that the CO extract can be used as an easily accessible source of natural antioxidants with a potential therapeutic role, which is relatively safe from toxic effects. (COI:No)

## 2P-148

### Comparative proteomics analysis reveals the effect of phulorotannin from *Sargassum carpophyllum* on activation mast cells

Takuya Matsui[1], Chihiro Ito[2], Toshiyuki Shibata[3], Masataka Itoigawa[4] (*[1]Dept Physiol, Sch Med, Aichi Med Univ, Japan, [2]Faculty Pharm, Meijo Univ, Japan, [3]Grad Sch Bioresources, Lab Marine Food Chem, Mie Univ, Japan, [4]Sch Sports and Health Sci, Tokai Gakuen Univ*)

Mast cells play a crucial role in inflammation such as allergic and fibrotic lesions, and their regulation can lead to inhibition of such pathological conditions. Recently, functional food has garnered much attention as a reliever of inflammation. Previously, we reported the isolation of compounds from plants such as *Rutaceae*, which is used as a fruit, flavoring agent, and traditional medicine, that could suppress the release of chemical mediators from mast cells. *Sargassum carpophyllum* is an edible seaweed, commonly consumed in Japan, and known to contain phlorotannins, which are phloroglucinol polymers with reported anti-inflammatory activities. In the current study, we investigated the anti-allergic effects of phlorotannin.

Rat mast cell lines (RBL-2H3) that were pre-treated with three different phlorotannins were stimulated with dinitrophenol-human serum albumin (DNP-HSA). The anti-allergic effects of phlorotannin on intra-cellular $Ca^{2+}$ levels, reactive oxygen species (ROS) levels, and $\beta$-hexosaminidase release in activated mast cells were investigated by spectrofluorometry and enzyme assay. Additionally, to identify the varied intra-cellular proteins affected by phrolotannins, an iTARQ-based comparative proteomics analysis was performed between phulorotannins-pre-treated cells and cells stimulated DNP-HSA alone using liquid chromatography-mass spectrometry.

Phlorotannin significantly and dose-dependently reduced $\beta$-hexosaminidase release ($IC_{50}$ 49 - 56 $\mu$M). At concentration of 40 $\mu$M, phlorotannin reduced the ROS levels by ~50% compared with those in cells stimulated with DMP-HSA alone and non-stimulated cells, although the intracellular ROS levels did not differ between the DNP-HSA-stimulated and non-stimulated cells. The intra-cellular $Ca^{2+}$ levels in phlorotannin-treated cells were marginally reduced after DNP-HSA stimulation. The proteomics analysis revealed that phulorotannins treatment upregulated the expression of 27 proteins and down-regulated that of 35 proteins compared with that in cells stimulated with DNP-HSA alone.

These results demonstrate that phlorotannin reduces ROS levels and $\beta$-hexosaminidase release, making it potentially useful for attenuating immediate hypersensitivity. (COI:No)

## 2P-149

### Developmentally regulated KCC2 phosphorylation is essential for dynamic GABA-mediated inhibition and survival

Miho Watanabe[1], Kristopher Kahle T.[2, 3], Atsuo Fukuda[1] (*[1]Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]Dept Neurosurgery, Yale Sch Med, New Haven, CT, [3]Depts of Pediatrics and Cell. and Mol Physiol; Centers for Mendelian Genomics, Yale Sch Med, New Haven, CT*)

Despite its importance for GABA inhibition and involvement in neurodevelopmental disease, the regulatory mechanisms of the $K^+/Cl^-$ cotransporter KCC2 (encoded by SLC12A5) during maturation of the central nervous system are not entirely understood. Here, we applied quantitative phosphoproteomics to systematically map sites of KCC2 phosphorylation during CNS development in the mouse. KCC2 phosphorylation at Thr906 and Thr1007, which inhibits KCC2 activity, underwent dephosphorylation in parallel with the GABA excitatory-inhibitory sequence *in vivo*. Knockin mice expressing the homozygous phosphomimetic KCC2 mutations T906E/T1007E (*Kcc2*$^{E/E}$), which prevented the normal developmentally regulated dephosphorylation of these sites, exhibited early postnatal death from respiratory arrest and a marked absence of cervical spinal neuron respiratory discharges. *Kcc2*$^{E/E}$ mice also displayed disrupted lumbar spinal neuron locomotor rhythmogenesis and touch-evoked status epilepticus associated with markedly impaired KCC2-dependent $Cl^-$ extrusion. On the other hand, knockin mice expressing the homozygous dephosphorylation of KCC2 mutations T906A/T1007A (*Kcc2*$^{A/A}$), which prevented normal phosphorylation of these sites in early developmental period, exhibited reduced anxiety, deficit in social novelty recognition, and reduced startle response. These data identify a previously unknown phosphorylation-dependent KCC2 regulatory mechanism during CNS development that is essential for dynamic GABA-mediated inhibition and survival. (COI:No)

## 2P-150

### Declining of olfactory ability and volumes of hippocampus subfields in elderly subjects

Natsuko Iizuka[1, 2], Yuri Masaoka[1], Masahiro Ida[3], Masaki Yoshida[4], Akira Yoshikawa[1], Nobuyoshi Koiwa[5], Satomi Kubota[1, 2], Motoyasu Honma[1], Yumika Kokudai[1, 2], Sayaka Wada[1, 6], Kenjiro Ono[2], Masahiko Izumizaki[1] (*[1]Dept Physiol, Showa Univ, Sch Med, Tokyo, Japan, [2]Dep Neulolo, Showa Univ, Univ, Sch Med, Tokyo, Japan, [3]National Hospital Organization Mito Medical Center, [4]Dept Ophthal, Jikei Med Univ, Tokyo, Japan, [5]Dept of Health and Sci, Univ Human Arts and Sci, Saitama, Japan, [6]Dept Ophthal, Showa Med Univ, Tokyo, Japan*)

It is now well recognized that impairments in olfaction often provide the first sign of neurodegenerative disorders such as Alzheimer's disease (AD). In AD, pathological changes, including accumulation of senile plaques and neurofibrillary tangles, occur first in the parahippocmapus (paraHI), which are key areas for olfaction. Previous our study showed that the decrease of olfactory ability was associated with small paraHI volume, especially that of the left hemisphere in healthy elderly subjects. The paraHI is an important relay area projecting hippocampus to retrieval of the memory. In this study, we measured paraHI volume and each hippocampus subfields volume (subiculum, dentate gyrus, CA1, CA3) to investigate how paraHI volume associated with each subfield. All 18 elderly subjects underwent magnetic resonance imaging to measure anatomical brain volume (Freesurfer, Version 6), and subjects were assessed using tests of olfactory acuity and cognitive function. Multiple linear regression analysis revealed that olfactory ability was associated with the left paraHI and left dentate gyrus. Subjects with lower olfactory ability showed small volume of the paraHI and dentate gyrus. Volume of the paraHI included entorhinal cortex which is the gateway to the dentate gyrus, and also project back to the orbitofrontal cortex. Volume changes in entorhinal cortex and dentate gyrus may be due to pathological changes, and that may be primarily associated with declining of olfactory function before actual decrease of cognitive function in elderly subjects. (COI:No)

## 2P-151

### Brain and autonomic nerve activity preceding spontaneous perceptual switching of bistable apparent motion

Arata Ichiki, Tamami Nakano (*Grad Sch Frontier Biosci, Osaka Univ, Japan*)

The neural mechanisms involved in spontaneous perceptual switching of bistable stimuli remain unclear. A previous study reported that the arousal level increases at perceptual switching; however, it appears to be associated with the button response. Therefore, this study examined changes in arousal state several seconds preceding spontaneous perceptual switching. The autonomic nerve activity was measured based on pupil diameter, heart rate, and skin conductance under two conditions of bistable apparent motion: the rivalry condition in which perceptual images alternated endogenously and the replay condition in which perceptual images alternated exogenously. Reduction in pupillary diameter was observed approximately 3 seconds before perceptual switching in the rivalry condition compared with that in the replay condition, suggesting that the arousal level decreased before spontaneous perceptual switching. Next, we examined brain activity related to this change in arousal state preceding spontaneous perceptual switching using an electroencephalograph. The theta and delta band power in the left frontal region and the delta band power in the parieto-occipital region increased significantly in the rivalry condition compared with that in the replay condition approximately 4 seconds before perceptual switching. Moreover, we observed that the more increase in the delta power in the left frontal region, the more decrease in the pupillary diameter before spontaneous perceptual switching. Our study suggests that increases in the power of the slow frequency band with decreases in the arousal level are involved in triggering spontaneous perceptual switching of bistable apparent motion. (COI:No)

## 2P-152

### Causal role for integrating expected value in risky choice in macaque ventrolateral prefrontal cortex

Yuki Tamaki[1], Ryo Sasaki[2], Norihiro Takakuwa[2], Tadashi Isa[2,3] ([1]*Sch Med, Kyoto Univ, Kyoto, Japan,* [2]*Dept Neurosci, Grad Sch Med, Kyoto Univ, Kyoto, Japan,* [3]*Institute for the Advanced Study on Human Biology, Kyoto Univ, Kyoto, Japan*)

Recent studies have shown that many brain areas represent positive and/or negative reward-related parameters like expected value (EV), reward probability, size and risk etc. However, little is known about where and how those parameters are integrated to make a decision.

To answer this question, we investigated how macaque monkeys handle their risky choice when they might get reward with high risk and high return or low risk and low return condition. We defined the risks as the reward probabilities and the returns as the reward sizes. We also compared this in the condition with different EVs. Two monkeys were required to choose one of the two color targets presented simultaneously by a saccade. Each color target was assigned a certain combination of EV and risk/return. We found that monkeys preferred risky choice as default mode which is consistent with other previous studies (McCoy and Platt, *Nat Neurosci.* 2005; Stopper et al., *Neuron* 2014). Interestingly, we also found that monkeys preferred higher risk when EV was relatively small. These results suggest that monkeys made a choice by integrating risk and EV.

We next investigated the causal role of a variety of brain areas in the behavioral choices by reversible inactivation with microinjection of a $GABA_A$ receptor agonist, muscimol. We identified and localized the target area using MRI image with injection of gadolinium in advance. When muscimol was injected into the bilateral ventrolateral prefrontal cortex (vlPFC) while monkeys were performing the task, the sensitivity to risky choice was gradually weakened, although the sensitivity to EV was nearly unchanged. Interestingly, the interaction between risk and EV was also weakened. Our results suggest that the integration of risk/return and EV might be accomplished in vlPFC. We will also discuss about the role of other brain areas like OFC, ACC and VTA. (COI:No)

## 2P-153

### Effects of cortical temperature on neural excitatory/inhibitory balance

Mizuho Gotoh[1,2], Kazuaki Nagasaka[1,3], Mariko Nakata[1], Ichiro Takashima[1,2], Shinya Yamamoto[1] ([1]*AIST, Tsukuba, Japan,* [2]*Univ. of Tsukuba, Tsukuba, Japan,* [3]*Niigata Univ. of Health and Welfare, Niigata, Japan*)

Changes in brain temperature affect various brain functions. However, little is known about how temperature affects neural information processing when activities of individual inputs mediated by different neurotransmitters are integrated. Herein, we examined whether and how local brain temperature affects post-synaptic potentials in anesthetized rats. Evoked potentials triggered by electrical stimulation to the midbrain dopamine area (VTA/SNc) were recorded in the frontal cortex while local cortical temperature was manipulated. Peak amplitudes of evoked potentials decreased/increased as temperature increased/decreased at >～16℃; that is, local temperature and amplitude were negatively correlated. This was inconsistent with the traditional view that brain cooling inactivates neural activity. We then found that glutamate receptor antagonists (NBQX and (R)-CPP) reduced the amplitude while it remained negatively correlated with temperature; dopamine receptor antagonists (SCH-23390 and raclopride) did not affect the amplitude. Interestingly, GABA receptor antagonists (gabazine) increased the amplitude, which was positively correlated with temperature in a high concentration of gabazine. These results suggest that the excitatory (glutamatergic) and inhibitory (GABAergic) input (E/I) balance is temperature-dependent, with inhibitory inputs dominating at higher temperatures and excitatory inputs dominating at lower temperatures. Previous studies showed that brain temperature changes dynamically within ranges of >1℃, and even that 1℃ changes can alter neural activity. Our results thus indicate that brain temperature potentially mediates neural information processing through E/I balance adjustment. (COI:No)

## 2P-154

### Effects of voluntary wheel running on conditioned fear learning and extinction learning

Hiroaki Kitagawa, Keita Nakaji, Kenny Daun, Anri Kuroda, Natsu Koyama, Seiji Hitoshi (*Dept Physiol, Shiga Univ of Med Sci, Japan*)

Voluntary exercise or environmental enrichment have been shown to increase adult hippocampal neurogenesis and improve learning ability. The current experiment investigated the effects of voluntary wheel running on not only conditioned fear learning but also extinction learning.

**Method:** C57BL/6 male mice were used for current experiment. The exercise mice were reared in the cage equipped with running wheel and shelter for four weeks, and non-exercise control mice were reared in normal cage. Pavlovian classical fear conditioning has been widely used as an experimental paradigm for investigating mechanisms underlying fear memory formation and extinction. In fear conditioning, a neutral contextual and auditory-cue stimulus (conditioned stimulus; CS) is paired with an aversive foot-shock experience (unconditioned stimulus; US). After three days of conditioning training, contextual test and cued test were performed, and the effects of associative learning were evaluated as freezing response. In extinction training, CS was loaded unpaired with US.

**Results:** At recent memory one day after conditional learning, freezing time in exercise mice was significantly longer than non-exercise control mice. But there was no statistical significance in remote memory four week after conditioning between exercise group and non-exercise group. On the other hand, freezing time in exercise group was significantly shorter than non-exercise control mice at the memory after extinction learning. Taken together, these findings indicate that voluntary wheel running enhances both fear conditioning learning and extinction learning. We will discuss about exercise related-neurogenesis in subependymal zone (SEZ) and subgranular zone in dentate gyrus (SGZ). (COI:No)

## 2P-155

### Neuronal activity of the monkey prefrontal cortex in a duration estimation and production task

Atsushi Chiba[1], Kazunori Morita[2], Ken-ichi Ohio[1], Masahiko Inase[1] ([1]*Dept Physiol, Facult Med, Kindai Univ, Osaka-Sayama, Japan,* [2]*Dept Physiol, Iwate Med Univ*)

To investigate whether the same neuronal mechanism works for duration estimation of a visual stimulus and for time production in motor preparation, neuronal activity in prefrontal cortex (PFC) of the monkey was examined during a duration estimation and production task. A green square (C1) was presented on the center of the monitor for 0.8, 1.6, or 3.2 sec. Following a 1sec delay period, a red square (C2) was presented on the monitor and kept on until the end of the trial. The monkey was trained to release the hold key and to press a target button during the allowed press period that was indicated by C1 duration. When the C1 was presented for 0.8 sec, the subject needed to press the button between 3.2 and 4.8 sec after the start of the C2 presentation. When the C1 duration was 1.6 or 3.2 sec, the allowed press period was 1.6 to 3.2 sec, or 0.8 to 1.6 sec after the C2 onset, respectively. Response times between the C2 onset and the hold key release were differentially distributed among the short, middle, and long C1 trials. Of 297 recorded PFC neurons, 70 neurons showed the task-related activity that was probably involved in interval timing. A group of PFC neurons exhibited gradually increasing activity during the latter part of long C1 presentation. The activity may function as an accumulator to estimate C1 duration. Another group of neurons showed build up activity increasing to the start of the allowed press period. This activity may be related to duration production for the button press preparation. These results suggest that different groups of PFC neurons are likely to work for the duration estimation of visual stimulus and for the time production in motor preparation. (COI:No)

## 2P-156

### Correlational analysis of c-Fos expression during rubber tail task in mice

Makoto Wada[1,2], Masakazu Ide[1], Kouji Takano[3], Yoshitake Sano[4], Yo Shinoda[5], Teiichi Furuichi[4], Kenji Kansaku[3,6,7] ([1]*Dev Disorders Sect, Dept Brain Rehab, Res Inst of NRCD,* [2]*Dept Informatics, Shizuoka Univ,* [3]*Sys Neurosci Sect, Dept Brain Rehab, Res Inst of NRCD,* [4]*Dept App Biol Sci, Fac Sci and Tech, Tokyo Univ of Sci,* [5]*Dept Environ Health, Sch Pharm, Tokyo Univ of Pharm and Life Sci,* [6]*Cent for Neurosci and Biomed Eng, The Univ of Electro-Communications,* [7]*Dept Physiol & Biol Info, Dokkyo Med Univ Sch of Med*)

We previously found that mice responded as if their own tails were being touched when the rubber tails were grasped after synchronous strokes to their tails and rubber tails (Rubber tail task; Wada et al., 2016), and we also found that the response was weakened in the *Caps2*-KO mice, autism spectrum disorder model mice (Wada et al., 2019). Moreover, we found that c-Fos positive cell densities were significantly lower in the posterior parietal cortex (PPC) and primary somatosensory cortex (S1) in the Caps2 KO mice, compared to WT mice that received the same synchronous visuotactile stroking (Wada et al., 2019). In this study, we investigated the correlational relationship of c-Fos expression between brain regions during the task.

After data matrix of c-Fos positive cell density were combined among the Caps2 KO mice (n= 8) and wild type (n= 6) mice. Correlations with seed regions where significant difference was observed between the groups were calculated at each data point.

When the S1 was set as the seed region, we found that c-Fos positive cell densities in the motor cortex, PPC, auditory and visual areas were significantly correlated ($P<0.05$, uncorrected). In contrast, when the PPC was set as the seed region, we found that c-Fos positive cell densities in the insular cortex, motor cortex, S1, retrosplenial cortex and hippocampus were significantly correlated ($P<0.05$, uncorrected). In addition, c-Fos positive cell densities in these regions were generally low in the KO mice.

The results suggest that functional connections among the sensory areas exist, while functional connections between the posterior parietal cortex and limbic system existed during the synchronous visuotactile stimulation. And they were partly overlapped.

We speculate that the functional connections are related to the multisensory integrations and the change of body ownership after the integrations, respectively. (COI:No)

## 2P-157

### The role of monkey orbitofrontal cortex in reward value computation in cost-benefit-based decision-making

Tsuyoshi Setogawa[1,2], Takashi Mizuhiki[1,2], Narihisa Matsumoto[3], Barry Richmond J.[4], Munetaka Shidara[1,2] (*[1]Faculty of Medicine, Univ. of Tsukuba, [2]Grad Sch. of Comprehensive Human Sci. Univ. of Tsukuba, [3]Human Informat. Res. Inst., AIST, [4]Lab. Neuropsychol., NIMH/NIH*)

When faced with having to choose one from some alternatives, animals, including humans, will normally choose more valuable options than less valuable ones. Previous studies have reported that neuronal activities in orbitofrontal cortex (OFC) are related to the subjective values of offered options. Here we studied whether 1) neurons in OFC encode the difference in value between offered options, and 2) there is a causal link between OFC neuronal activity and choice.

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, a 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. The monkey could choose one of them, and then the chosen reward schedule started.

We recorded 256 single neurons from OFC (Monkey P: 137, Monkey H: 119). For 56/256 (21.9%) of the neurons, the neuronal firing during the second target presentation period was significantly correlated with the subtraction between the first CT value and the second CT value, suggesting that these neuronal responses encode the difference in value between two CTs. To examine the causal relationship between these neural activities and choice, we injected muscimol into a small regions of OFC rich in neurons coding for choice-related values. Inactivating this tissue led the monkeys to choose slower and more likely to choose the less valuable alternative, when the difference in value was small. These results suggest that OFC neurons code for value information that could be used to guide choices, and these signals have a direct influence on the choice. (COI:No)

## 2P-158

### Perceptual bias for elapsed time caused by a self-initiated action in primates

Kei Mochizuki, Akira Murata, Masahiko Inase (*Dept Physiol, Facult Med, Kindai Univ, Osaka-Sayama, Japan*)

It is known that human has a cognitive bias to underrate the temporal interval between one's own action and its consequence. For instance, we usually perceive good synchrony between key presses and the appearance of characters on the computer even in the presence of inevitable mechanoelectrical delays. This bias in time perception ("intentional binding") is thought to arise from subject's involuntary tendency to temporally associate ("bind") own action to its consequence, and believed to be a charactaristic nature of the sense of agency.

To investigate the neurophysiological mechanism of this biased time perception, we established a new behavioral paradigm to study it in non-human primates. The monkey was trained to judge the delay between two auditory stimuli as short or long compared to a predetermined boundary. Stimuli were presented either passively or as a result of the monkey's spontaneous button press, in order to assess how precedent voluntary action would influence the perceived temporal delay between the stimuli. The delay was varied from 100 to 600 ms and changed in each trial.

As a result, the monkey judged longer delays as "short" when timing was spontaneously initiated. This indicates that the monkey tended to perceive the elapsed time shorter in trials with self-initiated action. Furthermore, among different sets of delay lengths tested on separate experimental sessions, this tendency was observed only in a limited set and range of potential time delays. This suggests that the increase of "short" response in self-initiated trials was not a mere byproduct of the difference of task demands, and that the attribution of outer event to one's own action is strictly sensitive to the absolute temporal discrepancy on the order of tens of milliseconds. Our new task paradigm enables future investigations of biological underpinnings of the sense of agency by neurophysiological experiments in non-human primates. (COI:No)

## 2P-159

### Brown adipose tissue is involved in antiobesity effects of Melinjo (Gnetum gnemon L.) seed extract in high fat diet-fed mice

Akira Terao[1], Takeshi Yoneshiro[2], Ryuji Kaede[2], Kazuki Nagaya[2], Manami Saito[2], Juria Aoyama[2], Elfeky Mohamed[2], Yuko Okamatsu-Ogura[2], Kazuhiro Kimura[2] (*[1]School of Biological Sci, Tokai Univ, Japan, [2]Dept Biochem, Grad Sch Vet Med, Hokkaido Univ, Japan*)

Dietary supplementation of Melinjo seed extract (MSE) has been proposed as an anti-obesity agent. However, the mechanism in which MSE modurates energy balance is unclear. In this study, we investivated the effects of dietary MSE on energy intake, physical activity, and brown (BAT) and white adipose tissue (WAT) thermogenesis in high fat diet (HFD)-fed mice. Twenty-four C57BL/6J male mice were provided with the different diets for 17 weeks: the normal diet (ND), HFD, and HFD added with 1% MSE. Food intake, spontaneous locomotor activitiy, body composition, hepatic trigriceride (TG) content, and blood parameters were examined. Expression levels of thermogenic molecules and inflammation markers in BAT and WAT were examined by using quantitative PCR and Western blotting analysis. HFD increased BAT and WAT weights and adiposity index compared with ND; however, HFD-induced gains in fat pad weight were decreased by dietary MSE. Dietary MSE did not affect energy intake or spontaneous locomotor activity, but significantly suppressed HFD-induced fat accumulation, hyperglycemia, and hyperinsulinemia. Homeostasis model assessment of insulin resistance score and hepatic TG content were both lower in the MSE-supplemented HFD-fed group than in the HFD-fed group, indicating reduced insulin resistance and a less fatty liver. Dietary MSE upregulated thermogenic uncoupling protein 1 (UCP1) and mitochondrial marker cytochrome c oxidase subunit IV protein expression in BAT; this was closely associated with sirtuin 1 mRNA induction. Obesity is closely associated with chronic inflammation in adipose tissue, accompanied by increased proinflammatory and decreased anti-inflammatory cytokine levels. mRNAs of adipose inflammatory markers, such as monocyte chemotactic 1 and interleukin-1, were induced by HFD but suppressed by MSE. Supplemental MSE activates BAT thermogenesis through the Sirt1-mediated mechanisms and improves obesity-associated adipose inflammation, hepatic steatosis, and insulin resistance. The MSE regimen would be applicable to the interventions aimed at activating BAT and preventing metabolic disorders. (COI:Properly Declared)

## 2P-160

### Effect of systemic acyl ghrelin administration on thermoregulatory behavior in rats in the cold

Yuki Uchida, Chinami Tsunekawa, Izumi Sato (*Fac of Hum Life and Env, Nara Women's Univ, Nara, Japan*)

**Introduction:** Ghrelin is known to be a peptide hormone to increase appetite; however, recent reports suggested another physiological effects. There are two types of ghrelin (acyl and des-acyl ghrelin). Des-acyl ghrelin facilities thermoregulatory behavior in rats in the cold (Uchida et al., 2018, Brain Res); however, the effect of acyl ghrelin on the response is unknown yet. We investigated the effect of acyl ghrelin on thermoregulatory behavior in rats in the cold.

**Methods:** Male Wistar rats received an i.p. saline or 24 $\mu$g acyl ghrelin injection, then exposed to 27℃ or 15℃ for 2-h with continuous body temperature, tail skin temperature, and thermoregulatory behavior (tail-hiding behavior) measurements.

**Results:** At 15℃, body temperature and the duration of thermoregulatory behavior were not different between the acyl ghrelin and control groups; however, tail skin temperature in the acyl ghrelin group was greater than that in control group.

**Conclusion:** These results indicated that acyl ghrelin might not affect thermoregulatory behavior; however, it affected tail skin temperature in the cold. (COI:No)

## 2P-161

### The influence of body weight loss caused by vitamin C deficiency on the ingestive behavior in rats

Toshiaki Yasuo, Takeshi Suwabe, Noritaka Sako (*Dept Oral Physiol, Asahi Univ Sch Dent, Japan*)

When annimals lack a required nutrient, they must detect it in their surrounding environment in order to ingest it. Vitamins are also essential nutrients. Most must be obtained from the diet because they cannot be synthesized in adequate quantities by an animals. Otherwise, the deficiency syndrome may occur. Vitamin C (VC) deficiency, called scurvy, causes body weight loss, anorexia, reduction of taste nerve responses (Yasuo et al., 2019), weakness, fatigue, depression, impaired wound healing, dysbasia, dental caries, dysfunctional dentine formation, gingivitis, and dry mouth. It is therefore important to understand how VC intake is controlled in annimals.

To investigate the mechanisms of ingesting VC, we conducted behavioral experiments using osteogenic disorder Shionogi/Shi Jcl- *od/od* (*od/od*) rats, which lack the ability to synthesize VC, and their wild-type controls osteogenic disorder Shionogi/Shi Jcl- +/+ (+/+) rats.

In the previous study, we demonstrated that VC-deficient these rats exhibited an increased consumption of VC solution and decreased consumption of water relative to normal rats in 48-hour two-bottle choice test.

However, it remains unclear whether VC deficiency-induced body weight loss is linked to VC intake or not.

To determine whether body weight loss influences VC intake in VC-deficient *od/od* rats or not. We found that the VC-deficient rats showed an increase in VC intake and a decrease in water intake; in contrast, BWC rats showed a decrease in water intake but no change in VC intake. These data suggest that the change in VC intake displayed by VC-deficient rats cannot be accounted for by changes in body weight. (COI:No)

## 2P-162

### In vivo labelling glucose excited neurons in ventromedial hypothalamus unveils its role in the regulation of systemic glucose metabolism

Ming-Liang Lee, Chitoku Toda, Kazuhiro Kimura (*Dept Biochem, Grad Sch Vet Med, Hokkaido Univ, Japan*)

Hypothalamus plays a critical role in regulating whole body glucose metabolism. Glucose excited (GE) neurons, one of the glucose sensing neurons, are excited by hyperglycemia and believed to reduce blood glucose level by arising glucose uptake of skeletal muscle and adipose tissue and inhibiting liver gluconeogenesis. However, due to a lack of biomarker for GE neurons, it is still a challenge to label and study actual role of these neurons in vivo. Here, we developed a method to label hypothalamic GE neurons in vivo by a technique called targeted recombination in active populations (TRAP), in which the tamoxifen-dependent Cre recombinase will be expressed by the neuronal activity-dependent promoter. We intraperitoneally injected large amount of glucose to activate GE neurons and injected 4-hydroxytamoxifen to trigger expression of a reporter protein in activated neurons. The neurons activated by glucose were therefore labeled and called TRAPed. The ex vivo calcium imaging experiments revealed these TRAPed neurons in the hypothalamus had higher neuronal activity under 2.5mM of glucose and lower activity under 0.2 mM glucose. To interrogate the physiological functions of the TRAPed neurons, we selectively ablated TRAPed neurons in the ventromedial hypothalamus (VMH) by AAV-delivered caspase3. Mice with ablated TRAPed neurons are glucose intolerant and insulin resistant compared to control. In line with this, chemogenetical activation of the VMH TRAPed neurons improved glucose metabolism. Thus, we successfully demonstrated the novel and reliable method to selectively label and manipulate GE neurons and that the GE neurons in the VMH regulates systemic glucose metabolism. (COI:No)

## 2P-163

### Production of anti-melanocortin-4 receptor antibodies to elucidate the mechanism of age-dependent obesity

Manami Oya, Kazuhiro Nakamura (*Dept Integrative Physiol, Grad Sch Med, Nagoya Univ, Japan*)

Obesity is often developed with age due to attenuation of energy expenditure including metabolic thermogenesis. However, the mechanism of age-dependent attenuation of whole-body metabolism is unknown. In this study, we focused on age-dependent alteration of the central neural circuit controlling metabolic thermogenesis in brown adipose tissue (BAT). Our in vivo physiological experiments revealed that skin cooling-induced BAT thermogenesis was attenuated in older (6 months old) male rats compared with younger (9 weeks old) ones. Neurons in the dorsomedial hypothalamus (DMH) are known to mediate thermogenic sympathetic outflow to BAT and express melanocortin-4 receptors (MC4Rs), which play essential roles in the regulation of appetite and energy homeostasis for anti-obesity. Thus, we hypothesized that melanocortin signaling mediated by MC4Rs in the DMH is altered in older animals. Supporting this hypothesis, nanoinjection of melanotan-2 (MT-2), an MC4R agonist, into the DMH induced blunted BAT thermogenesis in older rats, compared to younger rats. We conducted qPCR analysis and found that the MC4R mRNA level in the DMH was comparable between older and younger rats. To elucidate the mechanism of the age-dependent attenuation of MC4R sensitivity to melanocortin signals, we produced an anti-MC4R antibody. Using immunohistochemistry, we have successfully confirmed the specificity of the anti-MC4R antibody in cultured cells and brain slices. We are currently studying the mechanism of age-dependent attenuation of MC4R signaling by analyzing age-dependent alteration of the subcellular distribution of MC4R proteins with this antibody. (COI:No)

## 2P-164

### Impaired systemic glucose metabolism is a delayed onset response of social defeat stress in mice

Kan Kato, Kazuhiro Kimura, Chitoku Toda (*Laboratory of Biochemistry, Department of Veterinary Medicine, Hokkaido University.*)

Depression is a neuronal disorder which influences systemic glucose metabolism, and thus known as a risk factor of the type 2 diabetes mellitus in human. In mice, the chronic social defeat stress (CSDS) is used to promote depressive symptoms, such as the social avoidance and anxiety. Moreover, CSDS increases food intake, promotes obesity and deteriorates glucose tolerance and insulin sensitivity. However, the effect of CSDS on glucose metabolism is still controversial. To clarify the effect of CSDS on systemic glucose metabolism, we examined glucose tolerance test (GTT) in the middle of and after CSDS.

C57BL/6J mice were introduced to the cage housed male ICR mice for 10 minutes to have a physical interaction and kept in the same cage but separated from ICR mice by a wire netting for 24 hours. The same physical and psychological stress were applied to the C57BL mice for 10 days (day1-10). Open field test was done at two days after the end of CSDS (day12) to evaluate the depression. Food intake, body weight and glucose tolerance test were measured during and after the CSDS (day0-19).

Food intake and body weight were not changed in both CSDS and control non-CSDS mice throughout the experiment (day0-19), even though CSDS increased anxiety-like behavior in the open-field test compared to control mice at day12. Blood glucose levels were also not different between groups at day 10. However, the CSDS mice impaired glucose tolerance in GTT compared to control at day17.

Our data suggest that the abnormality of glucose metabolism by CSDS appears after the removal of the stress, which is not seen in the middle of the stress exposure. Thus, the CSDS is an unique stress protocol that affects systemic glucose metabolism with a delayed onset. (COI:No)

## 2P-165

### Effects of day-time feeding on murine skeletal muscle growth and synthesis

Shinya Aoyama[1, 2], Shuichi Kojima[2], Keisuke Sasaki[2], Takeru Shimoda[2], Kengo Takahashi[2], Rina Hirooka[2], Yu Tahara[2], Kazuyuki Shinohara[1], Shigenobu Shibata[2] ([1]*Dept Neuro Behavi, Grad Med Sci, Nagasaki Univ, Japan,* [2]*Faculty of Sci and Eng, Waseda Univ, Japan*)

Muscle mass is controlled by the balance between muscle synthesis and degradation. Although nutrition is important for the maintenance of muscle mass and growth, the effects of feeding time have remained unclear. In the present study, we aimed to evaluate the effects of day- or night-time-restricted feeding on the muscle volume using unilateral hypertrophy mouse models. The day- and night-time-restricted feeding was conducted from zeitgeber time 2 (ZT2) to ZT10 and ZT14 to ZT22, respectively. One week after time-restricted feeding, muscle hypertrophy (overloading) of the plantaris muscle was induced by unilateral surgical ablation of the distal tendons of the gastrocnemius and soleus muscles. Three or seven days after induction of muscle hypertrophy, plantaris muscle were collected at ZT6. The increase in muscle weight was significantly attenuated by the day-time-restricted feeding. The muscle protein synthesis, which was measured using SUnSET, was significantly increased on day 3 after overloading in the NRF group but not in the DRF group. However, on day 7 after overloading, the muscle protein synthesis was increased in the overloaded muscles of both groups, and there was no significant difference between the groups. Similar response was observed in the level of phosphorylated S6 protein, but not total S6 protein. The phosphorylation of S6 was increased by overloading, with the increase being less in the DRF group than in the NRF group. These results suggest that day-time feeding attenuated muscle growth via the inhibition of muscle synthesis. Feeding at an irregular time such as a late-night meal could be detrimental for muscle growth. (COI:No)

## 2P-166

### Effects of oral fatty acid sensitivity depending on menstrual cycle on fat intake in young women

Keiko Morimoto, Yuho Yamauchi, Yuri Mizukami, Haruka Nakayama, Kyoko Ueshima, Akira Takamata (*Dept Environ Health, Hum Life Environ Sci, Nara Women's Univ, Japan*)

Energy intake varies across the menstrual cycle in premenopausal women. Recent evidences suggest that oral detections of fatty acids released from dietary triacylglycerol is involved in fat intake. We examined whether oral sensitivity to fatty acid changes depending on menstrual cycle and affects fat intake in young healthy women. Subjects (n=21) underwent the experiments at 4 phases of menstrual, preovulatory, mid-luteal, and late luteal phases during the menstrual cycle. The oral sensitivity to fatty acid was examined using a three-alternative, forced-choice methodology for oleic acid. For the fat preference test, soups containing 4 different concentrations of canola oil were used for free-choice of the most preferred oil concentration. Furthermore, buccal mucosa was sampled to analyze the mRNA levels of the fatty acid, and estrogen receptors. In addition, they ate the laboratory-prepared lunch consisting of bread with butter and soup in the experimental day.

Oral detection threshold for oleic acid was significantly lower in the preovulatory phase than that in the menstrual phase or late luteal phase. However, there were no differences in fat preference and fat intake from the lunch among these four phases. In contrast, linear mixed-effect model analysis showed negative correlation between the oral fatty acid sensitivity and the fat intake. RT-PCR revealed that CD36 mRNA level in buccal mucosa changed depending on the menstrual cycle.

This study suggests that the oral fatty acid sensitivity changes depending on the menstrual cycle, and affects fat intake through oral CD36 mRNA level in young healthy women.(COI:No)

## 2P-167

### Molecular mechanism of APPL2 on NAFLD/NASH pathogenesis in zebrafish

Magdeline Carrasco [1], Ryoko Higa[1], Kenshiro Shikano[1], Ryohei Umeda[1], Masanori Inoue[2, 3], Kyoko Kiyota [3], Toshikatsu Hanada[2], Kenji Ihara[3], Reiko Hanada[1] ([1]*Dept Neurophysiol, Fac Med, Oita Univ, Japan,* [2]*Dept Cell Biol, Fac Med, Oita Univ, Japan,* [3]*Dept Pediat, Fac Med, Oita Univ, Japan*)

Currently, obesity and its related comorbidities, such as fatty liver disease, are being considered as an important public health problem because they account for the major mortality causes nowadays. In Oita University Hospital, a case of family members was found presenting an obese phenotype. A transcriptome analysis was performed to evaluate which genes could be related to this phenotype, then it was found a point mutation in the gene coding for the adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2 (APPL2). This mutation presented a change of cytosine for thymine in the position 1383 (C1383T). APPL2 and its homologous APPL1 are adaptor proteins that interact with the intracellular region of adiponectin receptors and mediate the mechanisms of adiponectin effect in glucose and fatty acids metabolism. Previous studies report a tight association between APPL2 and Non-Alcoholic Fatty Liver Disease (NAFLD), however, the molecular and physiological mechanisms are not yet elucidated. In this study, to reveal the pathophysiological role of APPL2 in NAFLD/NASH and obesity; we have established an APPL2 mutant zebrafish (Danio rerio). Elucidation of APPL2 molecular and physiological mechanisms in NAFLD/NASH and obesity entails a deeper understanding of these pathologies and their development which could allow for potential diagnostic and therapeutic approaches. (COI:No)

## 2P-168

### Alteration of $Ca^{2+}$ release via ryanodine receptors in the hippocampus of depression-like model mice

Emi Nakamura-Maruyama, Naoyuki Himi, Kazuhiko Narita, Risa Kai, Naohiko Okabe, Osamu Miyamoto (*Dept Physiol[2], Kawasaki Med Sch, Japan*)

Although depressive disorders are common diseases, their pathogenic mechanisms are not fully understood. We have previously reported the changes in the expression of ryanodine receptors (RyRs) in the hippocampus using depression-like model mice. In this study, in order to investigate the functional changes of RyRs in depressive condition, we examined the alteration of $Ca^{2+}$ release via RyRs.

We used the depression-like model mice (C57BL/6J, male, 11-12w) subjected to water immersion with restraint stress for 2 weeks. The hippocampal slices (350 $\mu$m) of the mice were made by using a vibratome, the fluorescence intensity changes of pyramidal cells in the dentate gyrus were measured as the amount of $Ca^{2+}$ release by introducing $Ca^{2+}$ ion indicators (Oregon Green 488 dye). Similarly, $Ca^{2+}$ release in the hippocampal slices of the mice which were treated with electro convulsive shock (ECS) was also measured. Induction of $Ca^{2+}$ release via RyRs was performed by flushing 200 $\mu$l of caffeine (40mg/ml) into the recording chamber (final concentration about 4mM). Concomitantly, actions of caffeine except for RyRs agonist were limited by bath application of selective A1 and A2A adenosine receptor competitive antagonist, 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) and SCH58261, respectively.

As a result, the number of cells that showed caffeine-induced $Ca^{2+}$ release decreased in the hippocampal dentate gyrus of the model mice, and the incidence of caffeine-induced oscillation also decreased compared with normal mice. On the other hand, these values of the model mice returned to the level of normal mice by ECS.

These results suggest that the ability of $Ca^{2+}$ release via RyRs decreased at the depressive condition and returned to the normal level according to the recovery from it. Ryanodine receptors might be a novel therapeutic target for depressive disorders. (COI:No)

## 2P-169

### Identification of a novel inhibitory factor for metastasis of mouse ovarian tumor cells

Naofumi Miwa, Hiromasa Satoh, Ryohei Saito, Mayu Hanaue (*Dept.Physiol., Saitama Med. Univ., Japan*)

We have previously characterized a novel egg-coating envelope protein that suppresses fertilization in Xenopus lavis (Hanaue and Miwa Sci Rep 2017). That protein (named dicalcin) binds to a glycoprotein, a constituent of the egg-coating envelope filament; regulates the orientation pattern of the filaments and the viscoelasticity of the entire envelope, thereby suppressing sperm-egg interaction. Cancer metastasis is a complex series of cellular processes involving glycoprotein and oligosaccharides. To contribute to the study of cancer metastasis, we investigated the action of dicalcin on the metastasis of mouse ovarian tumor cells. Extrinsically applied dicalcin remarkably binds to the cell surface as well as the cytoplasm of OV2944 cells. Pretreatment of OV 2944 cells with extracellularly applied dicalcin inhibited in vitro invasion using Matrigel chamber in a dose-dependent manner. It also suppressed the binding of OV2944 cells to Matrigel significantly; however, it unaffected the cell viability assessed by the MTT assay, which indicated that it suppresses in vitro invasion through its direct binding to OV2944 cells. Time-lapse imaging analyses discovered that the extracellular presence of dicalcin inhibited migration of OV2944 cells on the plastic plate. We next examined in vivo survival of mice that were injected with OV2944 cells. Concurrent injection with dicalcin-derived peptide significantly prolonged the survival days of the mice, indicating that dicalcin inhibited metastasis of OV2944 cells in vivo. In summary, our novel results elucidated the suppressive action of dicalcin on metastasis of mouse ovarian tumor cells through its binding to OV2944 cells. We believe that our present study will provide insight into molecular machinery of metastasis processes, and may lead to the development of potent bioactive compound that is capable of inhibiting cancer metastasis. There are no conflicts of interest to declare. (COI:No)

## 2P-170

### Effects of calcium-release activated calcium channel on signal transduction of mechanical stimulation to mouse synovial cells cultured in medium containing IL-6

Yu Okumura[1,2], Keiji Asada[1,3], Miyako Takaki[1] ([1]*Dept. of Orthopaedic Surgery, Nara Med. Univ., Kashihara, Japan,* [2]*Dept. of Phys. Ther., Fac. of Human Sci., Osaka Univ. of Human Sci., Japan,* [3]*Dept. of Rehab., Fac. of Health Sci., Suzuka Univ. of Med. Sci., Suzuka, Japan*)

**Purpose:** Rheumatoid arthritis (RA) is a chronic inflammatory disease mainly consisting of inflammation of the joint synovium, and interleukin-6 (IL-6) is involved in these symptoms. In addition, previous studies have reported that calcium-release activated calcium channel (CRAC) is involved in the inflammatory response of synovial tissue in RA. However, its mechanism has not been clarified. In the present study, we focused on the contribution of CRAC to the response of mechanical stimulation (MS) in mouse synovial cells cultured in medium containing IL-6.
**Methods:** Mouse synovial cells cultured in a control medium or a medium containing IL-6 were used in the experiment. Intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) of synovial cell was measured with loaded Fluo-3 AM by adding twice MS by a glass micropipette.
**Results:** Each MS elicited immediately $[Ca^{2+}]_i$ increasing response in the presence of extracellular $Ca^{2+}$. When the CRAC inhibitor (YM-58483) was treated, the ratio of the second response to the first one in synovial cells cultured in medium containing IL-6 was significantly smaller than that cultured in control medium.
**Conclusions:** The result indicated that CRAC possibly was involved in $[Ca^{2+}]_i$ increasing response to MS in mouse synovial cells cultured in medium containing IL-6. (COI:No)

## 2P-171

### Role of activated microglia/macrophages in brain edema formation after brain infarction

Yukie Murata[1], Kana Sugimoto[1], Chihpin Yang[1], Rina Gono[1], Teiji Harada[1], Yohei Miyashita[1], Kazuma Higashisaka[1], Kazuo Harada[1], Ryuichi Katada[1], Junya Tanaka[2], Hiroshi Matsumoto[1] ([1]*Dept Leg Med, Grad Sch Med, Osaka Univ, Japan,* [2]*Molecular and Cellular Physiology, Grad Sch of Med, Ehime Univ, Japan*)

Brain edema after brain infarction affects mobility and mortality. Aquaporin-4 (AQP4), a water channel protein, is expressed mainly in astrocytes and have increased after stroke in animal experiments. Therefore, AQP4 may be a therapeutic target in ischemic brain edema, but its expression mechanism is unclear. On the other hand, they have been reported in the animal stroke model that activated resident microglia (MG) and bone marrow-derived infiltrating macrophages (MPs) accumulate in the ischemic core and stimulate astrocytes in the ischemic core and penumbra after brain infarction and that IL-1$\alpha$, TNF, and C1q produced from activated MG are involved in astrocytes activation. Therefore, we investigated the role of MG/MPs on the expression of AQP4 in the transient middle cerebral artery occlusion (tMCAO) model rats and in the primary rat cultured astrocytes.
Brains were isolated at 0.25-7 days postreperfusion (dpr) in the tMCAO model. AQP4 mRNA level significantly increased in the penumbra at 3-7 dpr and core at 7 dpr. The change in AQP4 mRNA expression was well correlated with those of Iba1, IL-1$\alpha$, TNF, and C1q mRNA. Furthermore, the rats with high expression of AQP4 mRNA in the ischemic core at 7 dpr had high expression of IL-1$\alpha$, but TNF and C1q did not. In the primary rat cultured astrocytes, MG/MPs-conditioned medium or IL-1$\alpha$ treatment increased AQP4 mRNA expression. Addition of IL-1 receptor type I antagonist reduced the increase in AQP4 expression. MG "signature" genes such as P2ry12, Olfm13, Gpr34, and Pros1 were enriched in the ischemic core of $AQP4^{High}$/ IL-1$\alpha^{High}$ rats when compared to $AQP4^{Low}$/ IL-1$\alpha^{Low}$ rats, while MPs "signature" genes such as Ccr2 and Mybl2 were expressed at low levels. These findings suggest that IL-1$\alpha$ produced by the activated resident MG induces the AQP4 expression in the astrocytes in the ischemic core and penumbra. (COI:No)

## 2P-172

### Molecular mechanisms of Wischnewski spot development on gastric mucosa in fatal hypothermia: an experimental study in rats

Chihpin Yang, Kana Sugimoto, Yukie Murata, Rina Gono, Yuichiro Hirata, Yohei Miyashita, Kentaro Nakama, Kazuma Higashisaka, Kazuo Harada, Ryuichi Katada, Hiroshi Matsumoto (*Dept Leg Med, Grad Sch Med, Osaka Univ, Japan*)

Numerous dark-brown-colored small spots called "Wischnewski spots" are often observed on the gastric mucosa in fatal hypothermia, but the molecular mechanisms through which they develop remain unclear. We hypothesized that hypothermia may activate the secretion of gastric acid and pepsin, leading to the development of the spots. To investigate this, we performed experiments using organotypic rat gastric tissue slices culture. Stomachs were obtained from male Wistar rats (8 weeks old) after fasting for 6 hours. Gastric slices of 1-2 mm were taken from stomach between cardia and pylorus. The organotypic gastric slices were cultured in 37℃ (control) or 32℃ (cold) incubators. Cold loading for 6 h lowered the extracellular pH in the culture medium. The mRNA expression of gastrin, which regulates gastric acid secretion, increased after cold loading for 3 h. Cold loading increased the expression of gastric $H^+$, $K^+$-ATPase pumps in the apical canalicular membrane and resulted in dynamic morphological changes in parietal cells. Cold loading resulted in an increased abundance of pepsin C protein and an elevated mRNA expression of its precursor progastricsin. Collectively, our findings clarified that cold stress induces acidification by activating gastric $H^+$, $K^+$-ATPase pumps and promoting pepsin C release through inducing progastricsin expression on the gastric mucosa, leading to tiny hemorrhages or erosions of the gastric mucosa that manifest as Wischnewski spots in fatal hypothermia. (COI:No)

## 2P-173

### Mechanism of exercise-related sudden death under high temperature environment

Rina Gono (*Dept Leg Med, Grad Sch Med, Osaka Univ, Japan*)

Tokyo Olympic will open at 2020 summer. Recently, The International Olympic Committee announced that it was planning to move the Olympic marathon and race walking events to Sapporo, the north city of Japan, which will mean significantly lower temperatures for the athletes during the Olympic Games. Exactly, in the Utstein Osaka Project, 0.7% of out-of–hospital cardiac arrest (OHCA) of cardiac origin (222/31, 030) between 2005 and 2012 in Osaka Prefecture was related to exercise. Exercise increases core body temperature, leading to a possibility of heat stroke, one of important factors in the sudden death during exercise. We have showed that heat stress alone induced alteration of electrical rhythms using the heat stroke rat model and rat cardiomyocytes. Therefore, we hypothesized that exercise-induced heat stroke increases a possibility of onset of arrhythmia. Then, the aim of this study was to clarify the effect of exercise on onset of arrhythmia under high temperature environment. Male Wistar rats were initially subjected to an incremental speed exercise to evaluate their exercise capacity. On the following day, they were randomly divided into 4 experimental groups: (i) control, (ii) exercise, (iii) heat, and (iv) exercise and heat. Rats were exercised at 15 m/min speed for 15-30 min under temperate (22 ℃) or hot (37-40 ℃) environment condition. After exposure, the rectal temperatures and electrocardiograms were measured under anesthesia with isoflurane, and mRNA expression of various genes in heart was detected by quantitative real-time RT-PCR. The rectal temperatures and heart rates were higher in the exercise and/or heat groups than the control group. The exercise and/or heat stress significantly changed mRNA expression of autonomic nervous system-related genes. Therefore, these finding suggested that exercise-induced sudden death in the condition with high atmosphere temperature may be mediated by the change of autonomic nervous system mechanisms. (COI:No)

## 2P-174

### Effects of breast milk-derived probiotics on a mouse model with colorectal cancer induced by AOM/DSS treatment

Keizo Hiraishi[1,2], Haiyan Xu[3], Lin-Hai Kurahara[1,2], Heping Zhang[3], Katsuya Hirano[2], Ryuji Inoue[1] ([1]*Dept. Physiol., Sch. Med., Fukuoka univ., Japan,* [2]*Dept. Cardiovasc. Physiol., Fac. Med., Kagawa univ., Japan,* [3]*Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural univ., P.R.China*)

**Background:** In ulcerative colitis (UC), one of inflammatory bowel diseases (IBD), the risk of colorectal tumors increases due to repeated remission and relapse of inflammation. Whether this tumorigenic process involves intestinal flora has not been investigated yet. It has been reported that the longer period of breastfeeding is correlated with the lower risk of IBD and inflammatory tumors. In this study, we therefore examined whether breast milk-derived probiotics have an effect on inflammation-related colon polyp formation.
**Method:** A colorectal cancer model was prepared by treating 6-week-old C57BL/6NCrSlc mice with a single i.p. injection of azoxymethane (AOM : 12 mg/kg BW) and subsequent 2 sets of 7-day administration of 2% dextran sulfate sodium (DSS) in drinking water with one week intermission. Breast milk-derived probiotic *Lactobacillus rhamnosus* M9 (2 x$10^9$ cells/day/mouse) was administered to these mice by gavage in 2 sets of 7-day administration with one-week intermission.
**Result and summary:** Twenty weeks after AOM administration, AOM+DSS group exhibited an increased number of polyps, accompanied by a significant weight loss, bowel shortening, increased scores of pathological stool and colon pathology. These changes were significantly suppressed by M9 treatment. In addition, M9 suppressed the increase of interleukin-6/p-STAT3 and the Akt/NF-$\kappa$B signaling associated with tumorigenesis. The metagenomic analysis indicated a marked increase in *Bacteroides* and *Helicobacter hepaticus*; a marked decrease in *Eubacterium plexicaudatum* in the AOM + DSS group compared with the vehicle group, and these changes were suppressed by M9 treatment.
These results suggest that breast milk-derived *Lactobacillus rhamnosus* M9 suppressed inflammatory tumorigenesis by improving the intestinal flora and inhibiting inflammatory signaling in colon. (COI:No)

## 2P-175

### Influence of repeated restraint stress on the brain microglia and gut microbiota in the rat

Toshiyuki Saito[1, 2], Yui Tsuzuki[2], Chisako Kajiwara[2], Kurumi Yokoyama[1], Minori Shibutani[1]
([1]*Grad Sch Life Sci, Kyoto Sangyo Univ, Japan,* [2]*Dept Anim Med Sci, Fac Life Sci, Kyoto Sangyo Univ, Japan*)

The hypothalamic-pituitary-adrenal (HPA) axis is the major neuroendocrine system in response to physical and psychological stressors. It is well-known that repeated or chronic exposure to stressors may disturb regulation of the HPA axis, leading to neural diseases such as anxiety and depression. However, patho-physiological mechanism still remains unknown to cause the neuro-endocrine disturbance with the stress response. In this study, we examined a sign of pro-inflammatory events in the brain and any changes in composition of gut microbiota during repeated restraint stress. Male Wistar-Kyoto rats (180-220 g) repeatedly received the restraint (2 hours in a day, totally for 14 days). The level of the plasma corticosterone (CORT) increased on the day 1 and 7, but decreased on the day 14. In the paraventricular nucleus (PVN), there were observed the Iba-1 immuno-positive cells (microglia) with only several branches. Analysis of 16SrRNA in the feces (sampled on the day 14) shows tendency for decrease in the ratio for Lactobacillus in Firmicutes by the restraint, though the CORT level declined to that before the stress exposure (day −1). The present repeated restraint may change the gut microbiota with delayed onset. Further studies need to examine relation between microglial differentiation (pro-inflammatory or neuro-protective) in the PVN and change in composition of the gut microbiota by the repeated restraint stress. (COI:No)

## 2P-176

### Effect of homogenate extract from adult skeletal muscles on the proliferation and differentiation of myoblasts

Maki Yamaguchi[1], Takuhiro Kawahara[1], Kie Iida[2], Toshiko Yamazawa[1], Nobutake Akiyama[3], Shigeru Takemori[1]
([1]*Dept Mol Physiol, Jikei Univ Sch Med, Japan,* [2]*Dept Immunology, Jikei Univ Sch Med, Japan,* [3]*Dept Ophthalmology, Jikei Univ Sch Med, Japan*)

Residing next to mature skeletal muscle fibers, satellite cells are known to serve as the progenitor of myoblast when triggered by various stimuli including damage to the muscle fibers in vivo. Matsuoka et al. (2008) reported that the homogenate extract of various types of adult chicken skeletal muscles induced differentiation of primary culture of myoblast prepared from chicken embryo to express myosin heavy chain isoforms of the source skeletal muscle of the extract. Inspired from their work, we tested the effect of homogenate extract from adult mouse skeletal muscle on primary culture of satellite cells prepared from young mice. Unexpectedly, the extract seemed to enhance proliferation of the satellite cells without inducing obvious differentiation in the expression pattern of myosin heavy chain. However, its effect on the proliferation of satellite cells was unclear because of cellular contaminant such as fibroblast and adipogenetic cells. So we tested the effect of muscle extract on C2C12 myoblast. The proliferation of C2C12 myoblast was evaluated by the cytotoxic assay method. It was found that the extract enhanced the proliferation of C2C12 myoblast dose dependently. The mechanism of function of the extract will be discussed. (COI:No)

## 2P-177

### Changes in ventilatory and muscle oxygen dissociation states in subjects not accustomed to exercise with vocalization

Hajime Arikawa[1], Toshio Matsuoka[1], Teppei Takahashi[2], Tomoyoshi Terada[3], Seiichi Era[4]
([1]*Faculty of Sports and Health Sci, Chubu Gakuin Univ, Seki, Japan,* [2]*Dept Oral and Maxillofacial Surgery, Gero Hospital, Gero, Japan,* [3]*United Graduate School of Drug Discovery and Medical Information Sci, Gifu Univ, Gifu, Japan,* [4]*Clinical Laboratory Center, Japanese Red Cross Gifu Hospital, Gifu, Japan*)

**Purpose:** We had previously reported that vocalization during continuous upper-limb exercise at 80%$VO_2$peak by subjects accustomed to exercising with vocalization (such as *kendo* players) tended to increase the value of $FetCO_2$ (=$PaCO_2$) and to suppress the decrease of oxygen dissociation states in exercising muscles. However, vocalization during exercise also suppresses ventilations, which may make it difficult for those who are not used to exercising with vocalization to continue voicing or exercising. This study aimed to clarify the state of ventilation and active muscle oxygen saturation during continuous upper-limb exercise in persons who are not used to exercising with vocalization.
**Methods:** Eleven male subjects who were not used to exercising with vocalization participated in this study. They performed continuous upper-limb exercises using a hand ergometer at 80%$VO_2$peak for 3 min under two conditions: "with vocalization (Voc)" or "without vocalization (non-Voc)". We measured respiratory variables (such as $FetCO_2$), venous oxygenation index (VOI) of middle fingertip, and tissue saturation index (TSI%) of triceps brachii (using NIRS), before and immediately after exercise, and blood lactate concentration ($[Lac^-]$) before and 5 min after exercise.
**Results:** Voc value showed no difference in $\Delta FetCO_2$ compared to non-Voc value, but $\Delta$VOI tended to decrease more, $\Delta$TSI% tended to decrease less, and $\Delta[Lac^-]$ tended to increase more (no significant difference in any of them). In addition, some subjects were unable to continue vocalizing during exercise.
**Conclusions:** We observed that blood $CO_2$ concentration did not increase because of inaccurate vocalization during exercise. Even in this situation, the decrease in oxygen saturation of active muscles tended to be suppressed. From these results, we inferred that rhythmic vocalization with upper-limb exercise increased the rate of anaerobic energy supply and suppressed the increase in oxygen consumption in active muscles. (COI:No)

## 2P-178

### Age-related difference in autophagic adaptation and the effect of resistance exercise in rat soleus muscle atrophied with unloading

Hideki Yamauchi, Shigeru Takemori (*Div Phys Fitness, Dept Mol Physiol, The Jikei Univ Sch Med, Tokyo, Japan*)

**Aim:** We have reported that unloading selectively affected type I fibers in aged rats disrupting myofibrils with a decrease in sarcomeric proteins, forming inclusion body, and accumulating abnormal mitochondria. Here, we aimed to clarify age-related differences in autophagic adaptation and the effects of intermittent resistance-exercise (IRE) in unloaded muscles of rats. IRE is known to ameliorate sarcopenia.
**Methods:** Young (4 months, n=21) and aged (2 years, n=21) F344 female rats were randomly divided into control, unloading, and unloading + IRE groups. Rats of the unloading and unloading + IRE groups have their hindlimbs unloaded by tail-suspension. 10-min IRE was performed in unloading + IRE group 3 times per day every 4 hours in the dark period for 3 weeks. Soleus muscle was examined at the end of the intervention.
**Results:** Unloading-induced atrophy with a degenerative decrease in myofibrillar protein concentration was more prominently observed in aged than in young rats. Fbx32, a muscle specific ubiquitin ligase, increased along with an increase in ubiquitinated protein by unloading in both aged and young rats. LC3-II, an autophagy marker protein, and mitochondrial calcium uniporter (MCU), a key protein activating mitochondrial biogenesis and of signaling pathways for muscle hypertrophy, increased with unloading in young rats but decreased in aged rats. PGC1, playing similar roles to MCU, specifically decreased with unloading in aged than in young rats. Intermittent IRE ameliorated atrophy similarly in the rats of both ages, while the levels of LC3-II, MCU, and PGC1 were still lower than the control level in aged, but not in young rats.
**Conclusion:** Autophagic adaptation and myogenic response were critically different with age in rat soleus. These differences may be responsible for age-related muscle responsiveness to unloading and training. (COI:No)

## 2P-179

### Delayed umbilical cord clamping alters blood leukocyte profiles of neonatal rats

Ryo Ishiwata, Yuji Morimoto (*Dept Physiol, National Defense Medical College, Japan*)

**Background:** Delayed umbilical cord clamping (DUC) at birth is suggested to provide clinical benefits such as prevention of neonatal anemia and reducing the risk of late-onset sepsis. While it is reported that DUC increases blood supply and the hematocrit values of neonates, the effects of DUC on blood leukocyte profiles are not known.
**Methods and Results:** Full term Wistar rat fetuses at embryonic day 21 (e21) were used in the study. Fetuses were delivered by Caesarean section. In the control group, the placenta and the umbilical cord were immediately removed, while in the DUC group, the placenta and the umbilical cord were kept attached for 1h until the collection of peripheral blood. The collected blood samples were removed of red blood cells and were analyzed by flow cytometry. Compared with prenatal fetuses, granulocyte population in the blood was increased in the control group (1h after birth) and was further higher in the DUC group (8.1 ± 1.1%, 11.6 ± 1.7%, and 15.6 ± 2.2%, respectively, n = 5-6, $p$ <0.05).
Granulocyte-stimulating factor (G-CSF) is a cytokine mediating release of granulocytes into blood stream. Quantitative RT-PCR revealed that mRNA expression of G-CSF in umbilical vein was higher than that in umbilical artery (2.01 ± 0.37-fold, n=6, $p$ <0.05) and significantly increased by embryonic day (e21 vs. e19, 10.9 ± 2.03-fold, n=6, $p$ <0.01). Immunohistochemistry showed that G-CSF was predominantly expressed in umbilical vein endothelial cells in e21 rat fetuses.
**Conclusion:** DUC increased granulocyte population in rat neonatal peripheral blood. G-CSF that were highly expressed in umbilical vein endothelium may play a role in this process. (COI:No)

## 2P-180

### The roles of PAI-1 in the delayed bone repair induced by glucocorticoid

Kiyotaka Okada[1, 2], Takahiro Okamoto[2], Yoshimasa Takafuji[2], Masayoshi Ishida[2], Naoyuki Kawao[2], Osamu Matsuo[2], Hiroshi Kaji[2]
([1]*Dept Arts Sciences, Kindai Univ Facul Med, japan,* [2]*Dept Physiol Regenerat Med, Kindai Univ Facul Med, japan*)

Glucocorticoids (GCs) regulate numerous physiological processes in a wide range of tissues. GCs have been widely used for the treatment of chronic inflammatory diseases, such as the inflammatory bowel diseases, collagen diseases and rheumatoid arthritis for the anti-inflammatory effects. It is an important clinical task that GC treatment induces osteoporosis, and previous studies suggest that chronic GC treatment induces the delayed bone repair; however, the mechanisms by which GC induces the delayed bone repair have still remained unclear. Here, we investigated the roles of plasminogen activator inhibitor-1(PAI-1) in GC-induced effects on bone repair after femoral bone injury using female mice with PAI-1 deficiency and their wild-type counterparts. Dexamethasone (Dex) enhanced plasma PAI-1 levels and PAI-1 mRNA levels in the adipose tissues and muscles of wild type mice. PAI-1 deficiency significantly blunted the delayed bone repair induced by Dex in mice. Moreover, PAI-1 deficiency significantly blunted Runx2 mRNA levels and number of alkaline phosphatase-positive cells suppressed by Dex as well as Dex-induced osteoblast apoptosis at the damaged site 7 days after bone injury of mice. On the other hand, PAI-1 deficiency did not affect the ratio of receptor activator of nuclear factor $\kappa$B ligand / osteoprotegerin suppressed by Dex at the damaged site 7 days after bone injury of mice. Moreover, PAI-1 deficiency did not affect the adipogenic gene expression enhanced by Dex at the damaged site 7 days after bone injury in mice. In conclusion, we first showed that PAI-1 is involved in the delayed bone repair after bone injury induced by GC in mice. PAI-1 might influence early stage osteoblast differentiation and apoptosis during the osteoblastic restoration phase of bone repair process. (COI:No)

## 2P-181

### Significance of measuring the blood platelet-derived microparticles (PDMP) and GPIb after pediatric hematopoietic stem cell transplantation

Mariko Nakamura[1], Chisaki Tamayose[1], Satoru Hamada[2], Chiaki Kijima[1], Nobuyuki Hyakuna[2], Masanori Sunagawa[3], Koichi Nakanishi[2], Masayuki Matsushita[1] (*[1]Dept of Mol Cell Physiol, Grad Sch Med, Univ Ryukyus, Japan, [2]Dept Child Health, Grad Sch Med, Univ Ryukyus, Japan, [3]Grad Sch Nurs, Meio Univ, Japan*)

Pediatric hematopoietic stemcell transplantation has develop transplant-related complications: mucosal damage associated with pretreatment of anticancer drugs and radiation, organ damage, severe infections associated with immunodeficiency and GVHD. After more than 3 months of transplantation, life-threatening complications such as DIC, TMA, SOS are caused by vascular endothelial injury. Clinical parameters for early detection of such coagulation disorders and their measurement are expected. In this study, we focused on PDMP levels and platelet membrane GPIb $\alpha$ kinetics for the purpose of identifying early coagulation disorders after transplantation. As a result, an increase in PDMP value was observed in 4 out of 10 transplant patients at 4 weeks after transplantation, and the decrease in GPIb $\alpha$ expression, which was simultaneously measured by the FACS, was observed in 3 cases. Although PDMP did not increase, only GPIb $\alpha$ decreased in 2 cases. In the 5 cases with decreased GPIb $\alpha$ exhibited severe complications such as severe infection, severe GVHD, autoimmune hemolytic anemia, and nephrotic syndrome. In the 4 cases with increased PDMP, because proteases induced by complications (inflammation/infection) after pediatric allogeneic hematopoietic cell transplantation may act on the platelet membrane, these are released into the plasma from the platelet membrane. Since GPIb $\alpha$ has a binding site for thrombin and vWF, we performed in vitro experiments using GPIb $\alpha$ and thrombin. As a result, the released GPIb $\alpha$ was not detected by the FACS when bound to thrombin, indicating that the decrease in plasma GPIb $\alpha$ in patients with post-transplant complications is related to thrombin production in vivo. Post-transplant patients are likely to cause the complications when GPIb $\alpha$ decreases and PDMP increases. Early detection of plasma GPIb $\alpha$ decrease can detect early complications such as hypercoagulation after transplantation. Therefore, early detection of the dynamics of GPIb $\alpha$ and PDMP after transplantation is presumed to be useful for early treatment of complications after transplantation. (COI:No)

## 2P-182

### Endogenous hydrogen sulfide is essential to maintain eupneic respiration in the *in situ* arterially perfused preparation of rats

Minako Okazaki[1, 2], Saori Uozu[1], Tadachika Koganezawa[1, 3] (*[1]Dept Physiol, Fac Med, Univ Tsukuba, Tsukuba, Ibaraki, Japan, [2]Mstr Prog Med Sci, Grad Sch Comp Human Sci, Univ Tsukuba, Ibaraki, Japan, [3]Transborder Med Res Ctr, Univ Tsukuba, Ibaraki, Japan*)

Hydrogen sulfide ($H_2S$) is generally known as toxic gas but endogenously generated and has physiological roles in our body including the brain. However, roles of endogenous $H_2S$ to generate respiratory pattern in the respiratory center is not understood. The aim of this study was evaluation of functional roles of endogenous $H_2S$ in generating the respiration.

We performed *in situ* arterially perfused preparations of decerebrated rats and recorded the phrenic and vagus nerves activities. We administrated amino-oxyacetate hemihydrochloride (AOA: 10 mM) or hydroxylamine hydrochloride (HA: 2 mM) to inhibit $H_2S$ production *via* cystathionine $\beta$-synthase (CBS), DL-propargylglycine (PAG: 10 mM) to inhibit it *via* cystathionine $\gamma$-lyase (CSE), and S-adenosyl-L-methionine chloride dihydrochloride (SAM: 60 $\mu$M) to activate it *via* CBS. Further, we applied riluzole (5 $\mu$M) to block persistent sodium channels which can suppress pacemaker cells-based activity. And then, we compared the central respiratory pattern before and after administration of drugs.

By inhibiting the CBS with AOA or HA, the respiratory pattern was switched from three-phases eupneia into gasping-like respiration. Respiratory frequency and amplitude of phrenic and vagus nerves activities were significantly decreased by the inhibition. On the other hand, in the presence of both riluzole and AOA or HA, gasping was little observed. Neither inhibiting CSE with PAG nor activating CBS with SAM affected to eupneic respiratory pattern.

These results suggested that CBS-produced $H_2S$ has vital roles to maintain neuronal network to generate eupnea. The mechanism to generate respiratory pattern might be switched from network- to pacemaker cells-based systems depending upon $H_2S$ concentration. (COI:No)

## 2P-183

### The pathway transmitting hypoxia information from the solitary nucleus to the hypothalamus

Shigefumi Yokota[1], Noriyuki Hama[2], Kotaro Takeda[3], Masashi Fujitani[1] (*[1]Dept Anat & Neurosci, Shimane Univ Sch Med, Japan, [2]Dept Physiol, Shimane Univ sch med, Japan, [3]Fac Rehabili, Sch Healthcare, Fujita Hlth Univ, Japan*)

Obstructive sleep apnea decreases arterial oxygen pressure and then facilitates breathing as well as arousal from sleep. It is suggested that the parabrachial nucleus (PB) known as the pontine respiratory group is a key region in arousal by hypoxia. However, the neural circuits on this response are not fully understood. Under hypoxia, glutamatergic neurons in the medial part of the caudal solitary nucleus (cNTSm) are activated. Therefore in this study, we first examined the indirect pathway via the PB from glutamatergic neurons in the cNTSm to the perifornical area in the hypothalamus (PeF) where orexinergic neurons located. By using a combined methods of a genetically anterograde labeling of glutamatergic cNTSm axons and retrograde tracing, we found a dense plexus of axons originating from the cNTSm in the external lateral PB subnucleus (PBel) and additional distribution of many axons in the central lateral PB subnucleus (PBcl) and dorsal lateral PB subnucleus (PBdl). On the other hand, PeF-projecting neurons were observed in the PBcl and PBdl and they were overlapped to the distribution of cNTSm axons. We also found that CGRP-immunoreactive neurons, which are indicated as arousal-inducing neurons were embedded in the plexus of cNTSm axons in the PBel. Next, we demonstrated that some PeF-projecting neurons in the PBcl and PBdl, as well as many CGRP-immunoreactive neurons in the PBel, expressed Fos protein after exposure to 8% hypoxic condition, using a combination of immunohistochemistry and retrograde tracing. These results suggest that the glutamatergic cNTSm neurons may exert excitatory influence not only upon PB neurons projecting to the PeF but also upon CGRP neurons in the PBel to induce arousal by hypoxia. (COI:No)

## 2P-184

### Resting-State fMRI Connectivity Analysis between Hippocampus and Motor area in COPD patients

Akira Yoshikawa[1], Yuri Masaoka[1], Masaki Yoshida[2], Nobuyoshi Koiwa[3], Natsuko Iizuka[1, 4], Satomi Kubota[1, 4], Ryo Manabe[1, 5], Masahiro Ida[6], Masahiko Izumizaki[1] (*[1]Dept Physiol, Showa Univ, Sch Med, Tokyo, Japan, [2]Dept Ophthal, Jikei Med Univ, Tokyo, Japan, [3]Dept of Health and Sci, Univ Human Arts and Sci, Saitama, Japan, [4]Dep Neulolo, Showa Univ, Univ, Sch Med, Tokyo, Japan, [5]Dept Respiratory, Showa Univ, Sch Med, Tokyo, Japan, [6]Natl Hosp Org, Mito med Ctr, Tokyo, Japan*)

Chronic obstructive pulmonary disease (COPD) is a primary airway inflammatory disease characterized by irreversible airflow limitation which results in hypoxemia and hypercapnia. Meanwhile, it is also realized as a complex multi-component disorder. Cognitive impairment has been found as one of the important extrapulmonary manifestation in patients with COPD. In our previous study, we found that left hippocampus volume was smaller in COPD patients compared with those of control group. Resting-state functional magnetic resonance imaging (rs-fMRI) connectivity analysis is one of the valuable ways to observe functional changes in human brain, especially cognitive functions. In this study, we evaluated the functional connectivity (FC) between left hippocampal and other brain area in COPD patients.

20 COPD patients and age-matched 25 control subjects participated in the present study. Clinical 3T (Siemens) was used. Four minutes and thirty seconds rs-fMRI was recorded with cardiac pulse wave and respiration. We conducted a region of interest (ROI)-to-ROI FC analysis. The FC between the left hippocampus and other brain were calculated with CONN toolbox.

COPD patients had significant lower FC between the left hippocampus and the right sensorimotor area than controls. In addition, COPD patients also had significant lower FC between left and right hippocampus.

This study showed COPD having restricted physical activity due to respiratory problem accompanied with dyspnea, anxiety and sleep problem may affect hippocampus volume and reduced FC between hippocampus and motor-related areas. Since physical activity is a prognostic factor in COPD, reduced FC between hippocampus and motor-related areas could be one of an index for severity for restricted activity and poor quality of life. (COI:No)

## 2P-185

### Neural activity of place cell in various mazes in the same experimental room

Kana Mieno, Satoshi Hoshino, Kaoru Ide, Susumu Takahashi (*Grad Sch of Brain Science, Doshisha Univ, Japan*)

Various forms of maze such as the T-maze, plus maze, and radial maze are routinely used to examine learning and memory in animals. Place cells form a cognitive map because they fire only when an animal passes through a particular location. The firing pattern of hippocampal place cells tends to change when an animal moves to another room or the shape of the room changes. However, conventional mazes cannot easily change shape in the same physical location. Thus, it is difficult to complementarily compare and examine the results of experiments conducted in the various shape of the maze. Here, we developed a system that can reconfigure the maze shape in the same room. To examine how changes in cognitive maps affect learning and memory, we constructed several different shaped mazes using this system and examined whether it affects their running trajectory and the response of hippocampal CA1 place cell activity. First, we changed the shape of the maze from square to cruciform. As a result, place field location in the hippocampus was shifted. Next, we changed the shape of the maze from the cruciform back to the square, place field location returned to its original position. These findings suggest that the change of the maze shape in the same room caused different place cell representation. Besides, to examine hippocampal-dependent working memory, we trained rats to perform the delayed spatial alternation task for 10 days. As a result, the performance significantly improved with experience. Finally, we discuss the results of a preliminary experiment in which the shape of the figure 8 maze is scaled or zoomed in the vertical or horizontal directions. (COI:No)

## 2P-186

### In vivo calcium imaging with a single cell resolution using "cosmoscope", a new wide-field two-photon microscope

Keisuke Ota[1], Yasuhiro Oisi[1], Takayuki Suzuki[1], Tsubasa Ito[1, 2], Yoshiki Ito[1, 3], Kenta Kobayashi[4], Midori Kobayashi[1], Maya Odagawa[1], Chie Matsubara[1], Yoshinori Kuroiwa[5], Masaru Horikoshi[5], Junya Matsushita[6], Hiroyuki Hioki[7], Masamichi Ohkura[8], Junichi Nakai[9], Masafumi Oizumi[1, 3], Atsushi Miyawaki[1], Toru Aonishi[1, 2], Takahiro Ode[1, 10], Masanori Murayama[1] (*[1]Center for Brain Science, RIKEN, Saitama, Japan, [2]Tokyo Institute of Technology, Yokohama, Japan, [3]Univ. of Tokyo, Tokyo, Japan, [4]National Institute for Physiological Sciences, Okazaki, Japan, [5]Nikon Corporation, Yokohama, Japan, [6]Hamamatsu Photonics K.K., Iwata-shi, Japan, [7]Juntendo University Graduate School of Medicine, Tokyo, Japan, [8]Kyushu University of Health and Welfare, Miyazaki, Japan, [9]Tohoku University, Miyagi, Japan, [10]FOV Corporation, Yokohama, Japan*)

In neuroscience, in vivo functional imaging with a single-cell resolution over a wide field of view (FOV) is challenging. To accomplish this, we developed a wide-field two-photon microscope, which we named "cosmoscope", that equips a very large objective lens with low magnification and a high numerical aperture. We evaluated the optical resolution and aberrations of this microscope by means of simulation. Eighty percent of the energy was contained within a radius of 1.1 $\mu$m in all FOV. This result was almost equivalent to the performance at the diffraction limit, indicating that this microscope possesses the high efficacy of two-photon excitation and high spatial resolution in all three axes across the entire field of view. To further evaluate this microscope, we performed in vivo calcium imaging of L2/3 and L5 cortical neurons expressing GCaMP in an awake mouse. GCaMP fluorescence was observed in the cytoplasm, not in the nucleus, providing confirmation that this microscope possesses single-cell resolution. We also developed a low computational cost cell detection (LCCD) algorithm (Ito et al., bioRxiv 502153), because the data size acquired by our microscope was so large that the previously reported algorithms could not detect neurons within a practical period of time. LCCD enabled us to extract more than 16,000 neural activities of L2/3 cortical neurons. Finally, we identified the functional map of the neurons. Whereas sensory stimulus-evoked activity was localized to the responsible region, movement-related activity was globally distributed. Cosmoscope will open the door to monitor a great multitude of single cortical neurons. (COI:No)

## 2P-187

### The novel electroporation of a Water-in-oil Droplet was applied to cytomorphology modification

Rika Numano[1], kohjiro Matsumoto[2] (*[1]Toyohashi Univof Tech, Dept of Applied Chem and Life Science,, [2]Nepa Gene Co., Ltd.*)

Electroporation is the most widely used transfection method for delivery of cell-impermeable molecules into cells. We developed a novel gene transfection method, water-in-oil (W/O) droplet electroporation, using dielectric oil and an aqueous droplet containing mammalian cells and transgene DNA. When a liquid droplet suspended between a pair of electrodes in dielectric oil is exposed to a DC electric field, the droplet moves between the pair of electrodes periodically and droplet deformation occurs under the intense DC electric field.
During electrostatic manipulation of the droplet, the local intense electric field and instantaneous short circuit facilitate gene transfection.
This method has several advantages over conventional transfection techniques, including co-transfection of multiple transgene DNAs into even as few as 103 cells, transfection into differentiated neural cells. In addition, there have been improvements in W/O droplet electroporationelectrodes for disposable 96-well plates making them suitable for concurrent performance without thermal loading by a DC electric field. This technique will lead to the development of cell transfection methods for iPS cells and genome editiong. (COI:No)

## 2P-188

### Analgesic effects of voluntary running performed before or after the induction of inflammatory pain

Risa Yamauchi[1, 2], Hideshi Ikemoto[1], Naoki Adachi[1], Takayuki Okumo[1], Jun Oike[1], Yusuke Kunieda[1], Hiroyuki Horikawa[1, 2], Mana Tsukada[1], Tadashi Hisamitsu[1], Masataka Sunagawa[1] (*[1]Dept Physiol, Showa Univ, Sch Med, Tokyo, Japan, [2]Faculty of Arts and Sciences at Fujiyoshida, Showa Univ, Yamanashi, Japan*)

**AIM:** Some studies support the effectiveness of physical therapy for reducing pain and improving the physical function. These effects might differ depending on the modalities (e.g. forced or voluntary running) and the timing of physical exercises. In the present study, we investigated the influence of voluntary running before or after the induction of experimental inflammation on acute pain in rats.
**Methods:** *Experiment 1.* Male Wistar rats were divided into a control, a nonrunning before the injection of formalin (pre-NOR), and a voluntary running before the injection (pre-VR) groups. Inflammation was induced by injecting formalin (1%, 50 μl,) into the hindpaw. Rats in the pre-VR group were given free access to a running wheel for a week. The total time spent in pain-related behaviors was quantified for 60 min after the injection. *Experiment 2.* Rats were divided into a control, a non-running after the formalin injection (post-NOR), and a voluntary running after the formalin injection (post-VR) groups. Formalin was similarly injected, and then rats in the post-VR group were given free access to a running wheel for 12 days. The inflammatory sensitization was tested with the von Frey test. The expression of activated microglia in the spinal cord was analyzed by Western blotting and immunofluorescent staining. Microglia are thought to be involved in the prolongation and the chronicity of pain.
**Results:** Voluntary running before the induction of pain did not provide the analgesic effect (Exp. 1). However, running after the induction of pain significantly accelerated the recovery from pain, and significantly inhibited the expression of activated microglia (Exp. 2).
**Conclusions:** These results suggest that physical therapy early after surgery may promote the recovery from inflammatory pain by inhibiting microglial activation. (COI:No)

## 2P-189

### Preemptive analgesia provided by complementary approaches in acute inflammatory pain model rats

Nachi Ebihara, Hideshi Ikemoto, Naoki Adachi, Tamehiro Ebihara, Kayoko Ebihara, Jun Oike, Yusuke Kunieda, Yasunori Takayama, Tadashi Hisamitsu, Masataka Sunagawa (*Dept Physiol, Showa Univ, Sch Med, Tokyo, Japan*)

Preemptive analgesia is a strategy that is designed to prevent central sensitization and chronic postoperative pain. The present study aimed to elucidate the preemptive analgesia of complementary approaches, such as transcutaneous electrical nerve stimulation (TENS), electroacupuncture (EA), a Japanese herbal medicine Yokukansan (YKS), and a combination of EA and YKS, using rats with formalin-induced acute inflammatory pain.
Male Wistar rats were divided into six groups: a control, a formalin-treated (For), a pre-treatment of TENS+For (TENS), a pre-treatment of EA+For (EA), a pre-administration of Yokukansan (7 days)+For (YKS), and a EA+YKS+For (EA+YKS) group. Rats were injected subcutaneously with formalin (50 $\mu$l, 1%) into the hindpaw. TENS and EA were delivered at a frequency of 4 Hz for 30 min before the injection of formalin. EA was applied at the Zusanli (ST-36) acupoint. YKS was mixed with powdered rodent chow at a concentration of 3% and fed to the YKS-treated rats. The total time spent in pain-related behaviors was quantified for 60 min immediately after the formalin injections. We also observed the expression of phosphorylated extracellular signal-regulated kinase (pERK), which has been used as a marker of neural activation, in the spinal dorsal horn by immuno-staining. As a result, the duration of pain-related behavior was significantly increased following the injection of formalin; however, the increase was significantly inhibited in the TENS ($p<0.05$), EA ($p<0.05$), and EA+YKS groups ($p<0.01$). The number of spinal pERK (+) cells was also significantly increased; however, the change was significantly inhibited in the TENS, EA, and EA+YKS groups (each; $p<0.01$).
These results suggest that TENS, EA, and a combination of EA and the administration of YKS produce preemptive analgesia and inhibit the phosphorylation of ERK in the spinal cord. (COI:No)

## 2P-190

### Development of ASD screening algorithm in 5-year-old children using eye-tracking device (Gazefinder)

Manabu Saito[1, 2], Masaru Ninomiya[3], Shuji Hakoshima[3], Kenji Tsuchiya[4], Yui Sakamoto[1], Kazutaka Yoshida[1], Yuri Matsubara[1], Ai Terui[1], Shinya Ueno[2, 5], Kazuhiko Nakamura[1, 2] (*[1]Dept Neuropsychiat, Grad Sch Med, Hisosaki Univ, Japan, [2]Res Center for Child Mental Dev, Grad Sch Med, Hirosaki Univ, Japan, [3]JVCKENWOOD Corporation, [4]Center for Child Mental Dev, Grad Sch Med, Hamamatsu Med Univ, Japan, [5]Dept Neurophysiol, Grad Sch Med, Hisosaki Univ, Japan*)

Children with Autism Spectrum Disorder (ASD) have a characteristic eye movement due to social attention that depends on the bias of interest, compared to children with typical development (TD). It is necessary to detect early ASD for improving the symptoms, but it is not easy to diagnose ASD in early childhood. The aim of this study was to develop a screening algorithm by extracting gaze characteristics in pre-school 5-year-old ASD. 97 typical developmental children (TD) and 42 ASD children without coexisting other developmental disorder were just looking at 2-minute video with contents of face, preference, biological motion, and pointing. Statistics analysis was a principal component analysis on fixation rate at 89 Area of Interest (AOI) using diagnostic information and high sensitivity AOI based on correlation coefficient / p value / effect size. Considering the effects of gender, the analysis was divided into boys and girls. There was a significant difference between TD and ASD in specific AOI of both boys and girls. The fixation rate of girls (AOC=0.89) showed 86% sensitivity and 83% specificity, and the fixation rate of boys (AOC=0.73) showed 69% sensitivity and 69% specificity by a cut-off score. In all children, AOC was 0.78, and when the provisional cut-off score of the fixation rate was 50.4%, the sensitivity was 75% and the specificity was 75.8%. This result was appropriate as a screening algorithm. (COI:No)

# Poster Presentations

## Day 3

（March 19, 13:10～14:10）

| | |
|---|---|
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| **3P-019～3P-032**OU | Heart・Circulation（3） |
| **3P-033～3P-050** | Neuron・Synapse（3） |
| **3P-051～3P-062** | Sensory Function（3） |
| **3P-063～3P-072** | Behavior Science・Biorhythm（3） |
| **3P-073～3P-075** | Neurochemistry（3） |
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| **3P-082～3P-089** | Muscle Physiology（3） |
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| **3P-095～3P-099** | Endocrinology（3） |
| **3P-100～3P-102** | Kidney・Urination（3） |
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| **3P-109～3P-112** | Development・Growth・Aging（3） |
| **3P-113～3P-123** | Cell Physiology・Molecular Physiology（3） |
| **3P-124～3P-129** | Environmental Physiology（3） |
| **3P-130～3P-132** | Drug Actions（3） |
| **3P-133～3P-136** | Membrane Transport |
| **3P-137～3P-146** | CNS Function（3） |
| **3P-147～3P-154**OU | Nutrition・Metabolism・Thermoregulation（3） |
| **3P-155～3P-162**OU | Pathophysiology（3） |
| **3P-163～3P-165** | Physical Fitness・Sports Medicine（3） |
| **3P-166～3P-168** | Respiration（3） |
| **3P-169～3P-170** | Study Methodology（3） |
| **3P-171～3P-175**OU | Others（3） |

## 3P-001

### Molecular dynamics simulation of mutant type 1 ryanodine receptor

Toshiko Yamazawa[1], Haruo Ogawa[2], Takashi Murayama[3], Maki Yamaguchi[1], Hideto Oyamada[4], Junji Suzuki[5], Nagomi Kurebayashi[3], Kazunori Kanemaru[6], Katsuji Oguchi[4], Takashi Sakurai[3], Masamitsu Iino[6] (*[1]Dept Mol Physiol, Jikei Univ Sch Med, Japan, [2]The Univ Tokyo, Japan, [3]Juntendo Univ Sch Med, Japan, [4]Showa Univ Sch Med, Japan, [5]Univ California San Francisco, USA, [6]Nihon Univ Sch Med, Japan*)

In skeletal muscle cells, membrane depolarization is translated into intracellular $Ca^{2+}$ signals, and type 1 ryanodine receptors (RyR1), located in the sarcoplasmic reticulum membrane, play a key role in intracellular $Ca^{2+}$ release. Mutations in the RyR1 gene cause severe muscle diseases, such as malignant hyperthermia (MH), which is a disorder of $Ca^{2+}$-induced $Ca^{2+}$ release via RyR1 in the skeletal muscle. Thus far, more than 300 mutations have been reported in RyR1 in patients with MH, and most of those mutations have been found in three hotspot regions of RyR1. However, due to a lack of comprehensive analysis of the structure–function relationship of mutant RyR1, the mechanism remains largely unknown. Here, we combined functional studies and molecular dynamics (MD) simulation of RyR1 bearing disease-associated mutations at the N-terminal region. When expressed in HEK293 cells, the mutant RyR1 caused abnormalities in $Ca^{2+}$ homeostasis. MD simulations of wildtype (WT) and mutants were performed using crystal structure of the NTD monomer of RyR1. In WT, we found that the B(R283)-A(D61)-C(R402)-A(E40)-C(S406) hydrogen bonds/salt bridges network (B-A-C-A-C network) around R402. MD simulation of the mutant of R402 revealed that alterations of hydrogen bonds/salt bridges between NTD, consisting of A, B and C domains. The importance of R402 was verified by functional studies and MD simulations with Alanine mutants of E40 and D61 that form tight interaction with R402. Our results reveal the importance of inter-domain interactions within NTD in the regulation of the RYR1 channel and gain insights into the mechanism of MH caused by the mutations at the NTD. (COI:No)

## 3P-002

### Reexamination of the roles of LRRC8 and TTYH in the molecular identity of volume-sensitive outwardly rectifying anion channel VSOR

Yasunobu Okada[1], Toshiaki Okada[2], Kaori Sato-Numata[3,4], Tomohiro Numata[4] (*[1]Dept Physiol, Kyoto Prefectural Univ of Med, Kyoto, Japan, [2]Nat Inst Physiol Sci, Okazaki, Japan, [3]JSPS, Tokyo, Japan, [4]Dept Physiol, Fukuoka Univ, Fukuoka, Japan*)

Cell volume regulation (CVR) is fundamental to survival/death and functions of animal cells. CVR after osmotic swelling, called regulatory volume decrease (RVD), is attained by parallel activation of $K^+$ channels and $Cl^-$ channels. The most important $Cl^-$ channel directly activated by cell swelling and involved in RVD is the volume-sensitive outwardly rectifying anion channel VSOR (also called volume-regulated anion channel VRAC). Two membrane-spanning protein families, LRRC8(A+C/D/E) and TTYH(1-3), were reported to be the molecular identity of VSOR in 2014 and 2019, respectively. In the present study, the roles of these two protein families were reexamined in human epithelial cells by microarray assay and gene knockout/knockdown experiments. Actually siRNA-mediated quadruple knockdown of LRRC8A, 8C, 8D, and 8E or triple knockdown of TTYH1, 2 and 3 largely, though not completely, inhibited swelling-activated VSOR currents in HeLa cells. However, differential microarray studies showed that expression levels of mRNAs for LRRC8A-E and TTYH1-3 were not markedly different between VSOR-deficient KCP-4 cells and VSOR-rich parental KB cells as well as between KCP-4 and other VSOR-rich human epithelial cell lines including HeLa, HEK293T and Intestine 407. Furthermore, we produced a stable LRRC8A-deficient cell line, Clone-3, derived from HeLa cells by the CRISPR-Cas9 knockout method and then examined VSOR activity induced by reducing intracellular ionic strength under isotonic conditions (Low-IS) and by applying a hypotonic challenge. In Clone-3 cells, both Low-IS-activated and swelling-activated VSOR currents were still sizably observed, though considerably suppressed compared to those in wild-type HeLa cells. Moreover, siRNA-mediated triple knockdown of TTYH1-3 failed to abolish both types of VSOR currents in Clone-3 cells. Taken together, it is likely that some other core molecule is still missing, and thus further studies are warranted for the molecular identification of VSOR. (COI:No)

## 3P-003

### Action potential firing and Na currents in cerebellar Purkinje cells of class II ARF-deficient 'action tremor' mice: class II ARF may function as a Nav1.6 localizer at the AIS

Nobutake Hosoi[1], Koji Shibasaki[2], Teiichi Furuichi[3], Hirokazu Hirai[1], Tetsushi Sadakata[4] (*[1]Dept Neurophysiol & Neural Repair, Grad Sch Med, Gunma Univ, Japan, [2]Dept Mol Cell Neurobiol, Grad Sch Med, Gunma Univ, Japan, [3]Dept App Biol Sci, Faculty Sci Tech, Tokyo Univ of Sci, Japan, [4]Edu Res Support Center, Grad Sch Med, Gunma Univ, Japan*)

ADP-ribosylation factors (ARFs) are a family of small GTPases comprising six members categorized into three classes: class I (ARF1, 2, and 3); class II (ARF4 and 5); and class III (ARF6). Although class I and III ARFs are known to be the key regulators in vesicular membrane trafficking, the cellular function of class II ARFs remains unclear. Using double knockout technique, we produced the class II ARF hypomorphic mouse line carrying the $ARF4^{+/-}$/$ARF5^{-/-}$ genotype. It turned out that $ARF4^{+/-}$/$ARF5^{-/-}$ mice (class II ARF-deficient mice) show action tremor-like movement disorders. In the present study, we focused on the electrical properties of cerebellar Purkinje cells (PCs) which play an important role in motor control. Slice patch-clamp experiments revealed that class II ARF-deficient PCs cannot maintain high-frequency spike firing in response to larger current inputs. Spontaneous spike discharges were also reduced significantly in class II ARF-deficient mice. To elucidate the underlying mechanism, we examined voltage-gated Na currents in PCs. Although the voltage dependence of activation and inactivation of the Na currents in class II ARF-deficient PCs were similar to that in WT PCs, the mutant PCs showed a reduction not only in the typical fast transient Na current, but also in the resurgent Na current which is related to Nav1.6 pore-forming alpha subunit and contributes to repetitive high-frequency firing in PCs. Immunohistochemical analysis revealed that class II ARF-deficient mice exhibit reduced Nav1.6 localization to the PC axon initial segment (AIS) which is the action potential initiation site. On the other hand, outside-out patch recording from PCs and immunohistochemistry suggest no alteration in voltage-dependent K channels between WT and class II ARF-deficient mice. We conclude that class II ARF plays an important role in localizing Nav1.6 to the AIS of PCs. (COI:No)

## 3P-004

### Mouse ameloblasts express inwardly rectifying $K^+$ channels

Akiko Nakashima, Makoto Takano, Noriyuki Nakashima (*Dept. Physiol., Kurume Univ. Sch. Med., Japan*)

Enamel is a surface layer of teeth with extreme hardness comprising of mineralized structure. The enamel layer is formed by the secreting activity of ameloblasts. In addition to secreting machinery, ameloblasts are known to express ion transporters. Here, we further investigated the channel expression of ameloblasts. We first prepared the semi-intact preparation of mouse incisors at 300-$\mu$m thickness by frontal sectioning and performed a voltage-clamp patch clamp recording. We detected inwardly-rectifying channel activities, which were enhanced by the elevation of $K^+$ concentration in the recording pipette. In the whole-cell configuration, we confirmed the inwardly-rectifying currents, which were abolished by the application of 5 mM $Ba^{2+}$ in the bath solution. Based on these features characteristic to inwardly rectifying $K^+$ (Kir) channels, we investigated the mRNA expression patterns of Kir channel families by reverse-transcriptase PCR using the incisor tissues. As a result, we revealed that Kir 2, 4 and 6 subtypes were potentially expressed in the mouse incisor ameloblasts.

These results indicate that the membrane potential of ameloblasts is subject to the characteristic activities of Kir channels, e.g., in response to the extracellular $K^+$ concentration or the intracellular pH changes. (COI:No)

## 3P-005

### Coupling mechanism for $Na^+/H^+$ exchanger and $Cl^-/HCO_3^-$ exchanger in the intestine

Noriko Ishizuka, Naotaka Ikumi, Hisayoshi Hayashi (*Laboratory of Physiology, School of Food and Nutritional Sciences, University of Shizuoka*)

Intestinal NaCl absorption is considered to work by coupling a $Na^+$ transporter and a $Cl^-$ transporter. Although the $Na^+$ transporter has been shown to be $Na^+/H^+$ exchanger isoform 3 (NHE3) throughout the intestine, the $Cl^-$ transporter has a different form in each intestinal segment, which are $Cl^-/HCO_3^-$ exchanger slc26a6 in the small intestine and another $Cl^-/HCO_3^-$ exchanger slc26a3 in the large intestine. We have also shown that NHE3 changes coupling partner to $H^+$-coupled peptide transport (pepT1) when $H^+$-coupled peptide absorption is activated. However, the molecular mechanism of each coupling mode remains to be determined. To investigate this, we determined the involvement of slc26a3 in NaCl absorption in each intestinal segment of mice. Ussing chambers were used to measure transepithelial $^{22}Na^+$ and $^{36}Cl^-$fluxes across the intestinal membrane. In addition, we investigated a mode of coupling between slc26a3 and NHE3 by using intestinal organoids. The activity of NHE3 was determined as the rate of $Na^+$-induced intracellular pH recovery after acid loading. In the Ussing chamber experiments, the addition of NHE3 specific inhibitor S3226 to the luminal side induced simultaneous inhibition of net $Cl^-$ flux in the middle colon and cecum. However, the coupling ratio of NHE3 and slc26a3 is different between the middle colon and cecum. These results suggested that the mode of coupling of $Na^+$ and $Cl^-$ absorption is different along colonic segments. (COI:No)

## 3P-006

### Dynamic structure of transient receptor potential vanilloid 1 (TRPV1) cation channel observed by high-speed atomic force microscopy

Ayumi Sumino[1], Daichi Mukai[3], Zhao Yimeng[4], Mikihiro Shibata[1,2], Motoyuki Hattori[4] (*[1]WPI-NanoLSI, Kanazawa Univ., [2]Infiniti, Kanazawa Univ., [3]Coll. of Sci. and Eng., Kanazawa Univ., [4]Sch. of Life Sci., Fudan Univ.*)

Transient receptor potential vanilloid 1 (TRPV1) ion channel is a capsaicin receptor that induces burning pain. It is also the thermosensitive channel that opens over 43 ℃, functioning as thermosensor of the cells. Single-particle analysis using cryo-electron microscopy (Cryo-EM) have revealed high-resolution structure of the TRPV1 channel. However, molecular mechanism of the heat sensing and dynamic structural change upon gating is still unknown. Here, we applied high-speed atomic force microscope (HS-AFM) for observation of the dynamic structure of TRPV1. TRPV1 channels were solubilized and purified with detergent, then reconstituted into phospholipid bilayer on an AFM substrate. AFM image showed tetrameric structure like a windmill, whose diameter and protrusion from bilayer surface were about 15 nm and 3 nm, respectively. The cryo-EM structure shows similar structure with view from cytoplasmic side, suggesting that the structure imaged by AFM corresponds to cytoplasmic ankyrin repeat domains (ARD). The protruded height of the ARD is 2 nm shorter than the cryo-EM structure, implying that the ARDs might lie down on the bilayer surface. We will discuss how ligand binding and heat change the structure of ARDs and its fluctuation. (COI:No)

## 3P-007

### *CACNA1C*-E1115K Mutation Associated with Overlap Phenotype of Long-QT and Brugada Syndrome Disrupts Cav 1.2 Ion Selectivity in Patient-specific iPS Cell-derived Cardiomyocytes

Asami Kashiwa[1], Takeru Makiyama[1], Hirohiko Kohjitani[1], Thomas Maurissen[2], Yimin Wuriyanghai[1], Yuta Yamamoto[1], Jingshan Gao[1], Hai Huang[1], Tomohiko Imamura[1], Takanori Aizawa[1], Taisuke Ishikawa[2], Seiko Ohno[3], Futoshi Toyoda[4], Seiich Sato[5], Kazuhiro Takahashi[6], Knut Woltjen[2], Minoru Horie[7], Naomasa Makita[3], Takeshi Kimura[1]
([1]*Dept Cardiol, Grad Sch Med, Kyoto Univ, Japan,* [2]*CiRA, Kyoto Uni, Japan,* [3]*NCVC, Japan,* [4]*Dept Physiol, Med, Shiga Univ, Japan,* [5]*Okinawa Prefectural Nanbu Medical Center,* [6]*Kizawa Memorial Hospital,* [7]*Center for Epidemiologic Research in Asia, Shiga Univ, Japan*)

**Background:** A missense mutation, E1115K in *CACNA1C*, encoding L-type $Ca^{2+}$ channels (LTCCs), is located in the crucial site of ion selectivity (*Nature. 1993;366(6451):158-61*). Recently, the mutation was reported to cause diverse arrhythmogenic phenotypes including long-QT syndrome (LQTS) and Brugada syndrome.
**Objective:** The aim is to investigate the disease mechanism of *CACNA1C*-E1115K using an iPSC model.
**Methods and Results:** We generated iPSCs from a 12-year-old boy carrying heterozygous *CACNA1C*-E1115K, whose ECG presented QT prolongation and a drug-induced Brugada ECG pattern. Patch-clamp recording using differentiated cardiomyocytes (CMs) revealed that the peak $I_{CaL}$ densities of E1115K iPSC-CMs were reduced compared to controls ($4.9 \pm 0.8$ vs. $9.6 \pm 1.0$ pA/pF, $p<0.001$), which might contribute to Brugada phenotype. Impaired $Ca^{2+}$ selectivity was demonstrated by the negative-shifted reversal potential and marked outward currents through LTCC carried by monovalent cations in E1115K CMs. In action potential (AP) recordings using a dynamic clamp system injecting synthetic $I_{K1}$, E1115K CMs exhibited significantly longer AP durations (APD90 $444.0 \pm 12.3$ vs. $354.6 \pm 20.1$mV, $p<0.001$). We also evaluated drug effects on APDs using voltage-sensitive dye imaging. Nifedipine (10 nM) significantly shortened APDs in E1115K cells than controls (APD reduction rate: -45.8 vs. -36.1%, $p<0.05$). Furthermore, mexiletine and GS967, which can block sodium currents, significantly shortened APDs in E1115K cells (-11.1 vs. 0.7, $p<0.05$; -21.9 vs. -4.2%, $p<0.01$). These results suggest that late sodium currents are enhanced in E1115K cells, and might have an important role in APD prolongation.
**Conclusion:** We demonstrated impaired ion selectivity in *CACNA1C*-E1115K iPSC-CMs which might be associated with the patient's clinical phenotypes. Late sodium current blockers might be candidates to rescue QT prolongation in this mutation. (COI:No)

## 3P-008

### The electrophysiological analysis of TRPM5 channel

Kunitoshi Uchida[1, 2], Makoto Tominaga[2, 3], Jun Yamazaki[1, 4] ([1]*Dept Physiol Sci and Mol Biol, Fukuoka Dental Col, Japan,* [2]*Div Cell Signal, NIPS, Japan,* [3]*Thermal Biol, ExCELLS, Japan,* [4]*Lab Vet Pharmacol, NIhon Univ, Japan*)

TRPM5 is a monovalent cation-permeable channel activated by intracellular $Ca^{2+}$, and its activity is enhanced by temperature increase from 15℃ to 35℃. This channel is restrictedly expressed in taste cells, the pancreas, the brainstem and olfactory epithelium, and is thought to be involved in controlling membrane potentials. In taste cells, TRPM5 is expressed in type 2 taste cells, and involved in the signal transduction in the downstream of sweet taste receptor. In this study, we analyzed TRPM5 by using whole-cell patch-clamp recording. We found that TRPM5 is activated and irreversibly inactivated upon heat stimulation. The mechanism of temperature-dependent inactivation could be different from that of voltage-dependent inactivation. Next, we analyzed TRPM5 channel protein electrophysiologically by using planar lipid bilayer (PLB) method, which is one of the reconstitution systems. Thermosensitive activation and inactivation of TRPM5 channel protein were also observed in PLB method, suggesting that other molecules contained in cells could not be required for temperature dependent activation and inactivation of TRPM5. (COI:No)

## 3P-009

### Characterization of disease-associated CFTR-mutations identified in Japanese cystic fibrosis patients

Yoshiro Sohma, Rio Kimishima, Shogo Iwai, Hikaru Sohma, Nao Kobayashi, Kanako Nakao
(*Div Mol Therapeutics, Grad Sch Pharm, IUHW, Japan*)

Cystic Fibrosis (CF) is reported to be very rare among Asiansand previous reports suggested that the profiles of CF-caused CFTR mutations found in Japanese CF patients are different from Caucasians.
At present twenty-four Japanese CF patients have been definitely identified their disease-associated CFTR mutations. Twenty-two mutations were identified in the CFTR proteins. 11 out of 22 mutations seemed to be in the class II and 10 mutations in the class III or IV.
Among the Japanese CF mutations, a massively deleted mutation lacking the coding sequences along three exons without frameshift (dele 16-17b mutation) has been found with the highest frequency (13 alleles out of 28 CF alleles). CFTR protein derived from the dele 16-17b CFTR gene is expected to lack 153 amino acids from Gly970 to Thr1122 ($\Delta$(G970-T1122)-CFTR). Most importantly two non-consanguineous CF patients with homozygous dele 16-17b mutation have already been found out of all 29 Japanese CF patients, which suggests a small but significant population with heterozygous dele 16-17b mutation in Japanese.
In this study, we attempted to characterize effects of the Japanese-specific mutations on CFTR protein. (COI:No)

## 3P-010

### Identification of binding partners of the voltage-gated sodium channel Nav1.1

Ikuo Ogiwara (*Dept Physiol, Nippon Med Sch, Japan*)

Nav1.1 is a brain-type of voltage-gated sodium channel $\alpha$ subunit. Nav1.1 is predominantly expressed in parvalbumin-expressing (PV) cells, and plays critical roles in maintaining sustained fast-spiking from these inhibitory neurons. Mice with conditional deletion of Nav1.1 in PV cells display epileptic seizures and autism-like behaviors that resemble clinical features of human Dravet syndrome with mutations of the Nav1.1-encoding gene *SCN1A*. While Nav1.1 is also detectable in some excitatory cells, conditional deletion of Nav1.1 in excitatory cells exert a protective effect on seizure symptom. Nav1.1 interacts with sodium channel $\beta$ subunits and ubiquitously-expressed calmodulin. We here screened candidate proteins that could bind to Nav1.1 using immunoprecipitation with anti-Nav1.1 antibody and mouse brain extract, followed by tandem mass spectrometry. The list of proteins included sodium channel $\beta$ subunits, calmodulin and other proteins. We selected some Nav1.1-binding partner candidates, which were seemingly expressed in subsets of neurons on the basis of the Allen brain atlas database. We evaluated that two candidates, namely, A and B, were co-immunoprecipitated with Nav1.1, and vice versa, using mouse brain extract. The proteins A and B have been reported to be localized at the axons and post-synapses of neurons. We finally found that the proteins A and B both bound to the intracellular loop I of Nav1.1 using heterologous expressing systems with HEK293 cells. Our findings may suggest that these Nav1.1-binding partners may be involved in axonal localization of Nav1.1. (COI:No)

## 3P-011

### proton transport in endoplasmic reticulum membrane

Yoshimichi Murata, Taiki Okabe, Ruka Kobayashi, Yoshio Maruyama (*Dept Physiol, Grad Sch Med, Tohoku Univ, Japan*)

Many studies have revealed that pH regulation of intracellular membranous compartments is critical for their function. Each organelle has own pH regulation mechanisms and maintains their own pH for carrying out the function, although details are still unclear. Luminal pH of ER ($pH_{ERL}$) is thought to about 7.2, close to cytosolic pH. It looks like resulting of absent of active regulation of $H^+$ transport, i.e. $pH_{ERL}$ is always passively following the cytosolic pH. Though, regulation of $pH_{ERL}$ and its mechanisms are not well understood. We tried to reveal the $pH_{ERL}$ regulation agents using genetically encoded ratiometric fluorescent pH probe, mCherrySEpHluorin-ER in nuclear envelopes (NEs: containing peri-nuclear endoplasmic reticulum membrane). pH imaging of NE lumen was revealed that luminal pH followed pH change of cytosolic side, but the degree of pH change was diminished against the pH change of outside. For investigating the mechanisms of this proton transport, we carried out intra- and extraluminal ion substitution experiments. (COI:No)

## 3P-012

### Prostaglandin $E_2$ receptor EP4 induced $Ca^{2+}$ influx from the extracellular space via Orai1 and regulates the cell migration in oral cancer cells

Kohei Osawa[1], Masanari Umemura[1], Rina Nakakaji[1, 2], Kenji Mitsudo[2], Yoshihiro Ishikawa[1]
([1]*Dept CVRI, Grad Sch Med, Yokohama City Univ, Japan,* [2]*Dept OMS, Grad Sch Med, Yokohama City Univ, Japan*)

**Introduction:** The EP4 prostanoid receptors is one of the four receptor subtypes for Prostaglandin $E_2$ ($PGE_2$). EP4 may play an important role in cancer progression. Its inhibition is a potential strategy for cancer therapy. However, little information is available regarding the function and cellular signaling pathway of EP4 in oral cancer. In this study, we show that EP4 signal regulates cell migration and metastasis in oral cancer.
**Material and Method:** Human-derived tongue squamous cell carcinoma cell lines, HSC-3 and OSC-19 were used. Changes intracellular $Ca^{2+}$ level were measured by Fura-2, a $Ca^{2+}$-sensing fluorescent dye. Migration was examined with the scratch assay. Western blot was also performed. In order to ablate EP4 or Orai-1, shRNA was induced with lentiviral infection in HSC-3. In animal experiments, we established lung metastasis model mice to evaluate metastatic ability. Immunoprecipitation for EP4, Orai1 and transient receptor potential canonical 1 (TRPC1) were performed.
**Result:** The EP4 agonist (ONO-AE1-437), rapidly increased intracellular $Ca^{2+}$. EP4 agonist promoted cell migration and increased phosphorylation of ERK in HSC-3. In contrast, EP4-knockdown significantly reduced the cell migration. EP4-Knockdown decreased lung metastasis in mice. Orai1-knockdown also negated the EP4 agonist-induced $Ca^{2+}$ elevation. Immunoprecipitation showed that EP4 was colocalized and formed complexes to both Orai1 and TRPC1.
**Conclusion:** EP4 regulates intracellular $Ca^{2+}$ elevation via Orai1 and TRPC1, resulting in promoting cell migration of oral cancer. (COI:No)

## 3P-013

### Asparagine-linked glycosylation as a key regulator of gating properties in cardiac $Na_v1.5$ channels

Pu Wang, Yangong Liu, Mengyan Wei, Shinichiro Kume, Tatsuki Kurokawa, Katsushige Ono (*Deptment Physiopathology, Grad Sch Med, Oita Univ, Japan*)

Voltage-gated sodium channels are composed of a pore-forming $\alpha$ subunit and auxiliary $\beta$ subunits. SCN5A gene encodes a pore-forming $\alpha$ subunit of the voltage-gated sodium channel $Na_v1.5$ which is known to play an important role in human cardiac myocytes. In the $Na_v1.5$ channel, several potential glycosylation sites are postulated including the asparagine-linked (N-linked) glycosylation. Although the N-linked glycosylation is one of the common post-translational modifications, the detail of their functions on the $Na_v1.5$ channel are not been understood well. We applied the whole-cell patch-clamp technique to analyze the effects of the N-linked glycosylation inhibition on the human $Na_v1.5$ channel expressed in HEK293 cells. In our study we observed that the addition of tunicamycin, a specific and potent inhibitor of N-linked glycosylation, increased the $Na_v1.5$ channel current amplitude when the cells were incubated for 24 hours. Aslo a significantly shift of the steady-state inactivation curve to the hyperpolarization direction was observed, whereas the activation curve was unaffected. Recovery from inactivation was prolonged by tunicamycin treatment, where the fast phase ($\tau_{fast}$) was unaffected and the slow phase ($\tau_{slow}$) was prolonged. Decay of $Na_v1.5$ channel current was unaffected by tunicamycin. When the ubiquitin-proteasome pathway was inhibited with a proteasome inhibitor MG132 [N-[(phenylmethoxy)carbonyl]-L-leucy-N-[(1S)-1-formyl-3-methylbutyl]-L-leucinamide], tunicamycin could not modify Nav1.5 current any further. These findings suggest that maturation and degradation of $Na_v1.5$ channel protein depends on N-glycosylation for the channel kineties. (COI:No)

## 3P-014

### $\alpha$-mannosidase I-dependent N-linked glycosylation modifies distinct gating properties of the hERG channel

Mengyan Wei, Yangong Liu, Pu Wang, Shinichiro Kume, Tatsuki Kurokawa, Katsushige Ono (*Dept Morbid Physiol, Grad Sch Med, Oita Univ, Japan*)

Asparagine-linked (N-linked) glycosylation has emerged as an essential post-translational modification to control the number of channels embedded in the plasma membrane as well as to regulate their functional gating properties. The human ether-a-go-go related gene (hERG) channel is known to form the major subunits of the rapidly activating delayed rectifier potassium current ($I_{Kr}$) in cardiomyocytes. hERG channel proteins are initially synthesized in the endoplasmic reticulum, where they undergo N-linked core glycosylation to form immature channel proteins. Then the immature proteins are trafficked to the Golgi apparatus, during which they undergo the complex processes of glycosylation to become fully glycosylated mature proteins. Although many studies have reported functions of N-linked glycosylation, the exact role of $\alpha$-mannosidase I-dependent N-linked glycosylation processed in Golgi apparatus on the hERG channel is not well understood. We analyzed the effects of N-linked glycosylation inhibitions on the hERG channel under the whole-cell patch-clamp technique using the heterologous expression system in HEK293 cells. The whole inhibitions of N-linked glycosylation with tunicamycin, an inhibitor of the synthesis of dolichol-PP-GLcNAc, decreased hERG channel current amplitude when the cells were incubated for 48 hour. However, kifunesine, a selective inhibitor of class I $\alpha$-mannosidase, did not reduce the current amplitude; kifunesine modified the gating property of the hERG channel when the cells were incubated for 48 hour. More specifically, kifunesine reduced the tail current of hERG channel when pluse protocol was designed to evaluate the reactivation of the channel. These results lead to the conclusion that $\alpha$-mannosidase I-dependent N-linked glycosylation in the Golgi apparatus plays a key role in modifing the distinct gating property of the hERG channel. (COI:No)

## 3P-015

### N-glycosylation inhibition attenuates heart automaticity by deranging T-type $Ca_{2+}$current and HCN current

Yangong Liu, Pu Wang, Mengyan Wei, Shinichiro Kume, Tatsuki Kurokawa, Katsushige Ono (*Dept Physiol, Grad Sch Med, Oita Univ, Japan*)

Asparagine-linked glycosylation (N-glycosylation) is an essential post-translational modification for a large number of crucial proteins in numerous physiological processes, and 20 percent of the congenital disorders of glycosylation cases have been reported including cardiac complications, such as arrhythmias, cardiomyopathies, and structural defects. To know its cardiac functions, we studied the effects of N-glycosylation defect on rat hearts and cardiomyocytes using tunicamycin. The synthesis of glycoprotein and inhibition of protein glycosylation by tunicamycin were examined in *in vivo* and *in vitro* administration. We observed that the daily abdominal injection of tunicamycin (0.1 mg/100 g) caused a gradual slowing down of the heart rate and severe bradycardia in rats within days. Further electrophysiological study in neonatal rat cardiomyocytes revealed that tunicamycin application rendered an attenuation of cardiomyocytes automatic beating, as well as reduction of T-type $Ca^{2+}$current and the HCN current, which implies that N-glycans deficiency may cause bradycardia. Moreover, the calnexin and calreticulin chaperone system in the endoplasmic reticulum could be postulated as a mechanism for a well assignment of N-glycans existing in $Ca_V3.1$ channel, the dominant isoform of T-type $Ca^{2+}$current channels in the adult mammals. In conclusion, pacemaker-related ion channel currents, T-type $Ca^{2+}$current and HCN current, may therefore play roles in the pathogenesis of congenital disorders of glycosylation in the heart. (COI:No)

## 3P-016

### ATP- and voltage-dependent gating of P2X2 receptor analyzed by voltage-clamp fluorometry

Rizki Tsari Andriani[1,2], Yoshihiro Kubo[1,2] ([1]*Div Biophys and Neurobiol, Natl Inst Physiol Sci, Okazaki, Japan,* [2]*Dept Physiol Sci, SOKENDAI, Hayama, Japan*)

P2X2 is a ligand-gated ion channel activated by extracellular ATP. This receptor shows a complex gating depending on both [ATP] and voltage, in spite of the absence of a canonical voltage sensor domain. It remains unknown how the structural rearrangements occur during voltage-dependent gating. Thus, in the present study we aim to analyze the structural rearrangements of P2X2 receptor upon ATP- and voltage-dependent gating, by voltage-clamp fluorometry (VCF). We used a fluorescent unnatural amino acid (fUAA) named Anap as a fluorophore which can be directly incorporated into the channel protein in *Xenopus* oocytes. In addition to that, to improve the VCF recording optical signal by decreasing the intrinsic background fluorescent of oocytes, a small molecule kinase inhibitor named HG-9-91-01 (SIK inhibitor) was applied. We then observed Anap fluorescence intensity changes associated with voltage changes at Ala337 and Ile341 in the 2nd transmembrane domain (TM2). The changes upon step pulse application from Ala337Anap and Ile341Anap showed a much faster kinetic than the voltage-dependent current activation. Moreover, both changes showed a linear voltage- dependent behavior. These changes might indicate a phenomenon related to electrochromic effect, implying that there is an electric field convergence at the position of Ala337 and Ile341 during P2X2 receptor complex gating. Voltage-dependent fluorescence change at Ala337 was larger in the absence of ATP than in the presence of ATP, reflecting the ATP-dependent change of the focused electric field. Mutagenesis studies at Ala337 in TM2 and its possible counterpart, Phe44 in TM1, suggested that the interaction between Ala337 and Phe44 in the open (ATP-bound) state is important for the complex gating of P2X2 receptor. It is possible that Phe44 swings into the focused electric field and that the interaction between Ala337 and Phe44 upon ATP binding, which contributes to the opening, might be under the control of membrane voltage. (COI:No)

## 3P-017

### Modulations of ion channel function by Sigma-1 receptor, a multimodal membrane protein

Chang Liu[1,2], I-Shan Chen[1,2], Ruth Murrell-Lagnado[3], Yoshihiro Kubo[1,2] ([1]*Div. Biophys and Neurobiol, NIPS, Okazaki, Japan,* [2]*Physiol. Sci, SOKENDAI, Hayama, Japan,* [3]*School of Life Sciences, University of Sussex, Brighton, UK*)

Sigma-1 receptor (S1R) is a protein expressed mostly on the endoplasmic reticulum membrane. It is implicated in many psychiatric and neurological disorders including drug addiction, juvenile amyotrophic lateral sclerosis and Alzheimer's disease. S1R has been reported to directly or indirectly interact with ion channels such as $K_v$ channel family members and Kir2.1 channel, while the underlying mechanisms are unknown. To examine the influence of S1R on various $K_v$ and Kir channels, we performed experiments using *Xenopus oocytes* as an in vitro expression system and observed the following results. (1) By immunohistochemical staining, we confirmed the S1R expression in the oocytes injected with S1R cRNA. (2) By electrophysiological recordings, we observed that coexpression of S1R suppresses the current amplitude of Kir3.1/Kir3.2 in a S1R expression level-dependent manner. (3) We observed that coexpression of S1R diminishes the current amplitude of $K_v2.1$ but not those of $K_v1.1$ and $K_v1.3$. (4) Mutations of Ser583 or Ser586 in $K_v2.1$ to Ala are known to disrupt the clusters of $K_v2.1$ and to increase the total current amplitude. However, we observed that S1R still decreases the current amplitude of the mutants, suggesting that the effect of S1R on $K_v2.1$ is not relevant to the channel clustering. Taken together, our present data suggest that S1R modulates the function of Kir3.1/Kir3.2 and $K_v2.1$ channels, but not those of $K_v1.1$ and $K_v1.3$ channels. These results further our understanding of the modulation mechanisms of ion channels by S1R. (COI:No)

## 3P-018

### Asparagine-linked glycosylation modifies voltage-dependent gating properties of $Ca_V3.1$-T-type $Ca^{2+}$ channel

Shinichiro Kume, Yangong Liu, Pu Wang, Tatsuki Kurokawa, Katsushige Ono (*Dept Patphysiol, Grad Sch Med, Oita Univ, Japan*)

T-type channels are low-voltage-activated channels that play a role in the cardiovascular system particularly for pacemaker activity. Glycosylation is one of the most prevalent post-translational modifcations in protein. Among various glycosylation types, the most common one is asparagine-linked (*N*-linked) glycosylation. The aim of this study was to elucidate the roles of *N*-linked glycosylation for the gating properties of the $Ca_V3.1$-T-type $Ca^{2+}$ channel. *N*-linked glycosylation synthesis inhibitor tunicamycin causes a reduction of $Ca_V3.1$-T-type $Ca^{2+}$ channel current ($Ca_V3.1$-$I_{Ca.T}$) when applied for 12 h or longer. Tunicamycin (24 h) signifcantly shifted the activation curve to the depolarization potentials, whereas the steady-state inactivation curve was unafected. Use-dependent inactivation of $Ca_V3.1$-$I_{Ca.T}$ was accelerated, and recovery from inactivation was prolonged by tunicamycin (24 h). $Ca_V3.1$-$I_{Ca.T}$ was insensitive to a glycosidase PNGase F when the channels were expressed on the plasma membrane. These fndings suggest that *N*-glycosylation contributes not only to the cell surface expression of the $Ca_V3.1$-T-type $Ca^{2+}$ channel but to the regulation of the gating properties of the channel when the channel proteins were processed during the folding and trafcking steps in the cell. (COI:No)

## 3P-019

### Contradictory responses of aortic Beta and iliac-femoral Beta during diltiazem administration in rabbits

Shin-ichiro Katsuda[1], Yuko Horikoshi[2], Yuko Fujikura[3], Akihiro Hazama[1], Tsuyoshi Shimizu[4], Koji Shirai[5] (*[1]Dept Cellular & Integrative Physiol, Fukushima Medical Univ. Sch Med, Japan, [2]Dept of Lab Med, Fukushima Med Univ Sch Med, Fukushima, Japan, [3]6th-Year Med Student, Fukushima Med Univ Sch Med, Fukushima, Japan, [4]Inst Shimizu Space Physiol, Suwa Maternity Clinic, Simosuwa-machi, Japan, [5]Seijinkai Mihama Hospital, Chiba, Japan*)

**Background and purpose:**The regulatory mechanism of the stiffness of the elastic and muscular arteries during calcium channel blocker has not been clarified. By applying the theory of cardio-ankle vascular index (CAVI), we have established new arterial stiffness indices in the entire aorta (aBeta) and in the iliac and femoral arteries (ifBeta). The effect of diltiazem on aBeta and ifBeta was clarified using rabbits.
**Methods:** Fourteen male rabbits aged 10-12 months were studied under pentobarbital anesthesia. Pulse waves at the origin of the aorta (oA), distal abdominal aorta (dA) and distal end of left femoral artery (fA) and flow waves at oA were simultaneously recorded before and after the intravenous infusion of diltiazem at 50 $\mu$g/kg/min for 2 min via the ear vein. PWV in the entire aorta (aPWV), from dA to fA (ifPWV) and from oA to fA (afPWV) was determined by the difference in the rising time of two pulse waves and distance of two pressure sensors. Beta was determined as $\mathrm{Beta}=2\rho/\Delta P\times\ln(\mathrm{SBP}/\mathrm{DBP})\times\mathrm{PWV}^2$ ($\rho$: blood density, SBP, DBP and $\Delta P$: systolic, diastolic and pulse pressures).
**Results:** When diltiazem was administered, blood pressure and pulse rate decreased. aBeta increased significantly while ifBeta decreased significantly. Aortic-femoral Beta (afBeta) did not show significant change despite the infusion of diltiazem.
**Conclusion:** During diltiazem administration, contradictory responses of aBeta and ifBeta were observed, suggesting that there exist co-relating regulatory mechanism in which elastic artery of central site contracted responding to dilated peripheral muscular artery by diltiazem. (COI:No)

## 3P-020

### Effect of endurance exercise training on the development of diabetic cardiomyopathy in young Goto-Kakizaki rats

Hirotsugu Tsuchimochi[1], Takashi Sonobe[1], Takahiro Higuchi[2], James T Pearson[1] (*[1]Dept Cardiac Physiol, NCVC, Suita, Japan, [2]Julius-Maximilian's University of Wurzburg*)

The Goto-Kakizaki (GK) rat, a type 2 diabetes model, does not develop diabetes during early postnatal period, but as it develops, it exhibits fasted hyperglycemia and impaired glucose tolerance due to decreased insulin secretion. The purpose of this study was to investigate the effects of low-intensity endurance exercise on the development of diabetes in young GK rats. Four-week-old male GK rats (n=5) were subjected to low intensity endurance exercise 5 days a week for 8 weeks. At 12 weeks of age, an oral glucose tolerance test (OGTT), endurance exercise test, echocardiography, and left ventricular pressure-volume (P-V) relationship analysis were performed. Transcriptome analysis was performed using isolated left ventricular (LV) myocardium. A group of sedentary animals (n=7) were used as a sedentary control group, and male Wistar rats were used as a strain comparison group. Compared to Wistar rats, GK rats showed a mild increase in fasting blood glucose at 4 weeks of age. Endurance training improved endurance capacity in both GK and Wistar rats, but abnormal glucose tolerance in GK rats did not improve except for a slight decrease in blood glucose levels 30 to 90 minutes after OGTT. Echocardiography and P-V loop analysis showed no effect of exercise training on heart morphology and function in 12-week-old GK rats. Comprehensive analysis of LV myocardial mRNA expression showed that the change in mRNA expression level was much greater based on strain differences than the effect of exercise training. Since GK rats genetically develop diabetes, the effect of exercise training on the suppression of diabetes was limited. In order to examine the effect of exercise training on the prevention of the onset of diabetes, therefore, we conclude that it is necessary to investigate diabetes models that are caused by environmental factors. (COI:No)

## 3P-021

### Evolution of the coronary circulation hearts by shortening the elastic regions of connectin

Akira Hanashima[1], Yoshihiro Ujihara[2], Momoko Ohira[1], Misaki Kimoto[1], Aya Kodama[1], Ken Hashimoto[1], Satoshi Mohri[1] (*[1]First Dept Physiol, Kawasaki Med Sch, Japan, [2]Dept Elec Mech Eng, Nagoya Inst Technol, Japan*)

Vertebrate hearts are classified into two types: coronary circulation hearts and sinusoidal circulation hearts. Though the sinusoidal circulation hearts take oxygen and nutrients into spongiosa myocardium directly from heart lumen, the coronary circulation hearts take them into compacta myocardium via blood vessels, which enables heart to act as a strong pump in mammals and birds. The emergence of coronary circulation is thought to have promoted the vertebrate evolutions and the mammal births. However, the appearance time and the appearance reason of the coronary circulation are unknown because the hearts did not remain as fossils. The extensibility of myocardium, which regulates the pump function of heart, is determined by the passive tension of the elastic protein connectin. Therefore, we thought that these problems could be clarified by comparatively investigating the elastic region of connectin. In this study, we focused on connectin in cartilaginous fish hearts. Our analysis for gene and domain structures found that the elastic region of elephant shark connectin had shorter PEVK segment and fewer numbers of Ig domains than those in connectins of mammals and birds. However, the RT-PCR experiments elucidated that the most of these different components were spliced out in elephant shark heart, and become similar domain structure to that in adult heart of mammals and birds. Our microscopic observation found that the elephant shark hearts had coronary artery and consisted of the compacta myocardium, and the sarcomere structures and the connectin localizations were similar to those in mammals and birds. Therefore, it is possible that the coronary circulation of cartilaginous fishes appeared independently of mammals and birds, and the uniquely evolved elastic region of cartilaginous fishes connectin had been shortened by heart-specific splicing to make a similar compacta myocardium to mammals and birds as a result of the convergent evolution. (COI:No)

## 3P-022

### Carvedilol inhibits the emergence of waves resembling abnormal Q waves, and the spread of inflammation and fibrosis induced by isoproterenol toward the epicardium

Takeshi Yamaguchi[1], Ryuichiro Mukai[1,2], Chinatsu Hiraoka[1,3], Hideyuki Nakagawa[4] (*[1]Lab Anat & Physiol, Fac Nurs, Shikoku Univ, Japan, [2]Kidai Univ Hosp, Osaka, Japan, [3]Kitajima Taoka Hosp, Tokushima, Japan, [4]Dept Pharmacol, Fac Nurs, Shikoku Univ, Japan*)

**Purpose:** This study examined the involvement of carvedilol (Crv) in the emergence of abnormal Q waves and the spread of inflammation induced by isoproterenol (Iso).
**Materials & Methods:** Male SD rats were administered 20 mg/kg of Crv orally for 1 week. The control (Con) groups were administered DMSO. After 1 week, they were subcutaneously injected with 50 mg/kg of Iso. The groups continued to orally receive Crv or DMSO, respectively, for 1 week. ECG was recorded using standard lead II before and after Iso injection. ECG was recorded again after 1 or 4 weeks and rats were perfused with 4% paraformaldehyde. The heart was embedded in paraffin, and cardiac tissues were stained by HE (hematoxylin and eosin) and Masson-Goldner stain.
**Results:** Both groups exhibited almost the same ECG waves at 5 min after Iso injection. After 4 weeks, Con groups had developed waves resembling abnormal Q waves, but Crv groups had not. The amplitude of QR waves in the Con group was more than 20% of the R wave amplitude. However, in the Crv group, that of QR waves was less than 20% of that of R waves. The QRS intervals were prolonged after Iso injection in the Con groups, but not as prolonged in the Crv groups. The subendocardial tissues of the heart were infiltrated with inflammatory cells 48 h after Iso injection. Inflammatory cells were found near the epicardium after 4 weeks in the Con groups, but few cells were found in the Crv groups. On Masson-Golder staining, a positive area in the epicardium was observed in the Con group, but it was smaller in the Crv group.
**Conclusion:** These results suggest that Crv can inhibit the emergence of waves resembling abnormal Q waves and the spread of inflammation induced by isoproterenol toward the epicardium. (COI:No)

## 3P-023

### Coordinately early afterdepolarizations evoked in ventricular tissue trigger reentrant arrhythmias:in silico study

Takao Shimamoto[1], Kunichika Tsumoto[2], Yasutaka Kurata[2], Akira Amano[1] (*[1]Dept Bioinfo, Coll Life Scis, Ritsumeikan Univ, Japan, [2]Dept Physiol, Kanazawa Med Univ, Japan*)

Abnormalities of action potentials (APs) in cardiomyocytes and of its AP propagation lead to cardiac arrhythmias. It is believed that early afterdepolarizations (EADs) which transiently depolarizes during the AP repolarization phase triggers fatal arrhythmias such as Torsades de Pointes (TdP). Although, generative mechanisms of EADs have been intensively investigated numerous experimental and theoretical studies, the mechanism of EAD development-mediated arrhythmogenesis in the ventricle is not fully understood. To examine how EADs that evoked locally in ventricular tissue lead to arrhythmias, we constructed a mono-domain sheet model (6 cm × 6 cm) consisting of 360,000 human ventricular myocardial units and performed computer simulation of AP propagation. In the present study, we investigated how the numbers and locations of myocardial units evoking EAD in the myocardial tissue related the arrhythmogenesis. When myocyte units evoking EAD were located at the center of myocardial sheet as a square area and about 80% units in the myocardial sheet occurred coordinately EADs, the EADs occurrence followed by spiral wave-like excitations, i.e., reentrant arrhythmias, were triggered. Furthermore, we found that if EAD evoked myocyte units account for the off-center of the myocardial sheet, the reentrant arrhythmias can be triggered with fewer EAD evoked units (25% of the total myocyte units). These results suggested that not only the numbers of myocytes evoking coordinately EADs but also the location causing EADs may be involved in fatal arrhythmia onsets. (COI:No)

## 3P-024

### Role of a functional SNP of the gene coding brain serotonin synthesis rate-limiting enzyme Tph2 in dilated cardiomyopathy

Sachio Morimoto[1], Kengo Hayamizu[2] (*[1]Lab Physiol, Dept Health Sci at Fukuoka, IUHW, Japan, [2]Dept Anesthesiology and Crit Care Med, Kyushu Univ Grad Sch Med*)

Brain serotonin dysfunction is involved in depression, which has been shown to increase the risk of death in heart failure (HF) patients. Tryptophan hydroxylase 2 (TPH2) expressed specifically in the central nervous system is involved in serotonin synthesis in the brain as a rate-limiting enzyme. Inbred mice have a functional single nucleotide polymorphism (SNP) C1473G in Tph2, with the Tph2 activity being lower in BALB/c strain with G/G allele than in C57BL/6 mice with C/C allele. In this study, we examined the role of this SNP in the disease phenotype of knockin mice with a dilated cardiomyopathy (DCM)-causing mutation ΔK210 in cardiac troponin T (cTnT) by creating single-gene congenic strain with Tph2 1473C/C or G/G allele. The ΔK210-cTnT DCM mice on C57BL/6 background frequently suffered from sudden cardiac death (SCD) with no heart failure symptoms, whereas the ΔK210-cTnT DCM mice on BALB/c background mostly died of congestive HF instead of SCD. Introduction of Tph2 1473G/G allele into C57BL/6 background DCM mice caused congestive HF death while decreasing SCD and extending the life expectancy. On the other hand, introduction of Tph2 1473C/C allele into BALB/c background DCM mice caused SCD while decreasing congestive HF death and shortening the life expectancy. These results strongly suggest that brain serotonin function plays an important role in the disease phenotype of DCM. (COI:No)

## 3P-025

### Atrial arrhythmia induced by pilocarpine application on footpads of freely behaving mice

Shinichi Sato, Yosuke Okamoto, Kyoichi Ono (*Dept Physiol, Grad Sch Med, Akita Univ, Japan*)

**Purpose:** Previously, we reported that pilocarpine could penetrate through footpads of mouse and induce arrhythmia noninvasively by using a new multi-dry-electrode plate electrocardiogram (MDEP)-sensor system. In the present study, we discuss the characteristics of arrhythmia induced noninvasively in freely behaving mice.
**Methods:** Electrocardiogram (ECG)s of freely-behaving C57BL/6J mice were individually recorded for one h by the MDEP-sensor system following free walking in a cage on the paper soaked with 2% pilocarpine solution for 10 min. Fifteen ECG signals and one piezoelectric sensor signal from the MDEP-sensor were stored in a PC via a 16 channel A/D converter.
**Results:** Four mice out of seven mice examined showed arrhythmia from the beginning of ECG recording immediately after the pilocarpine treatment. A sudden increase in R-R interval up to approximately 160 – 180 % frequently appeared among the R-wave trains with a frequency of around 600 bpm. The relative incidence of arrhythmia declined about 30 min after the start of recording. The pilocarpine-induced arrhythmia is likely to be classified as atrial arrhythmia according to the observation of ECGs obtained by the MDEP-sensor system. In addition, some ECG traces may suggest the reentrant activity in the atria.
**Conclusion:** Pilocarpine acts as a muscarinic receptor agonist, which stimulates the secretion of saliva and sweat and is generally used to treat dry mouth and glaucoma. In the present study, we first demonstrate the usability of pilocarpine to induce arrhythmia in freely behaving mice. Pilocarpine should permeate the footpad skin and vascular walls and should be delivered by blood flow to the vicinity of the heart. However, it is not clear yet the role of pilocarpine for the induction of arrhythmia. Further study is needed to understand the mechanism of arrhythmia induction by pilocarpine. (COI:No)

## 3P-026

### The simulation based prediction method for early afterdepolarization in drug-induced arrhythmia

Akira Kimura, Shingo Murakami (*Dept. EECE, Faculty of Sci. & Eng., Chuo Univ.*)

Drug-induced arrhythmia is initiated by early afterdepolarization (EAD) occurring under IKr block and resulting prolongation of action potential duration (APD). Therefore, to predict drug-induced arrhythmia caused by $I_{Kr}$ blockers, action potential prolongation and $I_{Kr}$ block have been used. However, although there are $I_{Kr}$ blockers that prolonged the APD for the same amount of time, some cause EAD and some do not. For example, amiodarone causes few EAD and therefore is known to be a relatively safer drug. In contrast, terfenadine and bepridil cause drug-induced arrhythmia and EAD much more than amiodarone. Based on the above, we hypothesized that one needs to consider other factors than $I_{Kr}$ block to predict drug-induced arrhythmia. In the present study, voltage-dependent property of $I_{CaL}$ was adopted as a factor and we examined the effect of the voltage dependence on EAD, by using the mathematical model of human ventricular. Firstly, we confirmed that EAD is initiated under bradycardia and action potential prolongation in the simulation. Next, we confirmed that voltage-independent $I_{CaL}$ block suppressed EAD. Finally, we used the three $I_{CaL}$ block models of amiodarone and terfenadine and bepridil. The results showed that the model of amiodarone suppressed EAD but the others increased the occurrence of EAD. Analyzing the simulation results in detail, we found that the $I_{CaL}$ block being weak in hyperpolarization side by terfenadine and bepridil contributed to the increase of EAD occurrence and that by amiodarone did to the decrease. Therefore, when we predict drug-induced arrhythmia, not only APD prolongation but also voltage-dependent property of $I_{CaL}$ block should be checked. (COI:No)

## 3P-027

### Histamine Excites Neonatal Rat Intracardiac Ganglion Neurons Via Activation of Non-Selective Cation Channels and Inhibition of M-type K+ channels

Aya Sato[1, 2, 3] (*[1]Div Homeostatic Development, NIPs, [2]Sch Allied Health Sci, Kitasato Univ, [3]Dept Ped, Shiga Univ of Med Sci*)

Coronary spasm is a potentially life-threatening medical emergency that has been infrequently observed in allergy-related incidents in both children and adults. Here, its underlying mechanism is still poorly characterized although previous studies suggest that it is precipitated by histamine receptor 1 (H1 receptor) activation. Given that cardiac functions are greatly regulated by the autonomic nervous system and that intracardiac ganglia act as relay stations for all parasympathetic input to the heart, we therefore investigated the effect of histamine on neonatal rat intracardiac ganglion neurons in vitro. Under current-clamp conditions, application of 10 μM histamine depolarized the membrane potential and elicited accompanying repetitive action potential firing. This histamine response was almost completely abolished in the presence of the H1 antagonist triprolidine (1 μM), but not the H2 antagonist ranitidine (3 μM) or the H3 antagonist clobenpropit (3 μM). Furthermore, application of the H1 agonist 2-pyridylethylamine (30 μM) was able to mimic this histamine response. Under voltage-clamp conditions, histamine evoked inward currents when the membrane potential was held at -60 mV. The removal of extracellular $Ca^{2+}$ substantially increased current amplitude whilst the additional removal of extracellular $Na^{+}$ completely abolished it. This is consistent with the involvement of cation channels that are non-selective but show a greater permeability to $Na^{+}$ relative to $Ca^{2+}$. Separately, the amplitude of M-current deactivation, induced by a hyperpolarizing step from a holding potential of -20 mV to -60 mV, was reduced similarly by the application of either histamine or the M-current inhibitor XE-991. Notably however, XE-991 application had no effect on the resting membrane potential. Taken together, these results indicate that histamine facilitates the excitation of intracardiac ganglion neurons via distinct H1 receptor dependent signaling pathways that ultimately activate non-selective cation channels and inhibit M-type K+ channels. (COI:No)

## 3P-028

### Development of a new treatment for dilated cardiomyopathy by mutated troponin T replacement

Yuya Yamaguchi[1], Jun Tanihata[1], Susumu Minamisawa[2], Shunsuke Baba[2], Sachio Morimoto[3] (*[1]Division of Aerospace of Medicine, Department of Cell Physiology, The Jikei University School of Medicine, [2]Department of Cell Physiology, The Jikei University School of Medicine, [3]School of Health Science, International University of Health and Welfare*)

**Introduction:** Dilated cardiomyopathy (DCM) is characterized by cardiac dilation and pump failure. Fundamental therapy for DCM has not been established. Especially, DCM which develops in young age has poor prognosis. Cardiac troponin T (TNNT2) amino acid mutation (ΔK210) knock-in mouse ($TNNT2^{\Delta K210/\Delta K210}$) generated by Morimoto et al., has the similar phenotypes as human juvenile DCM. A previous report showed that overexpression of mutant TNNT2 in wild type mice caused DCM phenotype, suggesting that overexpression of normal TNNT2 in $TNNT2^{\Delta K210/\Delta K210}$ mice may improve DCM phenotypes.
**Purpose:** The aim of this study is to investigate whether replacing the mutant TNNT2 with normal TNNT2 overexpression would improve the DCM phenotypes caused by the mutant TNNT2.
**Methods and Results:** No difference was observed in the cardiac weight and function by echocardiography of human TNNT2 overexpression (Tg) mice compared to non-Tg mice. Next, we mated with Tg mice and $TNNT2^{+/\Delta K210}$ mice to generate $Tg/TNNT2^{\Delta K210/\Delta K210}$ mice. The life span of $Tg/TNNT2^{\Delta K210/\Delta K210}$ mice was slightly extended compared to $TNNT2^{\Delta K210/\Delta K210}$ mice. In the $Tg/TNNT2^{+/\Delta K210}$ mice, the cardiac weight was significantly lower compared with $TNNT2^{+/\Delta K210}$ mice at 7 weeks of age. Moreover, the expression levels of ANP mRNA in $Tg/TNNT2^{+/\Delta K210}$ mice were decreased compared with those of $TNNT2^{+/\Delta K210}$ mice at 7 weeks of age. Echocardiographic examination revealed that left ventricular end-diastolic dimension tended to be decreased and left ventricular fractional shortening was increased in $Tg/TNNT2^{+/\Delta K210}$ mice of 10-weeks-old compared to those of $TNNT2^{+/\Delta K210}$ mice.
**Conclusion:** These results suggest that DCM caused by mutant TNNT2 is partially improved by overexpression of normal TNNT2. In future, we will examine whether normal TNNT2 gene transfer using virus vectors could improve the DCM phenotypes caused by mutant TNNT2. (COI:No)

## 3P-029

### Cardiac pathology in a knock-in mouse model for human hypertrophic cardiomyopathy at early postnatal stages

Cheng-Kun Du[1], Dong-Yun Zhan[1], Sachio Morimoto[2], Tsuyoshi Akiyama[1], Mikiyasu Shirai[1], James Pearson[1] (*[1]Natl. Cereb. Cardiovas. Ctr., Suita, Japan,, [2]Int Univ Health & Welfare, Okawa, Japan*)

**Purpose:** Hypertrophic cardiomyopathy (HCM) is the most common monogenic genetic cardiac disease, with an estimated prevalence of 1:500 in the general population. However, little is known about the disease progression of HCM in children. In previous studies, we found that S179F HCM knock-in mice had a higher mortality rate before weaning. Therefore, in this study, we focused on disease progression of HCM during early postnatal stages.
**Methods:** Cardiac function was evaluated at 15 and 30 days old using echocardiography and pressure volume loop analyses. Protein levels were measured by western blotting assay.
**Results:** Echocardiography showed that LV internal diameter during end diastole and end systole of the HCM mice were less than WT, and LV ejection fraction was greater than WT, but LV wall thickness was not different between HCM and WT mice both at 15 and 30 days old. Echocardiography also showed a decrease in E/A ratio and an increase in isovolumic relaxation time at 15 and 30 days old HCM mice. In vivo cardiac catheter measurements showed a decrease in LV $dP/dt_{min}$ in 30 day old HCM mice. Myocardial disarray was observed at 15 and 30 days old, but fibrosis was observed only in 30 day old HCM mice. Further, we found decreases in protein levels of phosphorylated phospholamban at 15 and 30 days and in SR $Ca^{2+}$-ATPase of 30 day old HCM mice. There was however no change in proBNP expression at both time points.
**Conclusions:** In this study, we found that there was LV diastolic dysfunction in HCM but no increases in proBNP level and ventricular wall thickness that were both evident at 3 months of age. These suggest that targeted therapies for retarding disease progression in HCM would be more beneficial when commenced early in postnatal life. (COI:No)

## 3P-030

### Role of TRPC channels on single cell mechanics in mouse cardiomyocyte

Yohei Yamaguchi[1], Gentaro Iribe[1], Keiji Naruse[2], Akira Takai[1] (*[1]Dept Physiol, Med, Asahikawa Med Univ, Japan, [2]Dept Cardiovas Physiol, Grad Sch Med, Okayama Univ, Japan*)

An increase in preload induces a biphasic response in the heart. The short-term increase in preload rapidly augments contractile force owing to the Frank-Starling mechanism (FSM) that accelerates $Ca^{2+}$ sensitivity of the contraction protein. A further long-term increase in preload for several minutes to hours causes the increase in $[Ca^{2+}]_i$, leading to slow force response to stretch (SFR), a further increase in the contractile force. The authors' previous study identified the involvement of TRPC3 and TRPC6, known as mechanosensitive non-selective cation channels, in SFR. However, the participation of TRPC3 and TRPC6 in FSM remains unclear. To clarify the role of TRPC3 and TRPC6 in FSM, cardiomyocytes were enzymatically isolated from either wild-type (WT), TRPC3 knockout (KO), or TRPC6 KO mice. Both cell ends were held by two pairs of carbon fibers attached to both upper and bottom cell surfaces to apply stretch to the cells. The cells were paced at 4 Hz and superfused in normal Tyrode solution at 37 ℃. Cell length and active/passive force calculated from carbon fiber bending was recorded in six different preload conditions. The effect of each genetic deletion on cellular contractility and elastance was assessed by the slope of end-systolic force-length relation curve (ESFLR) and end-diastolic force-length relation curve (EDFLR). The slope of ESFLR was significantly steeper in TRPC6 KO mice than in WT mice, though it remained unchanged in TRPC3 KO mice. Meanwhile, the slope of EDFLR was not significantly different between WT, TRPC3 KO, and TRPC6 KO mice. These results suggest that TRPC6 regulates the contractile property via modulating the increase in the contractile force controlled by FSM, while TRPC3 is not involved in FSM. (COI:No)

## 3P-031

### Different EDH properties between rat gastroepiploic and mesenteric artery

Hiromichi Takano, Hikaru Hashitani (*Dept Cell Physiol, Grad Sch Med, Nagoya City Univ, Japan*)

Endothelium derived hyperpolarization (EDH) has more important role to regulate the vascular resistance than nitric oxide especially in the resistance arteries. The objective of this study is to compare properties of the EDH among the blood vessels. 3rd or 4th branch of mesenteric arteries (diameter 200 – 300 $\mu$m) and gastroepiploic arteries(diameter 100 - 200 $\mu$m) were dissected from rat. These preparation were pinned down to the bottom of the chamber (0.5 mL volume) and superfused with warmed (35 – 36 ℃) Krebs solution. The membrane potential was measured using a conventional microelectrode technique. The electrode was inserted from the adventitial side. Drugs were administered in the superfusate. All experiments were conducted in presence of N$\omega$-nitro-L-arginine (100 $\mu$M). The resting membrane potential was similar in both blood vessels (-53 ± 5 mV in mesenteric artery vs -55 ± 3 mV in gastroepiploic artery). Acetylcholine (ACh, 1 $\mu$M) produced an initial (10mV), followed by a second phase (4 mV) of membrane hyperpolarization in both types of blood vessels. dicrofenac Na (1 $\mu$M) inhibited the second phase of those hyperpolarization for both types of arteries. 1 $\mu$M TRAM-34, an intermediate conductance $Ca^{2+}$ activated $K^+$ channel inhibitor, inhibited the initial phase of hyperpolarization for gastroepiploic artery only. In presence of 0.1 $\mu$M apamin, a small conductance $Ca^{2+}$ activated $K^+$ channel inhibitor,, ACh failed to produce the initial phase of hyperpolarization for the mesenteric artery, while evoked smaller but significant hyperpolarization for gastroepiploic artery. In presence of diclofenac, TRAM-34 plus apamin, ACh produced no hyperpolarization for both types of blood vessels. These results suggest that 2 types of $K^+$ channels to produce EDH have different contribution for the hyperpolarization and that those contribution might be different between those types of blood beds. (COI:No)

## 3P-032OU

### Novel direct effect of SGLT inhibitor, Canagliflozin, on human myocardial redox state

Hidekazu Kondo[1], Akoumianakis Ioannis[2], Akawi Nadia[2], Kotanidis Christos[2], Antonopoulos Alexios[2], Channon Keith[2], Casadei Barbara[2], Antoniades Charalambos[2] (*[1]Department of Cardiology and Clinical Examination, Oita University, [2]Division of Cardiovascular Medicine, University of Oxford*)

**Background:** Recent clinical trials have demonstrated an effect of sodium glucose cotransporter 2 (SGLT2) inhibitors in improving cardiovascular outcomes of heart failure patients, but the exact mechanisms remain unknown.
**Aim:** We investigated the direct effects of Canagliflozin, a non-selective SGLT1/SGLT2 inhibitor on myocardial redox signalling in humans.
**Methods:** In Study-1, RAA samples from 51 patients were used in an ex vivo system to study the direct effects of canagliflozin on O2.- generation and understand its role in controlling the activity of NADPH-oxidases and uncoupled nitric oxide synthase (NOS). To further study the underlying mechanisms mediating our findings, we then used human cardiomyocytes (hCM) in a series of in vitro experiments (Study 2).
**Results:** Ex-vivo 1-hour incubation of human myocardium with Canagliflozin significantly reduced basal and NADPH-oxidase-derived O2.- and improved nitric oxide synthase (NOS) coupling as determined by the L-NAME inhibitable O2.-. The effects on NADPH-oxidases were mediated by AMP kinase-mediated reduction of membrane translocation of $p47^{phox}$, and suppression of Rac1 GTP-activation and consequent reduction of membrane translocation of Rac1. Canagliflozin also reduced tetrahydropterin oxidation, a NOS co-factor essential for maintenance of NOS coupling, resulting into improved NOS coupling. These findings were replicated in hCM, where canagliflozin was shown to regulate the AMP/ATP ratio, that could be upstream of AMPK activation.
**Conclusions:** We now demonstrate for the first time in humans that Canagliflozin suppresses myocardial NADPH-oxidases activity and improves NOS coupling through an AMPK/Rac1 -mediated pathway in the human myocardium. (COI:No)

## 3P-033

### Peripheral nerve injury-induced effect on BK channel modulation of GABAergic transmission in the superficial dorsal horn of mice

Teruyuki Fukushima (*Dept Physiol & Biol Inform, Dokkyo Med Univ, Tochigi, Japan*)

Large-conductance calcium-dependent potassium (BK) channels are found in tissues including nerves and smooth and cardiac muscles. Previously, we reported that BK channels located in presynaptic terminals reduce excitatory synaptic transmission in the superficial dorsal horn (SDH) and that functional downregulation of BK channels accompanies neuropathic pain induced by peripheral nerve injury. Here, we clarified the physiological significance of BK channels in the modulation of GABAergic synaptic transmission in the SDH, where nociceptive information is processed.
Spontaneously occurring GABAergic miniature inhibitory postsynaptic currents (sIPSCs) were recorded from GFP-negative neurons located in the SDH of spinal cord slices from glutamate decarboxylase 67-green fluorescent protein (GAD67-GFP) knock-in mice. The effect of a BK channel blocker, iberiotoxin (IbTX), on GABAergic mIPSCs was analyzed.
IbTX increased the frequency of GABAergic sIPSCs without affecting their amplitude in the sham-operated mice, suggesting that BK channels attenuated GABAergic synaptic transmission via presynaptic mechanisms.
We conducted similar experiments using sciatic nerve-ligated (SNL) neuropathic mice to clarify the role of BK channels in nociceptive information processing modulated by GABAergic transmission.
Before the IbTX application, the frequency of GABAergic mIPSCs in the SNL mice was significantly more than that in the sham-operated mice. During the IbTX application, the frequency in the SNL mice was similar to that in the sham operated mice. Those would suggest that SNL may induce functional downregulation of BK channels which suppress GABA release.
In the neurons that did not respond to IbTX, the both frequencies of GABAergic sIPSCs before and during the application of IbTX in the sham-operated mice were significantly more than those in the SNL mice, which imply that SNL may decrease GABAergic synaptic transmission in IbTX-unresponsive neurons by unknown mechanism except for BK channels.
Our results may clarify the underlying mechanisms of the nociceptive actions of BK channels in the SDH. (COI:No)

## 3P-034

### Positron Emission Tomography Tracer for AMPA receptors Characterizes Psychiatric Disorders in Human

Mai Hatano[1], Tomoyuki Miyazaki[1], Waki Nakajima[1], Tetsu Arisawa[1], Yuuki Takada[1], Yoshinobu Ishiwata[2], Masaki Sonoda[3], Naoki Ikegaya[3], Masaki Iwasaki[4], Naoto Kunii[5], Yuichi Kimura[6], Fumio Yamashita[7], Masataka Taguri[8], Hideaki Tani[9], Nobuhiro Nagai[9], Teruki Koizumi[9], Shinichiro Nakajima[9], Makoto Higuchi[10], Hiroyuki Uchida[9], Takuya Takahashi[1] (*[1]Dept Physiol, Grad Sch Med, Yokohama City Univ, Japan, [2]Dept Radiol, Grad Sch Med, Yokohama City Univ, Japan, [3]Dept Neurosurgery, Grad Sch Med, Yokohama City Univ, Japan, [4]Dept Neurosurgery, National Center Hospital of Neurology and Psychiatry, Japan, [5]Dept Neurosurgery, Grad Sch Med, Tokyo Univ, Japan, [6]Faculty of Biology-Oriented Science and Technology, Kindai Univ, Japan, [7]Division of Ultrahigh-Field, Institute for Biomedical Sciences, Iwate Medical University, Japan, [8]Dept Data Science, Yokohama City Univ, Japan, [9]Dept Neuropsychiatry, Grad Sch Med, Keio Univ, Japan, [10]Dept Functional Brain Imaging Research, National Institutes for Quantum and Radiological Science and Technology, Japan*)

The glutamate $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor(AMPAR) plays central roles in neuronal functions. However, clinical translation of AMPAR knowledge is limited due to the inability to visualize AMPAR in the living human brain. Here we developed a positron emission tomography(PET) tracer for AMPARs, named [11C] K-2, and showed its specific binding to AMPARs. Logan graphical analysis in first-in-human PET study with healthy participants revealed reversible binding of [11C] K-2. Further, [11C] K-2 revealed systemic reduction of AMPARs in patients with depression, while patients with schizophrenia exhibited focal decrease of AMPARs in parahippocampal and cingulate gyrus. These decreases were significantly correlated with their symptomatology scores in both disorders. Thus, [11C] K-2 could be a useful tool to study biological base of psychiatric disease, and expected to be a novel diagnostic drug in the clinical setting. (COI:No)

## 3P-035

### The mechanisms of synaptic imbalance in pathophysiological state of neuronal circuit at the prefrontal cortex in 15q11-13 duplication autism model mice

Fumihito Saitow[1], Toru Takumi[2], Hidenori Suzuki[1] (*[1]Dept Pharmacol, Nippon Med Sch, Japan, [2]RIKEN Center for Brain Science*)

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and interaction and repetitive behaviors. A chromosome 15q11-13 paternal duplication (*15q dup*) mouse corresponding to cytogenetically frequent CNV in ASD have core symptoms of ASD-like behaviors. Recently, we found that *15q dup* mice showed a hyposerotonergic state in the brain and a reduction in the neuronal activity of the dorsal raphe nucleus (DRN) neurons. Also, we found that administration of the SSRI fluoxetine during the early postnatal period normalized the serotonin (5-HT) level in adulthood. Together with the serotonin restoration, impairments of electrophysiological properties in the DRN and a social behavior were ameliorated. 5-HT is a well-known modulator of behavioral, physiological, and emotional functions at forebrain region. In this study, we investigated the regulatory mechanisms of synaptic excitatory/inhibitory (E/I) balance at the neuronal circuit in the prefrontal cortex (PFC). Using whole cell recording from layer V pyramidal neurons in acute adult brain slices of PFC (~3 month-aged), we found the E/I balance shifted toward the excitatory in *15q dup* mice, consistent with our previous study observed in the sensory cortex. The imbalance of E/I enhanced LTP of glutamatergic synaptic transmission. This enhancement was mimicked by partial blocking of $GABA_A$ receptors suggesting that the neuronal circuits in the PFC of *15q dup* mice were in a reduced state of GABAergic inhibition. We also found that excitatory synaptic inputs and $5\text{-}HT_2$ receptor-mediated modulation of parvalbumin-positive fast-spiking interneurons (FSINs) contributed to control of optimal E/I balance. Taken together, these results provide new insights into the cellular mechanisms underlying maintenance of optimal E/I balance in the PFC. (COI:No)

## 3P-036

### An optogenetic approach to investigate functional profiles of projection from the insular cortex to the nucleus accumbens

Kouhei Kitano, Yuka Nakaya, Masayuki Kobayashi (*Dept Pharmacol, Sch Dent, Nihon Univ, Japan*)

The nociceptive information in orofacial region is transmitted to the trigeminal spinal nucleus of caudalis, parabrachial nucleus, sensory thalamus, and cerebral cortex. Nociception is considered to be finally processed in several cortical regions: the primary and secondary sensory cortices, and limbic cortex including the anterior cingulate and insular cortices. Interestingly, the secondary somatosensory and insular cortices (S2/IOR) neurons send their axons to the nucleus accumbens (NAc), a center of pleasure, and therefore, this pathway is considered to modulate orofacial pain. However, little information is available in terms of the S2/IOR→NAc pathway including physiological and pharmacological properties. We hypothesized that acetylcholine that control neuronal activities in the NAc modulates synaptic transmission from S2/IOR neurons to NAc neurons, and the cholinergic modulation affects on limbic response to nociceptive stimulation in orofacial region. To explore this hypothesis, we used a whole-cell patch-clamp recording technique in combination with optogenetics. By injecting AAV5-hSyn-ChR2(H134R)-mCherry into S2/IOR, S2/IOR neurons expressed ChR2 one month after injection. Activation of ChR2 by blue light stimulation induced excitatory postsynaptic currents (EPSCs). Tetrodotoxin (TTX) application completely diminished EPSCs and application of 4-aminopyridine with TTX recovered EPSCs, suggesting that these EPSCs were mediated via monosynaptic connections. The glutamate receptor antagonist, DNQX, diminished EPSCs, indicating that S2/IOR→NAc connections are mediated by AMPA receptors. Carbachol, a cholinergic agonist, suppressed EPSCs, and this effect was blocked by atropine, a muscarin receptor antagonist. Thus, cholinergic effect on the S2/IOR→NAc connection is suppressive via musucarine receptors. This muscarinic effect might suppress limbic responses to oral pain. (COI:No)

## 3P-037

### Inhibitory local connections of parvalbumin-expressing neurons in the rat globus pallidus

Tetsuya Higashiyama, Fuyuki Karube, Yasuharu Hirai, Fumino Fujiyama (*Lab Neural Circuitry, Grad Sch Brain Sci, Doshisha Univ, Japan*)

The globus pallidus (GP) is known as a relay nucleus of the indirect pathway of the basal ganglia (BG) circuit. The GP plays an important role for the control of action. The GABAergic GP neurons can be classified into two subtypes on projection patterns. One type is a prototypic neuron that projects to the subthalamic nucleus and BG output nuclei. Another type is an arkypallidal neuron that solely projects to the striatum. Previous morphological and in vitro electrophysiological studies showed that GP neurons had local GABAergic axon collaterals. However, it is unclarified whether a strength and probability of the intra-GP connection depend on the subtypes and whether the connection affects the activity of each subtypes. To address this question, we examined local synaptic inputs from parvalbumin (PV) expressing GP neurons (PV-GP neurons) to the two subtypes with in vitro whole cell recording. PV-GP neurons are the most numerous population of prototypic neurons which account for 70% of GP neurons. For selective stimulation of PV-GP neurons, we combined Cre-dependent adeno-associated virus expressing channelrhodopsin-2 and PV-Cre rats in which PV containing neurons express Cre recombinase. We found that the photoactivation of PV-GP neurons elicited the optogenetically evoked inhibitory postsynaptic currents (oIPSCs) in both subtypes of post-synaptic GP neurons in voltage clamp recording. The oIPSCs were mediated by $GABA_A$ receptors. The connection probability and oIPSC amplitude were not significantly different between the subtypes of post-synaptic GP neurons. During 10Hz of the optical stimulations, PV-GP neurons suppressed the firing rate of post-synaptic GP neurons in current clamp recording. These results indicated that the inhibitory local connections of PV-GP neurons might modulate inhibitory outputs from prototypic neurons to downstream BG nuclei, and disinhibit the striatum via inhibition of arkypallidal neurons. (COI:No)

## 3P-038

### Optical measurement of glutamate release from multiple ribbon-type synapses at the terminal of goldfish retinal bipolar cell

Tomoko Oshima-Takago[1,2], Hirokazu Sakamoto [1], Shigeyuki Namiki[1], Kenzo Hirose[1], Masao Tachibana[1,2,3], Hideki Takago[1,2] (*[1]Dept. of Pharmacology, Grad. Sch. of Medicine, The Univ. of Tokyo, Tokyo, Japan, [2]Dept. of Rehabilitation for Sensory Functions, Research Inst., Nat'l Rehabilitation Ctr. for Persons with Disabilities, Saitama, Japan, [3]Ctr. for Systems Vision Science, Organization of Science and Technology, Ritsumeikan Univ., Shiga, Japan*)

Glutamate release at ribbon-type synapses in the retinal, cochlear and vestibular organs is optimized for transmitting continuous sensory signals with graded potential changes. At these synapses, presynaptic $Ca^{2+}$ signals that trigger glutamate release show heterogeneity within and across active zones (AZs). However, it is yet to be investigated whether glutamate release from AZs at ribbon synapses shows heterogeneity. Furthermore, these cells exhibit kinetically separate components of glutamate release: fast and slow components of release. Besides, accumulating evidence from total internal reflection fluorescence (TIRF) microscopic and/or electrophysiological studies indicates that ribbon-associated and ribbon-free AZs at the terminal of retinal bipolar cell might underlie fast and slow components of release, respectively. This outlier hypothesis remains to be further tested ideally by visualizing the dynamics of glutamate release at multiple AZs. Using a retinal bipolar cell (BC) specific enhanced glutamate optical sensor (BC-eEOS), which has been optimized from the original eEOS, we here report the establishment of optical measurement of glutamate signals from individual release sites at the terminal of goldfish retinal bipolar cell. This novel imaging technique using the BC-eEOS allowed us to find heterogenous nature of the fast and slow components of glutamate signals at the ribbons, which were visualized with 5-TMRIA-conjugated CtBP-binding peptide. First, the BC-eEOS fluorescence intensity for the fast component was variable across individual ribbons. Second, the slow component was not observed at all the ribbons. Rather, sites for the slow component appeared to be spatially confined. Third, the fast component occurred predominantly at ribbon-associated sites, whereas slow component appeared to occur at both ribbon-associated and ribbon-free sites. Taken these together, the novel imaging method using BC-eEOS might enable us to analyze the fast and slow components of glutamate release at multiple ribbon-associated as well as ribbon-free sites in the retinal bipolar cell terminal. (COI:No)

## 3P-039

### Mechanical stimulation-induced intercellular communication among trigeminal ganglion neurons

Tatsuhiko Yazaki[1,2], Sadao Ohyama[2], Wataru Ofusa[2], Haruna Toda[2], Hidetaka Kuroda[3], Asuka Higashikawa[2], Maki Kimura[2], Yoshiyuki Shibukawa[2], Tatsuya Ichinohe[1] (*[1]Dept Dent Anesthesiol, Tokyo Dent Coll, Japan, [2]Dept Physiol, Tokyo Dent Coll, [3]Dept Crit Care Med Dent, Kanagawa Dent Univ*)

Recently, paracrine communication between neurons and non-neuronal cells has been well described. There has been a little report on the intercellular communication between neurons, however. Although we have previously examined intercellular communication between trigeminal ganglion (TG) neurons by patch-clamp recordings, we could not record any responses from nearby TG neurons to the stimulated ones. To examine possible intercellular commnication via metabotropic receptors, we aimed to clarify whether the diffusible factors or substances are capable to be released from the neurons by direct mechanical stimulation to the single neurons or not using intracellular $Ca^{2+}$ imaging. We dissected TG from neonatal Wistar rats (7 days old) under pentobarbital sodium anesthesia (50 mg/kg with isoflurane). We then acutely isolated TG cells, and the cells were primary cultured (5% $CO_2$, 95% air, 37 ℃). The cells ware loaded with fura-2, and the intracellular free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) was recorded from the fluorescence (510 nm) intensity ratio at excitation wavelengths of 340 nm and 380 nm. Direct mechanical stimulation to the TG cells increased $[Ca^{2+}]_i$. In addition, not only directly stimulated neurons, but also the neurons which are located nearby stimulated neurons showed an increase in $[Ca^{2+}]_i$. The results suggested that neurons that received mechanical stimulation are capable to release diffusible intercellular transmitters to establish inter-neuronal communication via metabotropic receptor activation. (COI:No)

## 3P-040

### Microglia mediate peripheral nerve injury-induced plasticity to the thalamus

Yoshifumi Ueta, Sachie Sekino, Yoko Katayama, Mariko Miyata (*Dept Physiol, Sch Med, Tokyo Women's Med Univ, Japan*)

In the central nervous system, peripheral nerve injury reorganizes somatotopic representation. We have previously reported in mice that the whisker deafferentation induced by transection of the infraorbital nerve disrupts topographic projections from the brainstem to the thalamic ventral posteromedial nucleus (VPM) by increasing non-whisker-information carrying 'ectopic' axons in VPM. However a mechanism mediating peripheral nerve damages to this central reorganization is still unknown. Here we show the role of pathway- and region-specific microglial activation in the principal trigeminal nucleus (Pr5), a whisker sensory-recipient brainstem region. Systemic or local manipulation of microglia reveals that reactive microglia in Pr5 are necessary and sufficient for inducing ectopic axons in VPM. Microglia associate with deafferentation-induced Pr5 neuronal hyperexcitability. Inactivation of Pr5 neurons can suppress deafferentation-induced increase of ectopic axons in VPM in spite of microglial activation in Pr5. Moreover, microglial activity is necessary for deafferentation-induced ectopic hypersensitivity to lower jaw mechanical stimulation. Thus, brainstem microglial activity mediates nerve injury-induced plasticity to non-injured thalamic circuits and somatosensory-related behavior. (COI:No)

## 3P-041

### Contextual learning requires phosphorylation at Ser408-409 of GABAA receptor β3 subunit

Yuya Sakimoto, Ryota Sotomaru, Thein-Oo Paw-Min, Makoto Goshima, Dai Mitsushima (*Dept Physiol, Grad Sch Med, Yamaguchi Univ, Japan*)

Although contextual learning requires plasticity at both AMPA receptor-mediated excitatory and GABAA receptor-mediated inhibitory synapses in CA1 neurons, detailed mechanism of the learning-induced plasticity at GABAA receptor-mediated synapses has been unknown. We recently reported learning-induced increase in postsynaptic number of GABAA receptor channel (Sakimoto et al, Cerebral Cortex 2019), and phosphorylation of intracellular loop (Ser408-409) of GABAA receptor β3 subunit (Sakimoto et al., FASEB J 2019). To further examine the causal relationship among the Ser408-409 phosphorylation, synaptic plasticity, and the learning, we used cell permeable HIV tag peptide and synthesized with the novel peptide-based phosphorylation inhibitor targeting sites at Ser408-409 (Tat pep β3-SS). Under the freely-moving condition, we bilaterally microinjected the Tat pep β3-SS or site-specific mutated control (Tat pep β3-AA) into the CA1 region, and used them for the following behavioral test battery: contextual learning (IA task), emotion (open field task), perception (visual task) and social behaviors (pairing test). Tat pep β3-SS but not Tat pep β3-AA impaired the performance of retrieval test in IA task, while the effect was not observed in any other behavioral tests. Ex vivo whole cell patch clamp analysis further revealed that unilateral Tat pep β3-SS microinjection clearly blocked the learning-induced increase in the postsynaptic GABAA receptor-mediated Cl- current in CA1 pyramidal neurons.
These results suggest a causal relationship among the Ser408-409 phosphorylation, GABAA receptor-mediated synaptic plasticity, and the learning. Understanding the functional role of Ser408-409 phosphorylation of the subunit might be beneficial for the drug discovery and development for multiple cognitive disorders. (COI:No)

## 3P-042

### Reduced glutamate uptake in cerebellar Purkinje cells in Atp1a3 heterozygous knockout mice: glial compensation and its impacts on long-term depression

Keiko Ikeda[1,2], Shinlchiro Satake[3,4], Kiyoshi Kawakami[2] (*[1]Dept Physiol, Sch Med, Int Natl Univ Health Welfare, Japan, [2]Div Cell Biol, Center for Mo Med, Jichi Med Univ, Japan, [3]Natl Inst Physiol Sci, Okazaki, Japan, [4]Grad Univ for Advanced Studies, Fundamental Neurosci, Japan*)

Excitatory amino acid transporters (EAATs) are responsible for cellular uptake of neurotransmitter glutamate using the electrochemical gradients of sodium and potassium ions across the cell membrane. Na, K-ATPase (Sodium pump) plays a key role in the maintenance of the gradient. Mutations in the human Sodium pump alpha3 subunit gene, *ATP1A3*, have been identified as the cause for rapid-onset dystonia with Parkinsonism (RDP) and alternating hemiplegia of childhood (AHC). The alpha3 subunit protein is abundantly expressed in Purkinje cells (PCs), the sole output neuron of the cerebellar cortex. The pathophysiology of these disorders remains unknown. Here we focus whether EAATs relate to these sodium pump neurological disorders. We examined the EAAT activity in the cerebellum in *Atp1a3* heterozygous knock out mice (*Atp1a3*$^{+/-}$). We found a remarkable reduction of the glutamate uptake-coupled currents mediated by the EAAT4 subtype in PCs of *Atp1a3*$^{+/-}$ compared with those of wild type litter-mates. On the contrary, the amplitude of EAAT currents in the astrocyte Bergmann glia (BG) was profoundly higher in *Atp1a3*$^{+/-}$. Consistently, the protein levels of the glia-specific EAAT1 subtype in the cerebellum were increased. Furthermore, in *Atp1a3*$^{+/-}$, long-term depression (LTD, postsynaptic origin) was diminished at the excitatory synapses from parallel fibers (PFs) to PCs. The impaired LTD was rescued by application of the EAAT1 inhibitor UCPH102 and the mGluR1 agonist DHPG. Taken together, it is suggested that the enhanced glutamate uptake by BG submerged the weakened neuronal EAAT activity in *Atp1a3*$^{+/-}$, thereby attenuating the extracellular diffusion of the glutamate spilled out of the PF-PC synaptic clefts and reducing the activation of the perisynaptically distributed mGluR1s on PC dendritic spines. It is quite likely that these events interfere with LTD. Our findings would provide underlying mechanisms for the onset of dystonia symptoms in RDP and AHC. (COI:No)

## 3P-043

### Morphological study of motor neurons innervating the lumbar trunk muscles in rats

Minori Fukuda[1], Masako Ikutomo[2], Ken Muramatsu[3], Naomi Oshiro[1], Masatoshi Niwa[1]
([1]*Department of Occupational Therapy, Kyorin University, Tokyo, Japan,* [2]*Department of Rehabilitation, Tokyo Health Science University, Tokyo, Japan,* [3]*Department of Physical Therapy, Kyorin University, Tokyo, Japan*)

The lumbar trunk region has no ribs, and is thought to play an important role in maintaining the trunk in an upright position and performing anteroposterior and lateral bending. In this study, we aimed to clarify the localization and morphological feature of motor neurons innervating the lumbar trunk muscles in the first and second lumbar segments.
The retrograde labeling with fluorescently labeled dextran of motoneurons was performed on anterior branches(AB) of the spinal nerves that control the ventral side of the trunk muscles i.e. abdominal muscles, and posterior branches(PB) that controls the dorsal side i.e. back muscles from the first and second lumbar spinal cord in rats.
The anterior branch motor neurons were located in ventrolateral portion of anterior horn, the posterior branch motor neurons were located in ventromedial portion and there was no site where both were mixed. AB motor neurons were located over the range of stained segments and PB motor neurons on the rostral half of the stained segment. The average number and size of the AB motor neurons were 141 and 839 $\mu m^2$, they of the PB motor neurons were 82 and 819 $\mu m^2$ respectively. The size distribution of AB motor neurons was unimodal while the PB motor neurons was bimodal.
Previous studies have established that size distribution of hindlimb motor neurons show bimodal distribution and the smaller group is composed mainly of gamma motor neurons while the larger group is composed mainly of alpha motor neurons. Hence, difference of size distribution may reflect difference of gamma motor neuron content in AB motor nuclei and PB motor nuclei. The abdominal muscle and back muscle may have different modes of control by alpha and gamma motor neurons. (COI:No)

## 3P-044

### Loose coupling between SK and P/Q-type $Ca^{2+}$ channels in cartwheel cells of the dorsal cochlear nucleus

Tomohiko Irie (*Div Pharmacol, NIHS, Kanagawa, Japan*)

Small-conductance $Ca^{2+}$-activated $K^+$ (SK) and largeconductance voltage- and $Ca^{2+}$-activated $K^+$ (BK) channels are $Ca^{2+}$-activated $K^+$ channels that control action potential firing in diverse neurons in the brain. In cartwheel cells of the dorsal cochlear nucleus, blockade of either channel type leads to excessive production of spike bursts. In the same cells, P/Q-type $Ca^{2+}$ channels in plasma membrane and ryanodine receptors in endoplasmic reticulum supply $Ca^{2+}$ to BK channels through $Ca^{2+}$ nanodomain signaling. In this study, voltage-clamp experiments were performed in cartwheel cells in mouse brain slices to examine the $Ca^{2+}$ signaling pathways underlying activation of SK channels. As with BK channels, SK channels required the activity of P/Q-type $Ca^{2+}$ channels. However, this signaling occurred across $Ca^{2+}$ micro- rather than nanodomain distances and was ndependent of $Ca^{2+}$ release from endoplasmic reticulum. These differential modes of activation may lead to distinct time courses of the two $K^+$ currents and therefore control excitability of auditory neurons across different timescales. (COI:No)

## 3P-045

### Cell-cycle Length of Medial Ganglionic Eminence Progenitors Determines Interneuron Fate

Yong-Chun Yu, Min Wang, Yinghui Fu, Dan Shen (*Institutes of Brain Science, State Key Laboratory of Medical Neurobiology and Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200032, China.*)

Cortical interneurons represent a highly diverse population of cells with important roles in network function. How interneurons originating in the ganglionic eminences (GEs) become specified during development is an outstanding question. Although genetic mutations that disrupt the cell cycle of interneuron progenitors alter interneuron fate determination, the exact link between them remains poorly understood. Here, using a triple thymidine analog method to label dividing progenitors in vivo, we demonstrate that cell-cycle length of interneuron progenitors influences the fate determination of major subgroups of cortical interneurons. We found that short cell-cycle progenitors mainly locate in the middle region of the medial ganglionic eminence (MGE), whereas relatively long cell-cycle progenitors preferentially distribute in the ventral and dorsal regions of the MGE. A combination of cell transplantation and fate mapping studies further reveal that short cell-cycle progenitors mainly give rise to somatostatin-expressing interneurons, whereas relatively long cell-cycle progenitors generate predominantly parvalbumin-expressing interneurons. These results suggest that cell-cycle length of progenitors plays a pivotal role in the fate determination of MGE-derived interneuron subtypes. (COI:No)

## 3P-046

### Activity-dependent differentiation of axon initial segment in avian cochlea nucleus

Israt Jahan, Ryota Adachi, Hiroshi Kuba (*Department of Cell Physiology, Graduate School of Medicine, Nagoya University*)

The axon initial segment (AIS) is accumulated with voltage-gated sodium channels and involved in generation of action potentials. We previously showed in avian cochlear nucleus (nucleus magnocellularis, NM) that length of the AIS is determined in a tonotopic-region-specific manner; it is shorter in neurons with high characteristic frequency (CF) (~20 μm) than those with low CF (~25 μm), which plays a critical role in adjusting the output within the nucleus. However, the mechanism underlying this differentiation is not well understood. We addressed this issue in organotypic cultures of chicken brainstem, in which NM neurons are totally isolated from afferent input. At 14 days in culture, corresponding to the period after maturation, the AIS in high-CF neurons was shorter (~24 μm) than that in low-CF neurons (~30 μm). However, this difference disappeared when the cultures were incubated for 3-7 days with TTX and DNQX, due to an elongation of the AIS in high-CF neurons (~28 μm), suggesting that spontaneous activity shortened the AIS specifically in high-CF neurons, which caused the difference of AIS length between the neurons. In support, the AIS length was reduced in high-CF neurons, when spontaneous activity was augmented by either blocking Kv1 channels with dendrotoxin-I (~21 μm) or lowering $[K^+]_o$ with a chelator, sodium polystyrene sulfonate (~18 μm). Moreover, when we depolarized the membrane via elevation of $[K^+]_o$ (10.6 mM), the AIS (~20 μm) was shortened in high-CF neurons, but not in low-CF neurons. These results indicated that the activity-dependent shortening of the AIS occurs more efficiently in higher-CF neurons, which underlies the tonotopic differentiation of the AIS in NM. (COI:No)

## 3P-047

### Advantages of acute brain slices prepared at physiological temperature for investigating synaptic functions

Kohgaku Eguchi, Peter Koppensteiner, Ryuichi Shigemoto (*Shigemoto group, Institute of Science and Technology Austria, Am Campus 1, 3400 Klosterneuburg, Austria*)

Acute brain slice preparation has been developed as a powerful experimental model for investigating the characteristics of synaptic functions in brain. The acute slice preparation is readily accessible for electrophysiological and optical recordings and retains the cytoarchitecture and synaptic circuits in vivo except for the long-range projections. In general, to prepare acute slices, the dissected brain from an animal is immersed into the ice-cold cutting solution, and then sliced at ice-cold temperature (CT) to avoid the neuronal excitotoxicity during cutting. The acute slice preparation at CT, however, causes molecular and architectural changes of cellular components. Chilling of hippocampal slices induces disassembly of microtubules and eliminates dendritic spines. Re-warming of the hippocampal slices revives microtubule structures, but excessively reconstructs the dendritic spines resulting in higher density of synapses than that in intact brain (Kirov et al., 2004).
Recently, a method of acute slice preparation at physiological temperature (PT) has been developed to improve quality of cerebellar slices in aged rodents. In the warm-cut cerebellar slices, Purkinje cells (PCs) survived better without altered intrinsic electrophysiological features of the cells (Huang and Uusisaari, 2013). However, it hasn't examined whether the synaptic features are better preserved in the warm-cut acute slices compared to the cold-cut slices.
In this study, we investigated ultrastructural and electrophysiological features of synapses in mouse acute cerebellar slices prepared at CT and PT. In the slices prepared at CT, we found significant synaptic vesicle rearrangement and decrease in synaptic proteins, both of which were not detected in slices prepared at PT. Consistent with these structural findings, slices prepared at PT showed higher release probability and higher detectability of long-term depression after motor learning compared with slices prepared at CT. These results indicate substantial advantages of the slice preparation at PT for investigating synaptic functions in different physiological conditions. (COI:No)

## 3P-048

### WNK3 kinase maintains basal excitability by regulating inward rectification and intracellular chloride in layer V pyramidal neurons of mouse medial prefrontal cortex

Adya Saran Sinha[1], Tianying Wang[1], Yasushi Hosoi[1], Eisei Sohara[2], Tenpei Akita[1], Shinichi Uchida[2], Atsuo Fukuda[1] ([1]*Department of Neurophysiology, Hamamatsu University School of Medicine, Hamamatsu, Japan,* [2]*Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan*)

WNK kinases with their downstream phosphorylation cascades SPAK/OSR1 are critical for their regulation of cation chloride cotransporters namely NKCC1 and KCC2 in determining neuronal responses to GABA as either excitatory or inhibitory based on the status of intracellular chloride $[Cl^-]_i$ levels. Among the WNK family (WNK1-WNK4), WNK3 are highly expressed in brain. WNK1 is observed to be more active in immature neurons and WNK3 is proposed to regulate activity of WNK1 in mature neurons. Increased expression of WNK3 has been reported in pathological conditions of schizophrenia and temporal lobe epilepsy. Using genetically modified WNK3KO mice and WT litter-mates, we performed in-vitro patch clamp electrophysiology to understand the role of WNK3 kinase on basal and activity dependent regulation of excitability and $[Cl^-]_i$ levels in layer V pyramidal neurons from the medial prefrontal cortex (mPFC) between postnatal day (P)21-27. We found that loss of WNK3 in mature neurons significantly reduced intrinsic excitability, as evidenced by hyperpolarized resting membrane potential (RMP), decrease in input resistance thereby increasing the rheobase current for AP generation. Voltage-clamp recordings revealed the enhanced inwardly rectifying potassium (IRK) conductance caused the hyperpolarized RMP. Indeed, introduction of the active form of WNK3 kinase into the knockout neurons suppressed the enhancement indicating a phosphorylation dependent mechanism. In addition, gramicidin perforated patch clamp recordings from layer V pyramidal neurons exhibited a depolarized GABA response in comparison to WT neurons indicating higher $[Cl^-]_i$ levels. We also investigated by western blot analysis the downstream signaling cascade of WNK1, SPAK/OSR1and their phosphorylation status critical for regulation of NKCC1 and KCC2 function. Evaluation of synaptic currents also showed increased frequency of miniature inhibitory post synaptic currents (mIPSCs). Together, these observations indicate an important role of WNK3 in regulation of neuronal excitability of mature pyramidal neurons. (COI:No)

## 3P-049

### Proximodistal heterogeneity in learning-promoted pathway-specific plasticity at dorsal CA1 synapses

Paw-Min-Thein-Oo[1], Yuya Sakimoto[1], Hiroyuki Kida[1], Dai Mitsushima[1,2] (*[1]Department of Physiology, Yamaguchi University Graduate School of Medicine, Yamaguchi, 755-8505, Japan, [2]The Research Institute for Time Studies, Yamaguchi University, Yamaguchi, 753-8511, Japan*)

Contextual learning requires the delivery of AMPA receptors to CA1 synapses in the dorsal hippocampus. However, proximodistal heterogeneity of pathway-specific plasticity remains unclear. Here, we examined the proximodistal heterogeneity in learning-induced plasticity at the CA1 synapses with inputs from the entorhinal cortex layer III (ECIII) or from CA3. We subjected male rats to an inhibitory avoidance task and prepared acute hippocampal slices for whole-cell patch clamp experiments, where we stimulated ECIII-CA1 or CA3-CA1 input fibers to analyze evoked excitatory postsynaptic currents (EPSCs). Compared to untrained controls, trained rats exhibited higher AMPA/NMDA current ratios at proximal and intermediate, but not distal CA3-CA1 synapses, which suggested that region-specific plasticity occurred after learning. Moreover, trained rats exhibited higher AMPA/NMDA current ratios at intermediate and distal, but not proximal ECIII-CA1 synapses. These findings suggested the presence of proximodistal heterogeneity in pathway-specific postsynaptic plasticity. Regarding presynaptic plasticity, training slightly, but significantly increased the paired-pulse ratios of proximal and intermediate, but not distal CA3-CA1 synapses. Moreover, trained rats exhibited higher paired pulse ratios at intermediate and distal, but not proximal ECIII-CA1 synapses, which suggested region-specific presynaptic plasticity. Finally, learning was clearly prevented by the bilateral microinjection of a plasticity blocker in the proximal or intermediate, but not distal CA1 subfields, which suggested functional heterogeneity along the proximodistal axis. Understanding region- and pathway-specific plasticity at dorsal CA1 synapses could aid in controlling encoded memory. (COI:No)

## 3P-050

### Contextual learning promotes plasticity at the inhibitory synapses onto the granule cells in the dentate gyrus

Han-Thiri-Zin, Yuya Sakimoto, Dai Mitsushima (*Graduate School of Medicine, Yamaguchi University*)

Granule cells in the dentate gyrus of the hippocampus are thought to be essential to memory function by decorrelating overlapping input patterns (pattern separation). To examine the learning-dependent synaptic plasticity of granule cells, we subjected male rats to an inhibitory avoidance (IA) task and prepared acute hippocampal slices for voltage clamp analysis in trained and untrained animals. After addition of tetrodotoxin, we recorded the spontaneous miniature responses induced by a single synaptic vesicle of glutamate or GABA. Both miniature EPSCs (mEPSC at -60 mV) and miniature IPSCs (mIPSC at 0 mV) were sequentially recorded from the same granule cells. Compared with untrained control, IA-trained rats significantly increased mIPSC frequency, suggesting learning-induced plasticity at GABAA receptor–mediated inhibitory synapses. In contrast, IA training did not affect mEPSC amplitude and frequency. Moreover, specific stimulation of excitatory fibers from mossy cells, medial performant pathways, or lateral performant pathways failed to show significant changes in AMPA vs NMDA current responses, suggesting that the learning may not promote plasticity at AMPA receptor–mediated excitatory synapses. Since contextual learning strengthened both inhibitory and excitatory synapses onto the CA1 pyramidal neurons, learning-promoted plasticity seems to be cell type–dependent.

Keywords: AMPA receptor, $GABA_A$ receptor, glutamic acid, GABA, synaptic plasticity

(COI:No)

## 3P-051

### Membrane expression of mGluR6 transfected cultured retinal bipolar cells

Takumi Akagi, Atsushi Shimohata, Ikuo Ogiwara, Makoto Kaneda (*Dept Physiol, Nippon Med Sch, Japan*)

Metabotropic glutamate receptor 6 (mGluR6) is expressed at the dendritic tip of retinal ON-bipolar cells (ON-BC), and plays critical roles in the initial step of visual information processing. The localization of mGluR6 in ON-BC is restricted to the postsynaptic compartment at the dendritic tips where it mediates G-protein-dependent signaling cascades of 'light on-response' depending on glutamate release from photoreceptors. While the spatial restriction of mGluR6 localization is thought to be crucial for the precise visual signal transduction, the mechanisms of mGluR6 synaptic targeting remain elusive. In our previous study, we suggested that intracellular C-terminal domain (CTD) of mGluR6 may contain regulatory elements to control the membrane surface localization of mGluR6 in the heterologous expression system (HEK293, primary hippocampal neuron). In this study, to elucidate the molecular mechanisms of polarized distribution of mGluR6 in retinal ON-BC, we transfected the plasmid expression vector consist of cDNA encoding rat mGluR6 with V5 epitope tag located at the N-terminal end to primary cultured mouse retinal ON-BC, and examined localization of the expressed mGluR6. At DIV 7-10, cells with bidirectional neurites showed positive immunoreactivity for PKC $\alpha$, a rod bipolar cell marker. In these PKC $\alpha$-positive cells, we observed membrane surface expression of V5 tagged mGluR6. However, we could not observe the polarized localization of mGluR6. These observation suggest that mGluR6 possesses the ability to express itself to membrane surface of the primary cultured retinal bipolar cells, whereas an important additional mechanism is required for the polarized distribution in the cultured bipolar cells. (COI:No)

## 3P-052

### Estimation of stimulating electrodes in retinal prosthesis by recurrent neural network

Tomomitsu Miyoshi[1], Tomoyuki Naito[2], Ryota Imai[3], Takeshi Morimoto[4], Takashi Fujikado[3] (*[1]Dept Integrat Physiol, Grad Sch Med, Osaka Univ, Japan, [2]Lab Cogni Behav Neurosci, Grad Sch Med, Osaka Univ, Japan, [3]Grad Sch Frontier Biosci, Osaka Univ, Japan, [4]Dept Advanced Vis Neurosci, Grad Sch Med, Osaka Univ, Japan*)

We have been developed a novel retinal prosthetic system, Suprachoroidal Transretinal Stimulation (STS) for photoreceptor degenerating diseases. In the STS, stimulating electrode array is implanted in sclera so as to avoid damaging neural retina. To evaluate the prosthetic response in central nervous system, previously we investigated the response properties of single-unit activity in cat lateral geniculate nucleus. Here, we recorded prosthetic electrical evoked response of visual cortex with electrocorticogram (ECoG) electrodes, and investigated whether Long short-term memory (LSTM) network can recognize the position of the stimulating electrode from the ECoG response or not.
The STS array with 9 active electrodes was implanted into cat eye under general anesthesia in acute experiment. The size of single electrode in the array was 0.5 mm in diameter and 0.3 mm in height, which was the same as the device for clinical trials. The stimulation parameter was 500 uA amplitude, 0.5 ms/phase duration, biphasic, 1 Hz frequency. The prosthetic evoked response was recorded from cortical area17/18 surface by ECoG array with 16 electrodes.
Simple LSTM+3-layered fully connected layers network was trained with data of 250ms ECoG waveforms (from 50ms before trigger 200ms after trigger). 80% of full data was used for training and then, this trained LSTM classified the rest of waveforms. The accuracy of the classification was 0.63 (significantly higher than the chance level). This result shows that LSTM can discriminate the electrode position by ECoG with online single waveform.
(COI:Properly Declared)

## 3P-053

### Anti-inflammatory and anti-nociceptive effect of human gene-derived peptides

Rumi Nakayama-Naono[1], Hideki Funahashi[2], Yu Miyahara [2], Keiju Kamijo[1], Yusuke Ishida[1], Toshikazu Nishimori[2] (*[1]Dept of Anatomy and Cell Biol, Fac of Med, TMPU, Japan, [2]Dept Psychiatry, Fac of Med, Univ of Miyazaki, Japan*)

Endokinin peptides encoded by human TAC4 gene are a new member of tachykinin peptide family. Our previous study indicated that some of these peptides has a crucial role in nociceptive processing in the spinal cord. Indeed, endokinin peptides consist of four peptides, endokinin A, B, C and D, and endokinin A and B (EKA/B) elicits excitatory effects, while the function of endokinin C and D (EKC/D) remains uncertain. The pretreatment with EKC/D attenuated induction of nocicceptive-related behaviors by substance P (SP) indicating that EKC/D has an antagonistic role for the neurokinin-1 receptor (NK1R) that is the receptor of SP. This functional difference may contribute to that amino acids at the carboxyl-terminal of EKA/B or EKC/D are leucine and methionine, respectively. Since it is necessary to improve the anti-nociceptive effect of EKC/D, we synthesized EKC/D-derived peptides (D-EKC/Ds) having some of D-type amino acids. Then, to clarify whether the inhibitory effect of EKC/D is altered by replacement with D-type amino acids (D-EKC/Ds), the effect of these peptides was evaluated by SP-induced nociceptive behavior, and by the inflammatory nociceptive behavior. Pretreatment with D-EKC/Ds were long-lasting attenuated on SP-induced nociceptive behavior and inflammation which compared with EKC/D, and these effects were dependent on the number and position of D-type amino acid.These results indicate that, D-EKC/Ds were not only anti-nociceptive effect but also anti-inflammatory effect and some of D-type amino acids may have a crucial role of these effects. (COI:No)

## 3P-054

### Involvement of melanopsin on the photic excitation of neurons in the medullary dorsal horn and lacrimation

Akimasa Tashiro, Yuji Morimoto (*Dept Physiol, Natl.Defense Med Col.*)

Bright light can cause ocular discomfort and evokes protective reflexes such as blinking. Previously we reported that bright light activates trigeminal nerve activity through an intraocular mechanism driven by a luminance-responsive circuit (non-image visual functions) and increased parasympathetic outflow to the eye. Melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) are photoreceptors that mediate non-image visual functions.
To determine if melanopsin activation was necessary for light-evoked Vc/C1 excitation, melanopsin antagonist (Opsinamides) was injected systemically while recording light-evoked Vc/C1 unit activity. Under isoflurane anesthesia, units were recorded in laminae I-II at Vc/C1 under low ambient light. All units received convergent mechanical input from the ocular surface and facial skin. Units with a cutaneous receptive field (RF) were tested for responses to pinch stimuli. Light stimulation (300W/m2, 30s) was delivered from a thermal-neutral fiber optic source positioned 5 cm from the ocular surface at 20 min intervals. Light and mechanical evoked unit activity was recorded before and after 10 min after Opsinamides (1mg/kg, iv). Systemic administration of Opsinamides inhibited the light evoked unit activity. While, mechanical input from the convergent receptive field was not inhibited. Systemic administration of Opsinamides prevented light evoked increases in tear volume. These findings confirm that melanopsin plays a pivotal role in mediating light-evoked neural excitation at the Vc/C1 region and ocular-specific function such as reflex lacrimation. Activation of melanopsin may cause discomfort called dazzling sensation after intense light. (COI:No)

## 3P-055

### Role of olfactory tubercle in the weaning process of neonatal mice

Masahiro Yamaguchi, Yasutaka Chikuda (*Dept Physiol, Kochi Med Sch, Kochi Univ, Japan*)

There is a close relationship between feeding behavior and olfaction. Weaning of neonates is a typical example of feeding behavior development and learning, which proceeds between 2 and 4 weeks of age in mice. In the olfactory cortical regions, olfactory tubercle (OT) is thought to contribute to the odor-guided behavior learning, where the anteromedial domain drives attractive behavior and the lateral domain aversive behavior. Given that the OT develops structurally and functionally during the weaning period, we examined the role of the OT in the neonatal feeding behavior. By using transgenic mice expressing Cre recombinase in striatal neurons and viruses expressing toxins in a Cre-dependent manner, principal neurons in the OT domains were ablated during neonatal period. Cell ablation in the anteromedial OT domain impaired the ability of neonates to find food pellets hidden beneath the bedding, and prolonged their preference to lactating mother over food pellets. Ablation in the lateral OT domain did not induce these impairments. These results indicated a crucial role of the anteromedial OT domain in the physiological weaning process. (COI:No)

## 3P-056

### Activation mechanism of the olfactory tubercle in the odor-guided attracted behavior learning in mice

Yuriko Koga, Kazuko Yokoi, Masahiro Yamaguchi (*Dept Physiol, Kochi Sch Med, Kochi Univ, Japan*)

Odors induce various motivated behaviors, and most of the odor-guided behaviors are acquired through learning process. When mice are presented with a cue odor and food reward at the same time, they become attracted to the cue odor. On the other hand, when they are presented with the cue odor and noxious stimuli, they become aversive to the cue odor. Among various areas of the olfactory cortex, the olfactory tubercle (OT) is a crucial area to link odor cues with motivated behaviors. The anteromedial domain of the OT is activated when mice learn to take attractive behavior to the odor, while the lateral domain is activated when then learn to take aversive behavior. However, the plastic mechanism of the neural circuits of how specific domains of the OT become activated by odor-guided behavior learning remains elusive.
The OT receives inputs from various brain areas. In this study, we focused on the plastic change of synaptic inputs from the piriform cortex (PC), the largest area of the olfactory cortex, to the OT. By using optogenetics in mice, synaptic inputs from the PC to OT domains were activated and associated with food reward. Then the mice learned to take food-seeking behavior by the photostimulation. We are analyzing the synaptic terminals of the photostimulated synaptic inputs to the OT domains histologically in the brain sections, to see whether the synaptic inputs in the anteromedial and lateral domains are biased by the attractive behavior learning. (COI:No)

## 3P-057

### Olfactory marker protein buffers cAMP by direct binding to avoid depolarization-induced silencing of olfactory receptor neurons

Noriyuki Nakashima[1], Kie Nakashima[2], Akiko Nakashima[1], Akiko Taura[3], Harunori Ohmori[4], Makoto Takano[1] ([1]*Dept. Physiol., Kurume Univ. Sch. Med., Japan,* [2]*Grad. Sch. Biostudies, Kyoto Univ.,* [3]*Dept. Med. Engineering, Facult. Health Sci., Aino Univ.,* [4]*Dept. Physiol., Sch. Med., Kanazawa Med. Univ.*)

Sensory cells respond to external stimuli with resilient firing properties. Olfactory receptor neurons (ORNs) use odour- induced intracellular cAMP surge to gate cyclic nucleotide-gated nonselective cation (CNG) channels in the limited ciliary space. The prolonged exposure to cAMP causes adaptation of CNG channels and attenuates neural responses. On the other hand, the odour-source searching behaviour of animals requires ORNs to be sensitive to the odour while approaching the targets. How ORNs accommodate these conflicting aspects of cAMP responses remains unknown. Here, we show that the cAMP-buffering machinery swiftly bypasses surplus cAMP during signal transduction to maintain the sensitivity of ORNs. We discovered that the cytosolic olfactory marker protein (OMP) directly captured cAMP, which transiently desensitized CNG channel activity and prevented sustained membrane depolarization upon the application of sensory stimuli. Under repetitive stimulation, $OMP^{-/-}$ ORNs were immediately silenced after burst firing due to sustained depolarization and inactivated firing machinery. Consequently, $OM^{-/-}$ mice showed serious impairment during odour-source searching tasks. Therefore, cAMP buffering by OMP transiently desensitizes CNG channels by uncoupling the signal transduction, but maintains resilient sensory responses in ORNs. (COI:No)

## 3P-058

### Developmental changes in spike sequences of L4, L2/3 neurons in response to whisker stimulations in the rat barrel cortex

Fumitaka Kimura[1], Hiroshi Tamura[2] ([1]*Dept Mol. Neurosci, Grad Sch Med, Osaka Univ, Japan,* [2]*Grad Sch Frontier Bioscience, Osaka Univ*)

Spike timing-dependent plasticity (STDP) at L4-L2/3 cells plays a crucial role in deprivation-induced plasticity of sensory cortical maps. This is because sensory input produces L4 cell firing followed by L2/3 cell firing (L4-preceding-L2/3 firing) in the corresponding cortical column, which causes strengthening of this synapses under Hebbian STDP rule, while sensory deprivation produces sudden reversal of firings, or L2/3-preceding-L4 firing in the deprived column, which causes weakening of this synapses. Previously we found that parvalbumin-containing GABA neurons in L4 produce fast feedforward suppression on L4 and L2/3, which plays an important role in producing L4-preceding-L2/3 firing. However, such fast feedforward suppression completes around P13-P15 during development. In addition, we also found that L4-L2/3 STDP shows developmental switch from long-term potentiation only STDP (all-LTP STDP) to Hebbian STDP with LTP and LTD after P15 (Itami, 2012). Thus, we hypothesized that the switch of STDP and the completion of fast feedforward GABA circuit together initiate the critical period of map plasticity (Kimura, 2019). This hypothesis predicts that L4-preceding-L2/3 firing following sensory inputs occurs only after around P15. Present study was initiated to examine this prediction. For this purpose, we performed extracellular recording of cortical cell activity in response to whisker deflection with 32 ch electrodes covering from whole layers of rat barrel cortex under urethane anesthesia from P10 to P20. Cross-correlation analysis seem to indicate that our prediction was generally correct. (COI:No)

## 3P-059

### Auditory responses of the rat globus pallidus neuron subtypes

Yasuharu Hirai, Fumino Fujiyama (*Lab Neural Circuit, Grad Sch Brain Sci, Doshisha Univ, Japan*)

The globus pallidus (referring the external segment of that in primates) is the one of the basal ganglia nuclei, which receives inhibitory inputs from "indirect pathway" striatal projection neurons and makes GABAergic innervations to almost all nuclei of the basal ganglia. According to the classical scheme, the activation of indirect pathway suppresses the thalamocortical information transfer, or "movement" in the case of motor function, whereas the activation of direct pathway induces opposite effect on it. However, this go/no-go effect is still under the debate, and also it is unclear how the direct/indirect pathway framework relates to the sensory processing in the basal ganglia. To understand how the globus pallidus handles the sensory inputs as a part of the indirect pathway, we recorded the unit activity of urethane-anesthetized rat globus pallidus neurons during the acoustic stimulation. Tonal receptive fields were investigated with relation to the location of neurons within the nucleus, to the brain state (slow-wave sleep or cortical activation states), and to the neuron subtypes which can be classified by electrophysiological character, molecular expression, and axon projection target. We found that the most of neurons within the caudal part of globus pallidus responded to the sound mainly excitatory. It contradicts to the scheme but corresponds to the recent findings showing the existence of direct excitatory inputs from cortical pyramidal cells. The neurons showing high spontaneous firing responded to the sound even during slow-wave sleep states whereas other neurons were only during cortical activation states. These findings indicated that globus pallidus neurons in sensory region of basal ganglia responded to the cortical activity more than striatal activity, and there might be at least two processing neuron types; one is having high spontaneous activity and always responded to the sensory input and another is functional only during awaking. (COI:No)

## 3P-060

### Relationship between proprioceptive drifts and sense of ownership during robot hand illusion of elbow movements

Toshihiro Kawase[1, 2, 3], Toshimitsu Takahashi[1], Soichiro Fujiki[1], Kenji Kansaku[1, 4] ([1]*Dept of Physiol & Biol Inf, Dokkyo Med Univ Sch of Med, Mibu, Japan,* [2]*Inst Biomater & Bioeng, Tokyo Med Dent Univ, Tokyo, Japan,* [3]*Inst Innov Res, Tokyo Inst of Tech, Yokohama, Japan,* [4]*Cent Neurosci & Biomed Eng, Univ of Electro-Communications, Chofu, Japan*)

Understanding how we consciously experience our bodies is a fundamental issue in neuroscience. In former literatures, researchers have reported that the rubber hand illusion is accompanied by a change of perceived position of the hand towards the rubber hand, which is known as a proprioceptive drift, and some studies showed that the proprioceptive drift was correlated with the sense of ownership (SO) in moving rubber hand illusion, which used a rubber hand mechanically connected to participants' own hands (Kalckert and Ehrsson, 2014). In this study, we report that the same phenomena were observed in a robotic arm that moved synchronously with the participants' elbows.
Seven able-bodied participants were recruited. The robotic arm, using myoelectric control with one degree of freedom (elbow flexion and extension), consisted of a prosthetic glove and an actuator. The joint positions of the robotic arm were controlled continuously by means of the participant's muscular activity on the elbow flexor and extensor. The participants took part in the in-phase and out-of-phase movement conditions for 10 min each. Before and after each 10-min experiment, the participants pointed to indicate the sensed position of the right index finger with their left index finger with their eyes closed. We measured the position pointed to, and the proprioceptive drift in a vertical plane was then calculated by subtracting the two position measurements from each other. The participants answered a questionnaire to assess SO immediately after each experiment.
In the in-phase movement, the proprioceptive drifts were significantly correlated with the ratings of SO (Spearman: $r = 0.86$, $p = 0.024$), and in the out-of-phase movement, these were not significantly correlated. The results are consistent with the former studies of moving rubber hand illusion.
The preliminary results suggest that the robotic arm will contribute to our understanding of our experience on our bodies. (COI:No)

## 3P-061

### Spatiotemporal structure of spontaneous activity in the marmoset visual cortex

Teppei Matsui[1,2], Takayuki Hashimoto[1], Tomonari Murakami[1], Masato Uemura[1], Kouhei Kikuta[1], Toshiki Kato[1], Kenichi Ohki[1,3] (*[1]Dept. Physiol, Grad Scho Med, Univ Tokyo, Japan, [2]JST-PRESTO, [3]IRCN*)

Spontaneous neuronal activity in the resting-state is robustly observed throughout the human brain using functional magnetic resonance imaging and has been used widely to assess functional network structures at the scale of the whole-brain (Fox and Raichle, 2007). At the mesoscale, previous optical imaging in the visual cortex of cats and ferrets reported that spatial patterns of spontaneous cortical activity closely resemble spatial patterns of iso-orientation columns (Kenet et al., 2003; Smith et al., 2018) suggesting that spontaneous activity contain mesoscale network information. However, due to the lack of imaging technique applicable to primates, precise mesoscale structures of spontaneous neuronal activity in primates still remain largely unknown. Recently, genetically encoded calcium indicator applicable to common marmosets has been developed (Sadakane et al., 2013; Uemura et al., Society for Neuroscience Abstract, 2018). In this study, we utilized this technique and conducted widefield and two-photon calcium imaging of spontaneous cortical activity in a large field-of-view (6.5 x 6.5mm) spanning the primary (V1) and secondary visual cortex (V2) of the marmoset monkey. Wide-field imaging of spontaneous activity in the marmoset V1 and V2 indeed revealed rich spatiotemporal structures at various spatial scales. At a large spatial scale, wave-like spontaneous activity propagating across the cortex was readily observed. Importantly, at a smaller spatial scale, columnar patterns resembling orientation maps were frequently observed and were often embedded within the wave-like activity. Cellular scale imaging with two-photon microscopy confirmed that the columnar spontaneous activity reflected spontaneously active clusters of neurons. Spike triggered average of spontaneous activity (Tsodykes et al., 1999) further showed significant overlap between the column-like spontaneously activity and iso-orientation columns. These results suggest that the analysis of spatiotemporal patterns of spontaneous activity provide useful information about the modular organization of primate neocortex. (COI:No)

## 3P-062

### A mechanism for the inhibition of GABAergic transmission by vasopressin V1a receptors at the reciprocal synapse in the mouse accessory olfactory bulb

Mutsuo Taniguchi, Yoshihiro Murata, Masahiro Yamaguchi, Hideto Kaba (*Dept Physiol, Kochi Med School, Kochi Univ, Japan*)

Central vasopressin (AVP) facilitates social recognition and modulates numerous complex social behaviors in mammals. Vasopressin neurons were reported to exist in the accessory olfactory bulb (AOB). The AOB has been demonstrated to be a critical site for mating-induced mate recognition in female mice. The effect of AVP, however, on the synaptic transmission between dendrites in the AOB of female mice is largely unknown.

To address this issue, we previously measured synaptic currents (IPSCs) from mitral cells in the AOB. We have demonstrated that AVP significantly reduced the IPSCs through AVP V1a receptors. The reciprocal transmission, however, contains both glutamatergic transmission from mitral to granule cells and GABAergic transmission from granule to mitral cells. Thus, it is unclear whether AVP acts on the excitatory and/or the inhibitory transmissions.

In the present study, in order to conduct further investigation on the role of V1a receptor in the GABAergic trasmission, AOB slices were prepared from 23- to 35-day-old Balb/c mice. Using the patch-clamp technique in whole-cell configuration (holding potential, –70 mV), the current responses of mitral cells were recorded in the presence of antagonists for glutamatergic transmission, CNQX (10 $\mu$M) and AP5 (50 $\mu$M). An extracellular application of vasopressin did not affect the magnitude of the response of mitral cells to GABA (10 $\mu$M and 100 $\mu$M), whereas it slightly suppressed voltage-activated $Ca^{2+}$ currents in the granule cells. The present results suggest that AVP can modulate the synaptic transmission from granule to mitral cells through a presynaptic mechanism. (COI:No)

## 3P-063

### Pontine serotonergic system regulates body movement and respiratory rhythm coordination

Hirotaka Ooka[1], Chiaki Uchida[1], Akiko Arata[1], Youko Tsukamoto[2] (*[1]Dept Physiol, Hyogo Coll med, Japan, [2]Dept Food & Nutr, Hagoromo Univ.Intl. Studies, Sakai, Japan*)

Locomotor-respiratory coupling, phase-locking between breathing and stepping rhythms, occurs in many vertebrates (Daley MA et al. 2013). In the neural circuit level, respiratory rhythm is belonging to the medullary rhythm generator and body movement is belonging to the spinal rhythm generator. The group of serotonergic neurons are strongly activated during rhythmic activities and modulates the rhythm generator like respiratory and/or body movement. Parabrachial nucleus (PBN) is known about conjunctive system for sensory-motor co-ordination. However, the relationship between respiratory rhythm and body movement in the pons level has not been well investigated. In this study, we analyzed the relationship between respiratory rhythm and body movement in the pons using rat pons-medulla-spinal cord preparation; and examined the effect of serotonin on their co-ordination. Serotonin enhanced the respiratory rhythm-body movement synchronization with pons preparation, but not synchronized to both rhythms in the pons removal. In addition, we examined the distribution of optical signals in the pons triggered by body movement. We found the optical signals in the dorsal pons (probably PBN). When we add the 5-HT1A blocker to the preparation with pons, respiratory-body movement correlation weakened; therefore, the respiratory-body movement coupling was mediated by 5-HT1A receptor. According to immunochemistry approach, 5-HT1A receptors located in lateral PBN which was projected by optical imaging. Moreover, we examined the sensory-motor relationship between dorsal root stimulation in the C8 and respiratory rhythm entrainment. C8 dorsal root stimulation connected body movement to respiratory rhythm. These results suggested that the signal of body movement projected to the dorsal pons, and that serotonin activated the synchronization between respiratory rhythm and body movement through 5-HT1A receptor in the dorsal pons. (COI:No)

## 3P-064

### Male Elevated Gonadotropin Attracts Sexually Active Male Rats

Himeka Hayashi, Yui Shimomi, Kazuto Matsuda, Ryoko Kumagai, Yasuhiko Kondo (*Dept Animal Sci, Teikyo Univ of Sci, Japan*)

When simultaneously presenting odors of gonadally intact and of castrated males, sexually mature male rats show significantly longer exploration to castrated male odor than that of gonadally intact males. Our previous study suggested that this incentive to normal males is produced by elevated level of gonadotropin caused by disinhibition due to a lack of testosterone following castration. To further examine this hypothesis, we tested the effect of gonadotropin treatment in hypophysectomized castrates on the preference of sexually active males; hypophysectomy abolishes circulating luteinizing hormone (LH) and follicle stimulating hormone (FSH), so that the attractiveness of castrated males may decline.Then, we prepared 3 groups of stimulus males (n = 3, each), two of them subjected to hypophysectomy (Hx), and the one received castration and sham-Hx surgery (Sham). They were kept for a few weeks to be stable in hormonal levels before the testing. Six sexually vigorous Long-Evans males were selected as probe males through the mating sessions with hormone-primed receptive females and olfactory preference tests between odors of intact males and receptive females. Two hours before the tests, 3 Hx males were *i.m.* injected with 50IU human chorionic gonadotropin (HxhCG) and 3 Hx males and Sham males were injected with saline (HxSal and Sham, respectively). Probe males were tested for olfactory preference with 3 stimulus pairs, Sham vs. HxSal, Sham vs. HxhCG, and HxSal vs. HxhCG. Consequently, probe males showed significantly longer time spent exploration to HxhCG male odor than that of Sham or HxSal males, while the preference between Sham and HxSal male odors was equivalent. The current results clearly demonstrate that heighten circulating gonadotropin in males produces the attractiveness to same-sex, sexually active male rats. (COI:No)

## 3P-065

### In Vivo Monitoring Reveals Two Different Oscillators in Methamphetamine Treated Mice

Shota Miyazaki[1], Yu Tahara[2], Christopher S. Colwell[2], Gene D. Block[2], Wataru Nakamura[3], Takahiro J. Nakamura[1,2] (*[1]Laboratory of Animal Physiology, School of Agriculture, Meiji University, Japan, [2]Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, USA, [3]Department of Oral Chrono-Physiology, Graduate School of Biomedical Sciences, Nagasaki University, Japan*)

The central circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus, generates circadian rhythms responsible for organizing the timing of most behaviors and physiological events in mammals. However, there are other unidentified circadian oscillators. For example, chronic treatment of methamphetamine (MAP) induces locomotor activity rhythm in a circadian oscillation, independent of the SCN and driven by a pacemaker outside the SCN. However, it remains unknown whether MAP affects SCN rhythmicity in mice that display MAP-induced circadian locomotor activity rhythm (MCLA). To explore the effect of MAP, we employed in vivo real-time monitoring of multi-unit neural activity (MUA) in freely moving mice.

Three-month-old, male C57BL/6J mice were treated with 0.005% MAP dissolved in drinking water until they displayed MCLA. An MUA electrode was chronically implanted into the SCN or striatum, which is associated with motor functions. After recovery, we simultaneously recorded wheel running activity (WR) and MUA.

In the SCN, control mice showed diurnal and circadian rhythms of MUA which was antiphase with the rhythm of WR. Conversely, mice that displayed MCLA also showed robust MUA rhythms in light-dark and constant dark conditions, while WR gradually overlapped with the MUA. In addition, MUA in the striatum showed robust rhythms parallel to WR under all conditions.

These results indicate that MAP does not strongly affect the neural activity rhythm in the SCN, and that MAP-induced circadian oscillator and the SCN are completely independent of each other. (COI:No)

## 3P-066

### Factors affecting social hierarchy and glucose metabolism relationship

Momoyo Ibukuro, Rikako Ukichi, Yukari Takahashi, Yae Sugimura K., Fusao Kato (*Dept Neurosci, Jikei Univ Sch Med, Japan*)

Besides being regulated by the peripheral mechanisms, the blood glucose level is under potent influence of the central nervous system. For example, epidemiological studies suggest frequency of type 2 diabetes depends on socioeconomic status of patients (Rabi et al., 2006). As the first step toward understanding neuro-behavioral factors affecting the glucose metabolism, we evaluated the rank of individual mouse using the tube test (Wang et al, 2011) and compared with the glucose tolerance. The rank order of each of four mice in the same cage was evaluated repeatedly for 1-2 weeks and blood glucose level after glucose injection was measured at the end of the sessions. We analyzed influences of the following three elements: (1) diet, (2) sex and (3) systemic inflammation. (1) Effects of diet. We conducted the tube test in 10 sets of four mice fed with low-fat (5 sets) or high-fat diet (the rest). The diet did not essentially affect the general properties of the formed social hierarchy. (2) Influence of sex. We compared the hierarchy formation between male and female mice. While the stability of hierarchy structure was stable in both male and female, glucose metabolism depended on individual rank in male mice but not in female mice, suggesting that the relationship between the social rank and glucose metabolism is sex-dependent. (3) Impact of systemic inflammation on hierarchy. We examined how lipopolysaccharide injection (0.1-1 mg/kg, i.p.) affects the rank order and found its impact was limited with the present experimental conditions. Overall, these results suggest that the glucose metabolism is under influence of neuronal and behavioral factors, which would shed light on the novel approaches for improving glucose metabolism in diabetes patients. (COI:No)

## 3P-067

### Novel function of NMU/NMS system in formation of the fear memory

Akinobu Soda, Ryoko Higa, Kenshiro Shikano, Reiko Hanada (*Dept Neurophysiol, Fac Med, Oita Univ, Japan*)

Neuromedin U (NMU) and Neuromedin S (NMS) are neuropeptides with various physiological functions such as behavior and regulation of circadian rhythm. To investigate novel function of NMU/NMS system related to higher brain functions, we have established NMU/NMS double KO mice (dKO) and examined a series of behavior tests. In our preliminary experiment, dKO showed increasing of anxiety in feared conditioned test. In this study, we performed the passive avoidance test and analyzed the neuronal activation with c-Fos expression level. In the passive avoidance test, dKO showed statistical difference in fear memory formation compared with wild type mice (WT) in 1 day after electric foot-shock (EFS). The extinction of fear memory in 7 days and 28 days after EFS was shown in WT, however not in dKO. Furthermore, dKO had shown higher concentration of serum corticosterone level than WT. These data suggest that dKO were enhanced fear memory consolidation and reduced extinction of fear memory. Additionally, in 1 day after EFS, the number of c-Fos positive cells in the lateral amygdala nucleus significantly increased in dKO than WT. This indicates the lateral amygdala nucleus tightly contributes the fear memory formation in initial state. These results indicate that the NMU/NMS system is closely involved in the formation of the fear memory. (COI:No)

## 3P-068

### Involvement of glutamatergic inputs from the subfornical organ to the median preoptic nucleus in the water ingestion induced by angiotensin II in rats

Akihiko Ushigome[1], Makoto Takahashi[2], Shinichiro Takahashi[2], Junichi Tanaka[2,3] (*[1]Dept Judo Physical Therapy, Fac Human Care, Teikyo Heisei Univ, Tokyo, Japan, [2]Dept Disability Sci, Grad Sch Edu, Naruto Univ Edu, Tokushima, Japan, [3]Lab Neurosci, Naruto Univ Edu, Tokushima, Japan*)

The present study was conducted to examine the role of glutamatergic systems in the median preoptic nucleus (MnPO) in the water ingestion induced by administration of angiotensin II (ANG II) in the subfornical organ (SFO) in the rat. Microdialysis data demonstrated that microinjection of ANG II ($10^{-10}$ M) into the SFO significantly increased the release of glutamate (Glu) in the MnPO in the rats under the condition that water is available for drinking and the rats under the condition that water is not available for drinking. The amount of initial maximal increases in the Glu levels elicited by the ANG II injection was quite similar in drinking and non drinking rats, whereas the duration of the response was much longer in non drinking than in drinking rats. The amount of water ingestion in 20 min immediately after the ANG II injection was significantly enhanced by previous injections of N-methyl-D-asparatate (NMDA, 10 $\mu$ M) into the MnPO, while the ANG II-induced water ingestion was attenuated by pretreatment with the NMDA antagonist dizocilpine (MK-801, 10 $\mu$M). The amount of water intake elicited by the ANG II injection into the SFO was enhanced by previous injections of either the non-NMDA agonist kainic acid (KA, 50 $\mu$M) or quisqualic acid (QA, 50 $\mu$M) into the MnPO. On the contrary, the ANG II-induced drinking response was diminished by pretreatment with the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX, 10 $\mu$M) in the MnPO. These results suggest that the glutamatergic neural pathways to the MnPO may transmit the information for eliciting drinking in response to ANG II acting at the SFO and the ANG II-induced dipsogenic response may be mediated through both NMDA and non-NMDA receptor mechanisms in the MnPO. (COI:No)

## 3P-069

### Neurons in the medulla related to the regulation of sleep/wake cycles and autonomic nervous system

Yoshifumi Arai, Hayato Iwata, Tatsuya Suzuki, Kaname Mochizuki, Yoshimasa Koyama (*Faculty of Symbiotic Systems Science, Fukushima Univ, Japan*)

The medulla plays important roles in the promotion of rapid eye movement (REM) sleep and muscle atonia during REM sleep and contains the cardiovascular center, that regulates vasomotion and respiration. During REM sleep, rapid increases in blood pressure (BP) and heart rate (HR) are observed due to the turbulence of the autonomic nervous system. However, it remains to be known how the neurons in the medulla regulate sleep/wake cycles and BP fluctuation during REM sleep.

We recorded single neuronal activity and BP in head-restrained and unanaesthetised rats during sleep/wake cycles. Of 115 neurons recorded, 75.7% (87/115) showed increased activity during REM sleep, during REM sleep and waking, or during Slow wave sleep and REM sleep, which we term PS active neurons.

32.2 % of PS active neurons (28/87) started increasing their activity before the REM sleep onset. They are mainly located from central to the caudal parts of the medulla. Among them, the neurons showing tonic firing during REM sleep were distributed in the ventral parts, while the phasic firing neurons were found in the dorsal parts.

Of 87 PS active neurons, 32.2 % (28) showed firing in close relation with BP fluctuation during REM sleep, and 75 % of them (21/28) increased their firing rate before the BP fluctuation. The time preceding to the start of the blood pressure increase was longer in the neurons located in the rostral medulla compared to those in the caudal medulla. (COI:No)

## 3P-070

### Establishment of screening method for sleep/wakefulness in mice through forward genetics

Chika Miyoshi[1], Noriko Hotta-Hirashima[1], Satomi Kanno[1], Aya Ikkyu[1], Miyo Kakizaki[1], Mana Yamada[1], Staci Kim J[1], Shigeharu Wakana[2,3], Masashi Yanagisawa[1,4,5], Hiromasa Funato[1,6] (*[1]WPI-IIIS, University of Tsukuba, Tsukuba, Japan, [2]RIKEN BioResource Center, Tsukuba, Japan, [3]Institute of Biomedical Research and Innovation, Kobe, Japan, [4]University of Texas Southwestern Medical Center, Dallas, TX, [5]TARA, University of Tsukuba, Tsukuba, Japan., [6]Department of Anatomy, Faculty of Medicine, Toho University, Tokyo, Japan*)

The network of genes and molecules that govern sleep and wakefulness remains largely unknown. Forward genetics is a powerful approach in elucidating biological phenomena that cannot be predicted from the function of known genes. We conducted a large-scale screening in mice using the EEG/EMG-based sleep/wake monitoring. The search has succeeded in identifying several sleep regulatory genes.

Among the established mouse pedigree, the*Sleepy* mutant family shows a significant reduction in total wake time. *Sleepy* mice have mutation in the *Salt-inducible kinase 3 (Sik3)* gene which encodes a member of the AMP-activated kinase-related kinase. In addition to the hypersomnia, *Sleepy* has a tendency to become obese. This mouse pedigree will be a model animal not only for studying hypersomnia but also the obesity which are considered to be costly conditions for the society. (COI:No)

## 3P-071

### Generation of a genetically modified rat overexpressing BMAL1 dominant negative form

Yoichi Minami, Tomoko Yoshikawa, Mamoru Nagano, Satoshi Koinuma, Keisuke Ikegami, Atsuko Fujioka, Keiichi Furukawa, Yasufumi Shigeyoshi (*Dept Anatomy, Faculty Med, Kindai Univ, Japan*)

The circadian clock is the endogenous oscillator ticking about a day. At molecular level, the circadian clock is composed by a set of clock genes such as Per2, Cry1 and Bmal1. Genetical disruption of clock genes results in abnormal circadian rhythm. Because of technical advantages, mice are mainly used to generate molecular clock disfunction models. Of note, circadian period of mice behavioral rhythm is shorter than 24 hours, whereas that of humans and rats are longer than 24 hours. In this study, we focused on rats for the phenotype similarity to humans' circadian clock systems. We overexpressed BMAL1 dominant negative (DN) form which lacks C terminus CRY1 interaction domain specifically in neurons by using a mouse prion promoter (MoPrnP.XhoI). The transgene was injected to fertilized eggs collected from transgenic rats having destabilized firefly luciferase reporter gene driven by Per2 promoter (Per2-dLuc). We observed smaller amplitude of bioluminescence rhythm in BMAL1 DN transgenic rats using cultured SCN and rapid entrainment after an abrupt shift of the LD cycle. These findings suggest that the attenuation of the circadian rhythm brings rapid elimination of jet lag. (COI:No)

## 3P-072

### Withdraw

## 3P-073

### Microglia eliminate synapses by phagocytosis in mature brain; its physiological roles

Junya Tanaka (*Dept Mol Cell Physiol, Grad Sch Med, Ehime Univ, Japan*)

Microglia in the normal mature brain have long been recognized as resting cells that do not play anything until any kinds of pathologic events occur. However, currently it is well known that microglia constantly move their fine processes even in the physiological condition while monitoring, probably, the activities of synapses. In the developing brains, microglia take part in the construction of neural network by eliminating inactive synapses. In this paper, we will show the data on the involvement of microglial synaptic elimination in physiological functions. 1) Sleep and microglia. Decrease in synaptic density or activities during sleep have long been noted, whereas the mechanisms underlying the synaptic loss have not been known. We have recently found that microglia phagocytose synapses during sleep that binds eat-me signals such as complements or MFG-E8. The eat-me signal molecules should have bound to phosphatidylserine, therefore, eliminated synapses were apoptotic inactive ones. 2) Parkinsons disease model and microglia. Microglia may be involved in compensation of dopaminergic neuron loss by eliminating glutamatergic synapses from subthalamic nuclei in the 6-OHDA-induced rat Parkinsons disease model. Here, hyperactive synapses should have been eliminated. 3) Less active microglia in the attention deficit/hyperactivity disorder (ADHD) model rats. Recently, we have found that Lister hooded rats (LHRs), an inbred rat strain having black spots in the head and the back, display ADHD-like behaviors. Microglia in the frontal cortex of LHR with smaller somata expressed CD11b less significantly than those of Wistar rats. Synapsin I immunoreactivity was decreased in the prelimbic region in the frontal cortex compared to that in the Wistar rat brains. All these findings suggest that microglia may modulate various kinds of behaviors by eliminating synapses by phagocytosis. (COI:No)

## 3P-074

### Behavioral tests predicting striatal dopamine level in a rat hemi-Parkinson's disease model

Kazuya Miyanishi, Minori Watanabe, Rina Uchida, Emamussalehin Choudhury, Hajime Yano, Junya Tanaka (*Dept Mol Cell Physiol, Grad Sch Med, Ehime Univ, Japan*)

Parkinson's disease (PD) is one of the frequent neurodegenerative diseases causing bradykinesia, tremor, muscle rigidity and postural instability. Although progressive dopaminergic (DArgic) neuron loss in the substantia nigra is a main pathology of PD, motor deficits are thought not to become obvious until most DArgic neurons are lost, probably due to compensatory mechanisms that overcome the reduction of DA level in the striatum. Even in animal PD models, the compensatory mechanisms make it difficult to detect motor dysfunctions when most DArgic neurons are functional. In this study, we performed various behavioral tests (apomorphine-induced rotation, cylinder, forepaw adjusting steps (FAS), beam walking, rota-rod, and open-field test), using 6-hydroxydopamine (OHDA) and lipopolysaccharide (LPS)-induced hemi-PD model rats with various striatal DA levels, to find the best way to predict the DA level from earlier disease stages. Reduction in the striatal DA levels in the 6-OHDA-model was severe, while LPS-model was less significant. Among the behavioral tests, data from cylinder and FAS tests, which evaluate forelimb motor function, best correlated with decline of the DA level. The beam walking and apomorphine tests showed less significant correlation than the cylinder and FAS tests. Open-field and rota-rod tests were not detectable. Expressional levels of mRNA encoding tyrosine hydroxylase (TH), a marker of DArgic neurons, correlated well with the DA level. Metabotropic glutamate receptor 4 mRNA expression correlated with the striatal DA level and may be related to compensatory mechanisms. These results suggest that forelimb movements, or hands and forearms in clinical settings, rather than movement of the body or large joints should evaluate motor impairments of PD. The combination of cylinder and FAS tests may be the best way to evaluate the rat PD models, in which many functional DArgic neurons. (COI:No)

## 3P-075

### Activation mechanism of heat shock factor 1 induced after zebrafish optic nerve injury

Kayo Sugitani (*Div Health Sci, Grad Sch Med Sci, Kanazawa Univ, Japan*)

Generally, fish central nervous system (CNS) neurons can regenerate their axons even after nerve transection, whereas mammalian CNS neurons cannot. We have identified many nerve regeneration associated genes (RAGs) using the zebrafish optic nerve regeneration model. Heat shock factor 1 (HSF1) is one of the RAGs, which acts as a key regulatory molecule under the various acute stress conditions. Previous studies showed that HSF1 mRNA and phosphorylated-HSF1 protein rapidly started to increase in retinal ganglion cells within 30 min after optic nerve injury. However, the rapid activation mechanism of HSF1 has not been clearly elucidated. In breast cancer cells, activation of HSF1 is reportedly controlled by 17 $\beta$-estradiol (E2). RT-PCR analysis of HSF1 and brain specific aromatase B, as a biomarker of E2 exposure, showed very similar temporal expression pattern in retina and optic nerve after nerve injury. Here, we investigated the correlation of the levels of HSF1 and aromatase B during zebrafish optic nerve regeneration. (COI:No)

## 3P-076

### Localization of imidazoline 1 and $\alpha_2$-adrenergic receptors in newborn rat brainstem

Yukari Nagakura, Ryoji Ide, Chikako Saiki, Yoichiro Kitajima, Nana Hashizume, Toshio Imai (*Dept Physiol, Sch Life Dent at Tokyo, Nippon Dent Univ, Japan*)

**Introduction:** Dexmedetomidine (DEX), a $\alpha_2$-adrenergic receptor ($\alpha_2$-AR), has been used as a sedative and is also known to suppress blood pressure and heart rate through its stimulant effect on imidazoline 1 receptor ($I_1$R), as well as on $\alpha_2$-AR. However, compared to $\alpha_2$-AR, little is known about the distribution of $I_1$R in the area related to cardiorespiratory centers in the brainstem. Therefore, in this study we performed immunohistochemistry of newborn rat brainstem to detect $I_1$R and $\alpha_2$-AR-positive neurons in the ponto-medullary area to gain a further understanding of the effect of DEX on the cardiorespiratory regulation mechanism in the central nervous system.

**Materials and methods:** Newborn Wistar rats (n = 7, 8-11 g) were deeply anesthetized and after termination of breathing brainstem were dissected out. The dissected tissues were fixed in 4% paraformaldehyde, rinsed in phosphate-buffered saline containing sucrose, placed in compound, and stored at -80 ℃. Frozen serial sections (10 $\mu$m thick) were cut with a cryostat. The sections were incubated in a blocking solution and with primary and secondary antibodies. Immunofluorescence was visualized by using a spectral confocal microscope. As primary antibodies, we used anti-NISCH and anti-$\alpha_{2A}$-AR antibodies to identify $I_1$R and $\alpha_2$-AR, respectively, and anti-tyrosine hydroxylase (TH) antibody to identify both caudal ventrolateral medulla (CVLM) and rostral ventrolateral medulla (RVLM), in which the basic cardiorespiratory centers are located. In CVLM and RVLM, clusters of TH-positive cells exist in the regions corresponding to C1 and A1 areas, respectively.

**Results:** NISCH-positive neurons were detectable in brainstem, including CVLM, RVLM, and the area close to locus coeruleus (LC) of caudal pons. In addition, among the NISCH-positive neurons some neurons seemed to be co-expressed with $\alpha_{2A}$-AR.

**Conclusion:** Present results suggest a possibility that DEX influences central cardiorespiratory regulation mechanism by activating both $I_1$R and $\alpha_2$-AR in the ponto-medullary area. (COI:No)

## 3P-077

### Switching of autonomic cardiovascular regulation to emotional stimuli by the central nucleus of the amygdala in rats

Ko Yamanaka, Hidefumi Waki (*Dept Physiol, Grad Sch Health and Sports Sci, Juntendo Univ, Japan*)

Humans and animals can judge whether the current state is favorable or unfavorable, and select actions accordingly. To generate actions appropriate for the current state, autonomic cardiovascular tuning to supply energy to active skeletal muscles is important. However, the exact neuronal mechanisms of dynamic adjustments of autonomic cardiovascular responses in different states are still unclear. In this study, we hypothesized that the amygdala plays a role in autonomic cardiovascular tuning for adaptive behavioral control. We recorded the blood pressure and heart rate of rats during appetitive and aversive classical conditioning tasks in which they learned varying associations between conditioned stimuli (CS) and unconditioned stimuli (US) in three types of blocks: (1) in appetitive (REW) blocks, one tone cue (CS+, 10 kHz) preceded reward (US+, 5% sucrose) and another (CS-, 4 kHz) preceded non-reward (US-); (2) in aversive (AVE) blocks, the CS+ preceded an air puff (US+) and the CS- preceded no air puff (US-); (3) in neutral (NA) blocks, both CS tones preceded nothing. NA blocks were alternately deployed between REW and AVE blocks. In the REW blocks, blood pressure gradually increased during the CS-US interval, followed by a vigorous pressor response to the US+. In the AVE blocks, blood pressure showed phasic depressive responses to both CS+ and US+. The blood pressure response to the CS+ in the REW blocks was significantly higher than that in the NA blocks. The heart rate response to the CS+ in the AVE blocks was significantly lower than that in the NA blocks. Furthermore, bilateral inactivation of the central nucleus of the amygdala by microinjection of the GABAA receptor agonist muscimol significantly decreased the pressor responses during the REW blocks. These results suggest that the central nucleus of the amygdala may contribute to pressor response tuning evoked by emotional stimuli. (COI:No)

## 3P-078

### Effects of pharyngeal mechanical stimulation on skeletal muscle blood flow and blood pressure

Yudai Tanaka[1], Nobuhiro Watanabe[2], Harue Suzuki[2], Koji Fukui[1], Harumi Hotta[2] (*[1]Sys Eng & Sci, Grad Sch Eng & Sci, Shibaura Inst of Technol, Saitama, Japan, [2]Dept Auton Neurosci, Tokyo Metropol Inst Gerontol, Tokyo, Japan*)

It has been shown that a mechanical stimulation to pharynx induced a reflex increase in thyroxine and calcitonin secretion from thyroid gland by excitation of thyroid parasympathetic efferent nerve. In this study, we aimed to clarify whether the pharyngeal stimulation affects muscle blood flow, by modulating muscle sympathetic nerve activity. For this purpose, we compared the changes in muscle blood flow and mean arterial pressure (MAP) before and after cutting lumbar sympathetic trunks (LSTs). In the experiments, we used anesthetized male rats. We inserted a catheter into the common carotid artery and measured MAP. Rats were artificially ventilated via tracheal cannula. To measure muscle blood flow, we exposed soleus of left hindlimb and set a probe of the laser Doppler flowmeter on the muscle. The pharynx was mechanically stimulated by a small balloon every 10 seconds for a period of 1-5 minutes. LSTs were cut at a part of lumbar segments (L3-L5). When LSTs were intact, blood flow and MAP increased during pharyngeal stimulation. The percentage increase in blood flow during stimulation was 0-10% of the control value before stimulation. In the case of MAP, increase was 10-20%. It could be assumed that when vascular diameter of muscle is constant, the percentage increase in blood flow should be same as that in MAP. However, we found response of blood flow was smaller than that of MAP. Therefore, before cutting LSTs, we considered blood vessels in muscle constrict during stimulation. After cutting LSTs, both increases in blood flow and MAP were changed to the same extent (0-10%). The results indicate that reaction of blood vessels induced by pharyngeal stimulation disappeared by cutting LSTs. Therefore, we concluded that pharyngeal mechanical stimulation altered skeletal muscle blood flow and blood pressure by modulating muscle sympathetic nerve activity. (COI:No)

## 3P-079

### Effects of appetite-boosting peptides on the superior salivatory nucleus neurons in rats

Yoshihiro Mitoh[1], Tadasu Sato[2], Takehiro Yajima[2], Masako Fujita[1], Motoi Kobashi[1], Hiroyuki Ichikawa[2], Ryusuke Yoshida[1] (*[1]Dept Oral Physiol, Okayama Univ Grad Sch Med Dent Pharm Sci, [2]Div Oral Craniofac Anat, Tohoku Univ Grad Sch Dent*)

The superior salivatory nucleus (SSN) is the primary parasympathetic center for the submandibular and sublingual salivary glands. Since copious salivary secretion is observed during feeding in various animals, it is possible that appetite-boosting peptides induce the salivation by increasing SSN neuronal excitability, in addition to facilitating feeding behavior. In this study, we mainly electrophysiologically investigated effects of some appetite-boosting peptides on SSN neurons. Whole-cell patch-clamp recordings were performed from neonatal rat SSN neurons retrogradely labeled with a fluorescent tracer. The membrane currents and potentials were analyzed in bath-application of the peptides. Among peptides tested, only orexins (OXs; OXA and OXB) affected SSN neurons. In current-clamp mode, application of OXs at resting membrane potential induced depolarizations and often produced action potentials. OXA generated larger depolarization than OXB. In voltage-clamp mode, OXA generated inward currents at -70 mV and the currents were completely inhibited by antagonists for OX1R and OX2R of the receptor subtypes, consistent with immunohistochemical data. The frequency of miniature excitatory postsynaptic currents scarcely increased, suggesting that the neurons are mainly activated via postsynaptic OX receptors. When a long depolarizing current pulse was given at -90 mV (activation of A-currents), SSN neurons were classified into two types from the firing pattern. We discussed the relationship between the responsiveness of two types of SSN neurons to OXA and their target organs. (COI:No)

## 3P-080

### Saliva chromogranin A is associated with night sleep efficiency evaluated by heart rate variabilities in young adults

Kentaro Taniguchi[1, 2], Shizuka Nakajima[1], Yuka Nomura[1], Yuuki Kudo[1], Naoya Okumura[2], Naoya Jinno[2], Akito Shimouchi[2] (*[1]Nagahama Inst, Bio-sci and Tech, [2]College Life and Heal Sci, Chubu Univ*)

Chromogranin A (CgA) is an acidic secretory protein belonging to the granin family, and saliva CgA is related to the psychological stress. Heart rate variability (HRV) is one of the major methods to obtain health information including sleep efficiency with non-invasive method. To examine the relationships between CgA and sleep efficiency, we enrolled 20 young adults. HRV and physical acceleration were obtained every minute for 24 hours during free moving. Saliva was obtained before and after night sleep. The ratio of low frequency (LF)/high frequency (HF) and HFnu were used as HRV indices. Subjects with low saliva CgA after waking had significantly lower modulation between sleep and after waking of LF/HF and HFnu. We considered that lower levels of saliva CgA after wake-up suggested lower efficiency of night sleep. (COI:No)

## 3P-081

### Effects of gastro-intestinal osmotic stimulation on afferent vagal nerve outflows in the stomach, intestine and liver in rats

Yuichirou Kimoto, Mamoru Tanida, Yasutaka Kurata (*Dept Physiol 2, Kanazawa Med Univ, Japan*)

Previously, it has shown that duodenal stimulation by hypertonic NaCl caused thermogenesis and increase in metabolic rate (Osaka et la., Journal of Physiol. 2004). Vagal afferent pathways in the gastro-intestinal organs connect to the brain for introducing various information in the gastro-intestinal organs, but it has not been cleared whether osmotic stimulation affects afferent vagal nerve activities in the stomach and intestine. Thus, we examined effects of injection of hypertonic NaCl solution into the stomach or duodenum on afferent vagal nerve outflows in anesthetized mice.

We measured the afferent signals of the vagal nerve in the stomach branch, celiac branch and hepatic branch, and the efferent signals of the renal sympathetic nerve in urethane and $\alpha$-chloralose-anesthetized mice. The hypertonic solution (1M, 10ml/kg) was administered into the stomach, intestine or portal vein. In addition, vagotomized mice by deletion of afferent vagal branch were used to investigate role of vagal afferent pathways. We firstly showed that hypertonic solution injection into the duodenum but not the stomach dose dependently activated the afferent vagal nerve activity of the celiac branch, and it lowered blood pressure 30 min after injection. In addition, it stimulated the afferent vagal nerve activity of the hepatic branch. In the vagotomized mice, stimulatory response of efferent renal sympathetic nerve to intra-duodenal injection of NaCl solution was attenuated. These lines of evidence suggest that osmotic stimulation in the intestine affects the vagal afferents and activates efferent discharge of the renal sympathetic nerve in mice. (COI:No)

## 3P-082

### Generation of EAD in heart cells involves reverse E-C coupling and reverse electrotonic conduction along T-tubules

Takao Shioya (*Dept Physiol, Fac Med, Saga Univ, Japan*)

Early after depolarization (EAD) is an abberant cardiac afterpotential that underlies the development of life-threatening ventricular arrhythmias. It is believed that the development of EAD is caused by the reactivation of L-type $Ca^{2+}$ current during the period of the action potential plateau; however the cellular mechanisms that underlie the development of EAD is still controversial. One favorable alternative is the depolarizing reverse-mode operation of the $Na^+/Ca^{2+}$ exchanger, which is activated by aberrant $Ca^{2+}$ release from the sarcoplasmic retuculum in the process of reverse E-C coupling. Since EADs develop preferentially in damaged heart cells with abnormal $Ca^{2+}$-signaling, here I studied the causal link between the development of EADs and aberrant intracellular $Ca^{2+}$ level ($[Ca^{2+}]_i$) dynamics in mouse heart cells, using nystatin "superforated-patch" technique and $Ca^{2+}$ imaging by fluo-3 AM. My results show: 1) The generation of EADs was preceded by the development of depolarizing membrane potential ($V_m$) fluctuation. 2) The depolarizing $V_m$ fluctuation occured concurrently with a local brief $[Ca^{2+}]_i$ elevation, and the $V_m$ fluctuation was eliminated when $Ni^{2+}$ was used to block the $Na^+/Ca^{2+}$ exchanger. 3) The generation of the $V_m$ fluctuation and EADs were suppressed after the T-tubule system of the cell was detubulated. 4) Abbreviating the T-tubules' length constant by increasing extracellular $K^+$ level suppressed the development of the $V_m$ fluctuation and EADs accordingly. Taken together, I conclude that EADs are caused by the depolarizing $V_m$ fluctuation, which is induced locally in the T-tubule membrane by aberrant $[Ca^{2+}]_i$ elevation and is conducted back electrotonically along the T-tubules. Hence, the membrane potential waveform of the T-tubule membrane might be totally different from that of the surface plasma membrane. (COI:No)

## 3P-083

### Modulation of myosin II function causes thin filaments disarrangement through cross-bridge independent pathway in skinned smooth muscle

Masaru Watanabe[1], Naoya Nakahara [2], Yukisato Ishida[1] (*[1]Grad Sch Health Sci, Tokyo Met Univ, Japan, [2]Dept Mol Physiol, Jikei Univ Sci Med, Japan*)

In the skinned (cell membrane permeabilized) guinea pig taenia caecum, blebbistatin, a selective inhibitor of skeletal and smooth muscle myosin II (Limouze et al., 2004, Katayama et al., 2006), suppressed both myosin light chain phosphorylation-dependent- and -independent contraction, and also disrupted contractile filament organization (Watanabe et al., 2010). To clarify the mechanisms of the smooth muscle filaments disorganization by blebbistatin, we investigated lattice like organization and periodic repeat of myosin and actin molecules of beta escin skinned taenia cecum using X-ray diffraction technique (Watanabe et al., 1993, and Watanabe et al., 2009). The X-ray diffraction experiments were made at BL-6A in the Photon Factory, the Institute of Materials Structure Science, Tsukuba, using the small-angle X-ray camera. Diffraction patterns were recorded on GE healthcare imaging plates with a specimen-to-plate distance of 220 cm. The size of the beam on the specimen was 1.0×0.5 mm. The exposure time was three times of 180 s. In the resting state, blebbistain weakened 14.4 nm meridional reflection from the thick filament as previously reported (Watanabe et al., 2009). Also, blebbistatin extended an equatorial reflection at a Bragg spacing of 11.4 nm originated from lattice like arrangements of the thin filaments, but had little effects on the third, fifth and higher order meridional reflections from collagen. In the absence of nucleotides in the intracellular solution, blebbistatin did not show the extension of the 11.4 nm equatorial reflections. These results suggest that disruption of thick filaments by blebbistatin treatment causes thin filaments disarrangement through cross-bridge independent pathways in skinned taenia cecum, since blebbistatin is known to affect myosin nucleotide binding but dose not have direct effects on actin. (COI:No)

## 3P-084

### Actin filaments render considerable heat capacity to skeletal muscle sarcomere

Naoya Nakahara (*Dept. Mol. Physiol., Jikei Univ. Sch. Med., Japan*)

In biological tissue, crowding proteins considerably interfere with hydrogen bonding and molecular motion of neighboring water molecules to form so called bound-water as well as hypermobile water. Energetic consideration clearly indicates that functional proteins including myoproteins utilize entropic free-energy derived from restriction and liberation of a cluster of water molecules to drive biophysical reactions such as muscle contraction. One of the methods to detect the interaction between proteins and water is differential scanning calorimetry (DSC), which records heat capacity change with temperature. We analyzed skeletal muscle fibers of sartorius muscle from *Rana Catesbeiana* to analyze interaction of myoproteins with water molecules. Demembranated fibers at rigor condition were used to avoid osmotic movement of water molecules across the cell membrane with freezing. With gradual increase in temperature (1℃/min), latent heat absorption peaks were observed at -24, -21, 0, 46, 65℃. The peaks at 46℃ and 65℃ would represent irreversible denaturation of myosin and actin, respectively, because selective removal of myosin and actin decreased the corresponding peak. On the other hand, the -24℃ peak was largely affected by actin removal, and the -21℃ peak was affected by the removal of actin as well as myosin. Integrated heat capacity in the range from -80 to +20℃ was significantly decreased by denaturation of actin. The decrease in the integrated heat capacity with actin removal amounted to 65% on a per-myosin basis. These results indicate that actin filaments render considerable heat capacity to skeletal muscle sarcomere. (COI:No)

## 3P-085

### Inhibition of actin polymerization accelerates relaxation process in beta escin skinned smooth muscle

Satoko Mihashi (*Human Health Sci, Grad. Sch, Tokyo Metropolitan Univ, Japan*)

It is known that cytochalasin D, a fugal metabolite, and latrunculin B, a sponge toxin, are known to inhibit actin polymerization, and also to suppress smooth muscle contraction. We found that both cytochalasin D and latrunculin B inhibited the maximal Ca ion induced force at around 1 micro M, but enhanced submaximal force development induced by lower Ca ion concentrations (S-13, Watanabe and Mihashi), indicating that inhibition of actin polymerization enhances Ca ion sensitivity for the force. To investigate regulatory mechanisms of the thin filament liked Ca ion dependent pathway in detail, we examined the cytochalasin D and latrunculin B effects on Ca ion removal induced relaxation process in skinned (cell membrane permeabilized) taenia cecum and carotid artery from guinea pig. When 10 micro M Ca ion was removed from the intracellular solution, the contractile force was decayed bi-exponentially with initial short time lag as previously reported (Yoshino et al., 2005, Hashimoto et al., 2008). Both cytochalasin D and latrunculin B accelerated the relaxation process. The acceleration effect of the actin polymerization inhibitors was observed even in the absence of nucleotide in the solution. On the other hand, according to our preliminary results, myosin light chain inhibition with ML-7, a kinase inhibitor, might attenuate the accelerating effects of cytochalasin and latrunculin B on relaxation, indicating that modulation of actin function directly affect myosin light chain kinase activity. (COI:No)

## 3P-086

### Contractile properties of rat epididymal duct

Retsu Mitsui[1], Hikaru Hashitani[1], Lang Richard J.[2], van Helden Dirk F.[3] ([1]*Dept Cell Physiol, Nagoya City Univ Grad Sch Med Sci, Japan,* [2]*Sch Biomed Sci, Monash University, Australia,* [3]*Sch Biomed Sci and Pharmacy, The University of Newcastle, Australia*)

**Background:** The contractility of smooth muscle in the epididymal duct plays a fundamental role in transport of sperm from the testis to the vas deferens. Here, we examined spontaneous and sympathetic nerve-mediated contractions of the rat epididymal duct.
**Methods:** Short segments from two caudal regions (diameter: 200 $\mu$m and 400 $\mu$m) were isolated, and both ends were loosely tied with threads. Changes in the longitudinal distance between the threads were monitored with a video camera, and analysed using edge-tracking software.
**Results:** Small ducts (200 $\mu$m in diameter) exhibited rhythmic phasic contractions that were suppressed by yohimbine (1 $\mu$M), an $\alpha_2$-adrenergic receptor antagonist. Rhythmic contractions were abolished by CPA (10 $\mu$M, sarcoplasmic reticulum (SR) $Ca^{2+}$-ATPase inhibitor), 2-APB (100 $\mu$M, IP3 receptor inhibitor), niflumic acid (100 $\mu$M, $Ca^{2+}$-activated $Cl^-$ channel blocker) or lowering extracellular $Cl^-$ concentration (12.4 mM). Contraction frequency was decreased by nifedipine (1 $\mu$M, L-type $Ca^{2+}$ channel blocker) or ML218 (1 $\mu$M, T-type $Ca^{2+}$ channel blocker). Larger ducts 300-500 $\mu$m in diameter remained quiescent, but clonidine (100 nM), an $\alpha_2$-adrenergic receptor agonist, induced rhythmic contractions in a manner sensitive to yohimbine. In the larger ducts, electrical field stimulation (EFS; 50 $\mu$s duration, 2 Hz for 1 min, 5 min interval) induced a sustained contraction superimposed with rhythmic contractions. Prazosin (1 $\mu$M), an $\alpha_1$-adrenoceptor antagonist, or PPADS (10 $\mu$M), a purinoceptor antagonist, suppressed the sustained component, while yohimbine (1 $\mu$M) prevented the superimposed rhythmic contractions. Depletion of sympathetic neurotransmitters (10 $\mu$M guanethidine) largely diminished the EFS-induced contraction.
**Conclusion:** 1) Sympathetic nerve-derived noradrenaline acts on $\alpha_2$-adrenoceptor to enhance or induce rhythmic contractions. 2) Rhythmic contractions are likely to arise from SR $Ca^{2+}$ release and depolarisation through resultant opening of $Ca^{2+}$-activated $Cl^{--}$ channels. 3) EFS-induced contraction consists of $\alpha_1$-adrenoceptor- and purinoceptor-mediated sustained contraction and $\alpha_2$-adrenoceptor-mediated superimposed rhythmic contractions. (COI:No)

## 3P-087

### Quantitative evaluation of the decreased capacity of skeletal muscle hypertrophy and the ratio of the bone marrow cell transplantation (BMT) after the various total body irradiation (TBI)

Tsuyoshi Fukuzawa[1, 2], Ippei Yamato[2], Shuichi Soeda[2], Tetsuro Tamaki[2] ([1]*Radiation Therapy, Tokai Univ, Japan,* [2]*Muscle Physiol & Cell Biol Unit, Tokai Univ. Japan*)

The capacity of skeletal muscle hypertrophy was quantified after the various intensity of the TBI in comparison of normal mice.The rate of BMT was also examined and compared.A single TBI of 9.0(n=24), 5.0(n=6) and 2.5(n=5)Gy was delivered to C57BL/6mice, and the bone marrow stromal cells, which was obtained from GFP-Tg mouse, were injected from the tail vein(1x106 cells).Five weeks after the TBI, the compensatory hypertrophy was induced in the right plantaris(PLT)muscle by the surgical ablation(SA) of the synergistic muscles(gastrocnemius and soleus), and contralateral left-side was preserved as a control.Five weeks after the SA, blood and muscle samples were obtained, and the rate of BMT in the blood(GFP-chimaerism) and the ratio of muscle hypertrophy/contralateral-side was determined.The mean GFP-chimaerism were 93 ± 4.9% in9Gy, 88 ± 7.8% in5Gy, and 0.5 ± 0.1% in2.5Gy group following the TBI intensity, and reducing ratio between 9Gy and 5Gy was small, but that was very large between 5Gy and 2.5Gy with almost no replacement of bone marrow cells occurred.In contrast, the muscle hypertrophy ratio was decreased following inverse correlation to the TBI intensity as 2.3 ± 15 in 9Gy, 35.6 ± 11.5 in 5Gy and 44.5 ± 12.3 in 2.5Gy groups. In this respect, we have found that the normal mouse SA model always showed 71.4 ± 5.6% hypertrophy, which bring out the almost maximum capacity of skeletal muscle.Therefore, when the value of normal mouse sets out the 100%, the TBI9Gy reduced 97% the capacity of muscle hypertrophy, 5Gy reduced 50%, and 2.5Gy reduced 45%.Therefore, we concluded that the influence of TBI is basically different from the bone marrow and skeletal muscle. (COI:No)

## 3P-088

### Effect of TRPC knockout on pupil diameter adjustment

Toshiyuki Kaneko, Akira Takai (*Dept Physiol, Asahikawa Med Univ, Hokkaido, Japan*)

Since vision is an important sensory organ for mammals, mechanisms to properly capture visual information are developed very well. The iris has a role of adjusting the size of the pupil to adjust the amount of light incident on the retina, the pupillary sphincter contained therein is only involved in the miosis and is dominated by parasympathetic nerves. On the other hand, the pupil dilator muscles involved in mydriasis are dominated by sympathetic nerves, and the pupil diameter is adjusted antagonistically by these intraocular smooth muscles. To maintain the light intensity properly, the iris needs to rapidly change the diameter of the pupil (rapid phase) and maintain the pupil for a long time (sustained phase). Extracellular calcium influx is required for intraocular smooth muscle, but there are some molecular entities and mechanism of action, but details are unknown. So far, we have shown that calcium influx in sustained phase is mediated by two nonselective cation channels (NSCC) with different unit conductance by experiments in bovine ciliary muscle. Expression of TRPC1, TRPC3, TRPC4, TRPC6, Orai1, etc. has been confirmed as a molecule candidate, but it is difficult to apply gene knockdown to bovine material, details of the relationship with NSCC have been clarified absent. Therefore, experiments were carried out using TRPC 3 and TRPC 6 knockout mice and double knockout mice based on these, as experimental materials, using mice which are relatively easy to genetically modify. As a result, the pupil diameter during strong light stimulation decreased significantly with the TRPC3 knockout but did not change with weak light stimulation. These results suggest that TRPC3 may have some effect on the contraction of the pupil dilator muscles. (COI:No)

## 3P-089

### Differences between DOMS and muscle contusion in acute inflammation

Kazuyoshi Hisatsune (*Dept Musculoskeletal Functional Research and Regeneration, Grad Sch Biomedical and Health Sci, Hiroshima Univ, Japan.*)

Delayed onset muscle soreness (DOMS) and muscle contusion (MC) are often observed as skeletal muscle injury for athletes, optical treatments could be different at each injury and the progression. The purpose of the present study was to investigate histological and physiological differences between DOMS and MC in acute inflammation. Male SD rats were divided into the DOMS group induced by lengthening contraction and MC group drop-mass method, respectively. The transverse sections at 48 h after injury were stained with hematoxylin and eosin for histological observation. Additionally, the calf circumference, pain sensitivity, and motor function were evaluated at 24 and 48 h after injury. Necrotic areas with inflation of mononuclear phagocyte were wider in the MC group than DOMS groups. Pain sensitivities of the DOMS group were higher at 48 h than 24 h after injury. Conversely, the sensitivities of the MC group were higher at 24 h than 48 h after injury. There were no differences in the calf circumferences of the DOMS group between the intact and injured limbs at 24 and 48h after injury. The circumferences of the MC group were longer in the injured limb than the intact limb at 24 and 48h after injury. Downhill-walking speeds were almost the same between the DOMS and MC groups at 24 and 48h after injury. These results suggest that acute inflammation is occurred both in DOMS and MC, but the severity is remarkable in MC. The change in pain sensitivity by 48h after injury was different between the injuries, not necessarily correspond to the sign of swelling and motor function. Further research including measurement of the physiological signs correlated with pain sensitivity is needed to clear differences between DOMS and MC. (COI:No)

## 3P-090

### Mechanical stimulation-induced intracellular cAMP- and $Ca^{2+}$-signaling in human odontoblast

Mayumi Matsunaga[1, 2], Maki Kimura[2], Haruna Toda[2], Sadao Oyama[2], Wataru Ofusa[2], Asuka Higashikawa[2], Yoshiyuki Shibukawa[2], Tatsuya Ichinohe[1] ([1]*Dept. Dent. Anesthesiol., Tokyo Dent. Coll.,* [2]*Dept. Physiol., Tokyo Dent. Coll.*)

We previously reported that mechanical stimulation evoked $Ca^{2+}$ influx from extracellular medium through mechanosensitive transient receptor potential (TRP) channels in acutely isolated rat odontoblasts. In addition, mechanosensitive piezo 1 channels are involved in mechanosensory processes in rat odontoblast-neuron complex. The mechanosensitive $Ca^{2+}$ signaling in odontoblasts participates in reactionary dentin formation, and/or sensory transduction for the dentinal pain. We previously elucidated cyclic AMP (cAMP)-mediated crosstalk between TRP vanilloid subfamily member 1 (TRPV1) channels and cannabinoid 1 (CB1) receptors in odontoblasts. CB1 receptor activation stimulates adenylyl cyclase, resulting in facilitation of cAMP production. The increase in intracellular cAMP then activates TRPV1 channels, resulting in $Ca^{2+}$ influx. These findings suggested that the increase in intracellular cAMP concentration mediates $Ca^{2+}$ signaling elicited by mechanosensitive ionic channel activation, and intracellular cAMP might play key roles in dentin formation and/or sensory transduction. However, the detailed intracellular cAMP signaling pathway, the role and the participation of cAMP on the cellular function and the involvement of cAMP in mechanosensitive $Ca^{2+}$ signaling in odontoblasts remain unclear. In the present study, we measured intracellular cAMP and $Ca^{2+}$ concentrations following mechanical stimulation applied to human odontoblast (HOB) cells. Intracellular cAMP level and intracellular free calcium concentration ($[Ca^{2+}]_i$) was recorded by fluorescence from mNeon Green-based cAMP sensor and fura-2, respectively. In the presence of extracellular $Ca^{2+}$, mechanical stimulation increased $[Ca^{2+}]_i$ in both mechanically stimulated HOB cells, and neighboring HOB cells to the stimulated ones. The increases were not desensitized by repeated mechanical stimuli. Mechanical stimulation decreased intracellular cAMP level in HOB cells, but did not induce any desensitizing effects on the intracellular cAMP level. These results indicated that mechanical stimulation elicited intracellular $Ca^{2+}$ signaling in HOB cells, and the signal established intercellular odontoblast-odontoblast communication. The results also suggested that mechanical stimulation inhibited intracellular cAMP signaling in odontoblasts. (COI:No)

## 3P-091

### Influence S-PRG filler eluate on secretion of MMP-1 and MMP-3 in TNF-α stimulated human gingival fibroblasts

Hiroshi Inoue, Seiji Goda (*Dept Physiol, Osaka Dent Univ, Japan*)

The S-PRG filler is known to have a high cariostatic effect material. In this study, we examined the effect of S-PRG filler eluate on MMP-1 and MMP-3 secretion of human gingival fibroblasts (HGF). S-PRG filler eluate was added to HGF and we detected MMP-1 and MMP-3 secreted into the culture supernatant by immunoblotting. As a result, secretion of MMP-1 and MMP-3 were enhanced by S-PRG filler eluate. We assessed the effects of S-PRG filler eluate induced phosphorylation of p38, ERK 1/2 and JNK in HGF by immunoblotting. Results revealed that phosphorylation of p38 and ERK 1/2 occurred within 1 minute. However, phosphorylation of JNK was not enhanced by S-PRG filler eluate. Secretion of MMP-1 and MMP-3 increased by S-PRG filler eluate was slightly decreased by p38 inhibitor, but was markedly decreased by JNK inhibitor and ERK inhibitor. Secretion of MMP-1 and MMP-3 significantly increased by TNF-α stimulation was suppressed by S-PRG filter eluate. These results suggested that phosphorylation of p38, ERK 1/2 and JNK may be involved in the secretion of MMP-1 and MMP-3 by S-PRG filler eluate. Furthermore, it was suggested that S-PRG filler eluate may be suppressed the enhancement of MMP-1 and MMP-3 secretion by TNF-α stimulated HGF. (COI:No)

## 3P-092

### Effect of steroid-containing ointment in a rat oral ulcerative mucositis model

Mako Naniwa[1,2], Suzuro Hitomi[1], Izumi Ujihara[1], Takemi Shikayama[1,3], Kazunari Matsuda[4], Kentaro Ono[1] (*[1]Div Physiol, Kyushu Dent Univ, Japan, [2]Div of Oral Health Sci, Kyushu Dent Univ, Japan, [3]Div of Periodontol, Kyushu Dent Univ, Japan, [4]Daiichi Sankyo Healthcare Co. Ltd., Japan*)

Oral ulcerative mucositis (OUM) is a common oral disease and causes pain during meals and speaking. Ointment, which is composed of base and medicinal ingredient such as steroid, is frequently applied to the OUM region for healing. Since application of ointment on the oral cavity in experimental animals is technically difficult, little study have demonstrated the effects of topical treatment on OUM pain. In this study, we investigated the effect of steroid-containing ointment, which has high residence in the oral cavity, on OUM. First, we compared the physical and sensory properties of various ointment bases (vaseline, plastibase, traful ointment [TO] base and traful ointment pro-quick [TOPQ] base). TO and TOPQ have high adhesiveness, hardness and viscosity in measurement by a rheometer. TO and TOPQ showed higher adhesiveness and residue than vaseline and plastibase in human sensory evaluation. Next, we investigated the effect of triamcinolone-containing TO(TO+Tmc) on OUM region and OUM-induced pain. In rats, OUM was developed in the inferior labial fornix region by soaking in 50% acetic acid under anesthesia. Ointment was applied twice a day. On day 2, prolonged facial grooming behavior (a sign of spontaneous pain) was shortened by TO+Tmc application, compared with non-treatment group. The head withdrawal threshold to von Frey stimulation in the OUM region was significantly increased by TO+Tmc application. Moreover, mRNA level of the glucocorticoid receptor target gene GILZ was increased, and TNF-α and COX2 mRNA levels were decreased. These results indicate that steroid in the highly-residual ointments are effective for pain relief on OUM model rats. (COI:Properly Declared)

## 3P-093

### Difference between somatosensory and gustatory input on parasympathetic increase in blood flow in rat three major salivary glands

Toshiya Sato, Ratna Ramadhani, Kohei Mito, Hisayoshi Ishii (*Div. of Physiol., Dept. of Oral Biol., Sch. of Dent., Health Sci. Univ. Hokkaido*)

Salivary gland hemodynamics is generally considered to be important for secretion of salivary fluid. We have previously demonstrated that stimulation of trigeminal sensory nerves induces rapid increases in blood flow in three major salivary glands mediated by parasympathetic nerves, as well as salivation. This supports the importance of parasympathetic nerve activation linked with orofacial sensory input in not only salivary secretion but also blood flow in salivary glands. Relative secretion of the major salivary gland varies depending on the type of stimulus such as mechanical stimulus or chemical stimulation of the sense of taste. Thus, it is assumed that the glandular hemodynamics are also regulated in harmony with regulation of salivation, however, regulatory details remain unclear. To clarify this relationship between type of sensory input and parasympathetic increase in blood flow among three glands, we analyzed the glandular hemodynamics during electrical stimulation of the inferior alveolar nerve (IAN; somatosensory input) or lingual nerve (LN; somatosensory and gustatory input) with 20 V at various frequencies (1-40 Hz) for 20 s in urethane-anesthetized rats. IAN or LN stimulation induced frequency-dependent blood flow increases in three glands, and the increases were significantly inhibited by the intravenous administration of hexamethonium, autonomic ganglion blockade. The increase evoked by IAN stimulation was the highest in the parotid gland, whereas that evoked by the LN stimulation was the highest in the submandibular gland. Therefore, our results indicate that the parasympathetic increase in blood flow in parotid gland is involved in somatosensory input, while that in submandibular gland is activated with gustatory input, and suggest that these differences would be related in the differences in relative secretion rate of three salivary glands. (COI:No)

## 3P-094

### Different Effects Between Trigeminal Sensory and Vagal Visceral Input on Saivary Glands Blood Flow

Ratna Ramadhani, Kohei Mito, Toshiya Sato, Hisayoshi Ishii (*Division of Physiology, Department of Oral Biology, School of Dentistry, Health Sciences University of Hokkaido*)

Adequate blood supply to the three major salivary glands, submandibular (SMG), sublingual (SLG), and parotid (PG), is important for saliva production because the fluid in saliva originates from blood capillaries and the interstitial fluid. Salivary gland hemodynamics is regulated by the sensory and autonomic nervous system via several cranial nerves, including the trigeminal and vagus nerves. Trigeminal sensory input is essential for regulating hemodynamics and secretion of salivary gland in the orofacial area (Sato & Ishii, 2015). Although afferent electrical stimulation of the vagus nerve has been reported to induce salivation in anesthetized rodent (Ueda et al., 2016), the effect of vagal-visceral input on hemodynamics in salivary glands remains unclear. Therefore, the purpose of this study was to evaluate and compare the different effects of trigeminal sensory and vagal-visceral inputs on blood flow in salivary glands in deeply urethane-anesthetized rats.

The left AVN, LN, and CVN were stimulated by bipolar electrodes. The cervical sympathetic trunk (CST) on both side and the CVN on the right side were cut before the stimulation of the left abdominal vagus nerve (AVN). The left CVN and lingual nerve (LN) were stimulated after cutting the CST and CVN. The hemodynamics of the salivary glands (SMG, SLG, and PG) were recorded using a laser speckle imaging blood flow meter. Systemic arterial blood pressure (SABP) was recorded from the femoral catheter using a Statham pressure transducer.

Our results indicated that LN stimulation elicited an increase in both SABP and blood flow in the SMG, SLG, and PG. Furthermore, CVN and AVN stimulation induced an increase in SABP; however, blood flow increase in the three salivary glands was lower than that observed after LN stimulation. In conclusion, trigeminal sensory input rather than vagal-visceral input appears to be involved in the regulation of blood flow in the salivary glands. (COI:No)

## 3P-095

### Transcriptional kinetics altered by thyroid hormone during mouse cerebellar development

Hiroyuki Yajima[1], Sumiyasu Ishii[1], Wataru Miyazaki[2], Noriyuki Koibuchi[1] (*[1]Dept Integr Physiol, Grad Sch Med, Gunma Univ, Japan, [2]Dept Biosci Lab Med, Grad Sch Health Sci, Hirosaki Univ, Japan*)

**Background:** Thyroid hormone (TH) plays essential roles in the development of the cerebellum by regulating transcription of target genes. TH binds to TH receptor (TR) located in the cell nucleus and stimulates transcription through TH response element (TRE). The expression of many genes is temporary and spatially regulated by TH during cerebellar development. However, the mode of transcriptional regulation by TR may vary among target genes. In the liver, altered duration of TH exposure resulted in distinct gene expression profiles. To examine the mechanism of transcriptional regulation by TH in cerebellar development, gene expression profile induced by various TH exposure duration was studied.

**Methods:** Anti-thyroid drug propylthiouracil (250 ppm in drinking water) was administered to C57BL/6J mice from the gestation day 14 to postnatal day (P) 7 to generate perinatal hypothyroid mice. To study the effect of continuous TH exposure, TH was subcutaneously administered to hypothyroid pups from P2 to P7 (6 days group). To study the gene expression profiles induced by single TH administration, TH was injected on P7 and mice were sacrificed either 6 (6 hours group) or 24 hours (24 hours group) after injection. Cerebellar samples were collected to extract RNA and subject to microarray analysis. Microarray results were confirmed after injection by RT-qPCR.

**Results:** Compared with hypothyroid mice, TH injected groups induced an alteration of mRNA levels (upregulation, 1295 genes; downregulation, 1332 genes). Only 7.6% of the genes were overlapped in three groups among positively regulated genes, suggesting differential regulation of transcription stimulation in an exposure time-dependent manner of TH. In contrast, 57.2% of the genes were common in the negatively regulated genes.

**Conclusion:** TH distinctively regulates transcription of target genes depending on exposure schedule in mouse developing cerebellum. (COI:No)

## 3P-096

### Acute mono-arthritis activates the neurohypophysial system and hypothalamo-pituitary adrenal axis in rats

Haruki Nishimura[1], Makoto Kawasaki[1], Takanori Matsuura[1], Hitoshi Suzuki[1], Yasuhito Motojima[1], Kazuhiko Baba[1], Hideo Ohnishi[1], Yoshiaki Yamanaka[1], Teruaki Fujitani[1], Mitsuhiro Yoshimura[2], Takashi Maruyama[2], Hiromichi Ueno[2], Satomi Sonoda[2], Kazuaki Nishimura[2], Kentarou Tanaka[2], Kenya Sanada[2], Tatsushi Onaka[3], Yoichi Ueta[2], Akinori Sakai[1] (*[1]Dept Ortho, UOEH, Japan, [2]Dept Physiol, UOEH, Japan, [3]Dept Physiol, Jichi Med Univ, Japan*)

Various types of acute/chronic nociceptive stimuli cause neuroendocrine responses such as activation of the hypothalamo-pituitary adrenal (HPA) axis and hypothalamo-neurohypophysial (oxytocin [OXT] and arginine vasopressin [AVP]) system. Chronic multiple-arthritis activates the OXT/AVP system as well as the HPA axis, but the effects of acute mono-arthritis on the OXT/AVP system and HPA axis in the same animals have not been evaluated simultaneously. In the present study, we used an acute mono-arthritic model induced by intra-articular injection of carrageenan in a single knee joint of adult male Wistar rats. Acute mono-arthritis was confirmed by a significant increase in knee diameter in the carrageenan-injected knee and significant decrease in mechanical nociceptive threshold in the ipsilateral hind paw. Immunohistochemical analysis revealed that the number of Fos-immunoreactive (ir) cells in the ipsilateral lamina I-II of the dorsal horn was significantly increased, and the percentage of OXT-ir and AVP-ir neurons expressing Fos-ir in both sides of the supraoptic (SON) and paraventricular nuclei (PVN) was increased in acute mono-arthritic rats. In situ hybridization histochemistry revealed that levels of OXT mRNA and AVP hnRNA in the SON and PVN, CRH mRNA in the PVN, and proopiomelanocortin mRNA in the anterior pituitary were also significantly increased in acute mono-arthritic rats. Further, plasma OXT, AVP, and corticosterone levels were significantly increased in acute mono-arthritic rats. These results suggest that acute mono-arthritis activates ipsilateral nociceptive afferent pathways at the spinal level and causes simultaneous and integrative activation of both the OXT/AVP system and HPA axis, with a distinct pattern of upregulated gene expression compared to that of chronic multiple-arthritis. (COI:No)

## 3P-097

### Circadian rhythm of PVN neurons regulates glucose tolerance

Toshiya Yamazaki[1,2], Rika Kita[1], Boyang Zhang[1], Masanori Nakata[1] ([1]*Dept Physiol, Wakayama Med Univ, Japan,* [2]*Dept Clinical Acupuncture, Kansai Univ of Health Sciences, Japan*)

Physiological functions, including feeding, body temperature, and hormone release, show circadian patterns. Furthermore, impairment of the circadian patterns in these functions is thought to be causally implicated in diverse diseases including obesity and diabetes. Especially the paraventricular nucleus (PVN) in the hypothalamus integrates afferent signals from the hypothalamus including SCN, the region of master clock, and plays a role in the regulation of energy metabolism. In this study, we aimed to clarify the function of circadian rhythm of PVN on glucose metabolism. We generated and analyzed PVN-preferential Bmal1 KO mice. KO mice resulted in impairment of glucose tolerance without affecting feeding and energy expenditure. Interestingly, KO mice also exhibited decreases in insulin secretion without insulin resistance. On the other hand, insulin release from isolated islets and increases in cytosolic $Ca^{2+}$ concentration of isolated pancreatic $\beta$-cell were maintained in KO mice as well as Cre mice. These results suggested that the circadian rhythm in PVN neurons maintain neuronal regulation of insulin secretion.These results demonstrate that the circadian rhythm of PVN neurons drives neuronal regulation of insulin secretion, which is required for glucose homeostasis. (COI:No)

## 3P-098

### Identification of allopregnanolone-biosynthesizing cells in adrenal grand and developmental change in GABA signaling machinery in adrenal medullary cells

Keita Harada[1], Hidetada Matsuoka[1], Yuchio Yanagawa[2], Masumi Inoue[1] ([1]*Dept Cell and Systems Physiol, UOEH, Fukuoka, Japan,* [2]*Dept Genetic and Behavioral Neurosci, Gunma Univ Grad Sch Med, Maebashi, Japan*)

GABA is assumed to function as an autocrine or paracrine factor in adrenal medullary (AM) cells. We have reported that GAD67, an isoform of GABA synthesizing enzyme, and $\alpha$3-containing $GABA_A$ receptors are expressed in rat AM cells and allopregnanolone, a neuroactive steroid which is known to be synthesized in the adrenal gland, produces an increase in $GABA_A$ receptor channel activity in guinea-pig AM cells. However, it is not clear how this GABA signaling is established in the adrenal medulla. Before the birth, AM cells are not innervated by the preganglionic sympathetic nerve fibers and do not form a compact mass at the center of the adrenal gland. Thus, GABA signaling machinery might be established during the development of the adrenal medulla after the birth, possibly under the influence of the sympathetic innervation and/or adrenal cortical steroids. The present experiment was undertaken to identify the cells where allopregnanolone is synthesized and investigate whether GABA signaling in AM cells develop after the birth or not. 3$\alpha$-hydroxysteroid dehydrogenase (3$\alpha$HSD) and 5$\alpha$-reductase, enzymes involved in allopregnanolone biosynthesis, were immunohistochemically detected in mouse adrenal cortical (AC) cells, but not AM cells, and 3$\alpha$HSD-like immunoreactivity (IR) was co-localized with calreticulin-like in AC cells, a marker protein of the endoplasmic reticulum. To investigate a developmental change in GABA synthesis, GFP-GAD67 knock-in mice were used. GFP-expressing cells were located in the adrenal medulla, but not in the adrenal cortex. The number of GFP-expressing AM cells in five-week old mice were much larger than that in one-week old mice. On the other hand, $GABA_A$ receptor channel activities in newborn hamster AM cells did not differ from those in adult AM cells. These results indicate that allopregnanolone is synthesized in AC cells and expression of GAD67 and $GABA_A$ receptors in AM cells is differently regulated. (COI:No)

## 3P-099

### The gastrin-releasing peptide system in the medial preoptic area controls male sexual activity in rats

Sho Maejima[1], Rei Nomura[1], Keiko Takanami[1,2], Tatsuya Sakamoto[1], Hirotaka Sakamoto[1] ([1]*Ushimado Marine Institute, Fac Sch, Okayama Univ, Japan,* [2]*Natl Inst Genetics*)

In male rats, gastrin-releasing peptide (GRP) neurons in the spinal cord control male sexual function such as erection and ejaculation, and project axons into the brain. Central GRP is known as a modulator of many physiological functions including itch, circadian rhythms, food intake, fear memory consolidation. However, the role of GRP in male sexual behavior remains to be uncovered. In this study, we investigated the involvement of central GRP neuron system in regulatory mechanism of male sexual behavior. We generated a transgenic (Tg) rat that expresses a human diphtheria toxin (DT) receptor and a monomeric red fluorescent protein (mRFP) depending on GRPR gene promoter in GRP receptor (GRPR)-expressing neuron. In the morphological analysis using this Tg rat, we found that GRPR-expressing neurons are distributed in several nuclei involved in male sexual functions such as the medial preoptic area (mPOA; a regulatory center of male sexual behavior), bed nucleus of the stria terminalis, ventromedial nucleus of the hypothalamus, and medial amygdala. A subpopulation of GRPR-expressing neurons exhibits a male-biased sexual dimorphism in the mPOA, and c-Fos expression in these neurons was significantly increased following to ejaculation. Then we examined the lesion effect of GRPR-expressing neurons on sexual behavior by administration of DT into the mPOA of Tg rats. Sexual activity of DT-administered males was significantly attenuated in compared with before DT-administration. Subsequently, GRPR agonists and antagonists were injected locally into the mPOA and male sexual activity was studied to verify the role of endogenous GRP. Pharmacological stimulation of GRPR by agonists did not show any effects on male sexual activity, whereas the inhibition of GRPR by antagonists significantly reduced male sexual activity. These results suggest that the GRP system in the mPOA plays an important role in male sexual activity in rats. (COI:No)

## 3P-100

### Renal tissue kallikrein may be involved in the regulatory Ca transport along the kidney distal nephron

Yukiko Yasuoka[1], Tomomi Oshima[1], Yuichi Sato[2], Noriko Takahashi[1], Hiroshi Nonoguchi[3], Katsumasa Kawahara[4] ([1]*Dept Physiol, Sch of Med, Kitasato Univ. Sagamihara, Japan,* [2]*Dept of Mol. Diagnostics, Kitasato U. Sch. of Allied Health Sci., Sagamihara, Japan,* [3]*Internal Med., Kitasato U. KMC Hospital, Kitamoto, Japan,* [4]*Dept of Health and Nutrition, Sendai Shirayuri Women's College, Sendai, Japan*)

Tissue kallikrein (TK), a serine protease in the distal nephron including the collecting ducts (CDs) and Henles loops (HLs) (Chen LM et al, 1995), may be a key to regulate the renal calcium (Ca) reabsorption (Picard N et al, 2005). To understand the molecular basis of the regulatory Ca transport, a segmental and functional expression of TK, TRPV5 (luminal Ca transporter), $CaBP_{28K}$, (Calbindin D28K: cytosolic Ca buffering protein) was semi-quantitatively estimated in conjunction with blood and urine analysis.
**Methods:** Male mice (10 weeks) were treated with either 1% Ca-citrate diet (control) or low (0.05%)-Ca diet (LCa) for 1 week. Expression and localization of the TK, TRPV5, and $CaBP_{28K}$ mRNAs and proteins were estimated by using *in situ* hybridization and immunohistochemistry.
**Results:** Plasma pH and $Ca^{2+}$ concentration were unchanged in mice with LCa (pH7.38 and 4.4 mg/dl (LCa) vs. pH7.36 and 4.5 mg/dl (control)), since the urinary excretion of Ca and $NH_3$/$NH_4^+$ was contrarily decreased and increased, respectively, from 186.0 (control) to 60.3 μg/day (LCa) and 120.7 (control) and 1101.6 μg/day (LCa) ($P<0.005$, n=8-9). Importantly, expression levels of TRPV5 and $CaBP_{28K}$ proteins, co-localized in the distal convoluted tubule (DCT) and the early connecting tubule (CNT), increased similarly during LCa. More importantly, TK expression dramatically increased in the luminal membrane of the cortical and medullary thick ascending limb of the HLs (CTAL and MTAL), the upper stream of the DCT and CNT.
**Conclusion:** Segment-specific increases of the TK, TRPV5, and $CaBP_{28K}$ expressions during low Ca diet may be co-operatively involved in regulation of the renal Ca reabsorption along the distal nephron. (COI:No)

## 3P-101

### Thermal stimulation to lower back and rump skin improves voiding efficiency in urethane anesthetized rats

Kaori Iimura, Nobuhiro Watanabe, Harumi Hotta (*Dept Auton Neurosci, Tokyo Metropol Inst Gerontol, Japan*)

**Aim:** The aim of the present study is to clarify whether somatosensory stimulation can improve the voiding efficiency in underactive bladder.
**Methods:** We used female Fisher rats anesthetized with urethane. The cystometrogram was recorded via a bladder catheter inserted from bladder dome. Saline was continuously infused via the catheter until the rat spontaneously voided. The voiding efficiency was calculated based on infusion volume and voiding volume (the percentage of the voided volume relative to the infused volume). Somatosensory thermal stimulation was applied to the lower back and rump skin using a Peltier thermode from start saline infusion to voiding. During stimulation, the temperature of the thermode was changed between 35℃ and 25℃ with a speed of 1-2℃/second, repeatedly every 10 seconds. We compared voiding efficiency without and with thermal stimulation.
**Results:** Before applying thermal stimulation, voiding efficiency was approximately 24%. During thermal stimulation, voiding efficiency significantly increased to approximately 40%. There was 16% increase in voiding efficiency during thermal stimulation compared to control condition before stimulation. The maximum pressure of bladder contraction was not different between before and during stimulation. On the cystometrogram, we observed high frequency oscillations (HFOs) during voiding, which reflecting repetitive contractions of the external urethral sphincter. The duration of HFOs increased twofold from 1.1 ± 0.5 sec (mean ± SD) before stimulation to 2.4 ± 1.0 sec during stimulation.
**Conclusion:** The results suggest that thermal stimulation to the lower back and rump skin improved voiding efficiency by prolonging the duration of urethral sphincter contractions and extending the urine output time. (COI:No)

## 3P-102

### Effects of Docosahexaenoic acid- and Arachidonic acid-containing diet on Renal function of 5/6 nephrectomy rats

Hiroki Muramatsu, Kenji Sugibayashi, Masanori Katakura (*Nutr Phyol, Grad Phar Sci, Josai Univ, Japan*)

The kidneys are responsible for excretion, maintenance of pH, adjustment of blood pressure, and regulation of body fluid volume. The structure of the kidneys is highly complex, and it is very difficult to recover after injury. Therefore, it is important to prevent the kidneys from being damaged. Arachidonic acid (ARA), an $\omega$6 polyunsaturated fatty acid (PUFA), is involved in the development and maintenance of renal functions. Docosahexaenoic acid (DHA), an $\omega$3 PUFA, produces anti-inflammatory effects. In this study, we assessed the effects of consumption of different types of PUFAs on the development and progression of chronic kidney disease (CKD) using 5/6 nephrectomized rats. Male Sprague Dawley rats were randomly divided into four groups, which were fed ad libitum, either control, ARA, DHA, or ARA+DHA-containing diets for 4 weeks; then, 5/6 of each subject's kidney was removed. Four weeks after surgery, rats were housed in individual metabolic cages for 24 hours and urine was collected. Then, plasma and kidneys were collected for biochemical and histological analysis. Urinary albumin excretion was increased in the control CKD group compared to the control sham group, but it was attenuated by feeding the DHA diet. ARA and DHA levels in red blood cell were increased in groups.
fed diets containing ARA and DHA compared to the control CKD group. Peroxynitrite levels in the kidney decreased in ARA, DHA, and ARA+DHA groups compared to the control CKD group. Peroxynitrite levels in the kidneys had significantly negative correlation with creatinine clearance. Lipid peroxide levels in the kidneys were not increased in any group. In contrast, in the plasma, lipid peroxide levels increased in the control CKD group, the levels were attenuated by feeding the ARA+DHA diet. These results suggest that DHA, or a combination of ARA and DHA, inhibits the progression of early stage of CKD. (COI:No)

## 3P-103

### How do the basal ganglia control thalamocortical activity?

Satomi Chiken[1,2], Hiromi Sano[1,2], Kenta Kobayashi[2,3], Atsushi Nambu[1,2,3] (*[1]Div Syst Neurophysiol, Natl Inst Physiol Sci, Okazaki, Japan, [2]Dept Physiol Sci, SOKENDAI, Okazaki, Japan, [3]Sec Viral Vector Devel, Natl Inst Physiol Sci, Okazaki, Japan*)

The basal ganglia (BG) play a crucial role in controlling voluntary movements, and their dysfunction causes severe motor disturbance, such as Parkinson's disease and dystonia. The BG receive information from a wide area of the cerebral cortices. The internal segment of the globus pallidus (GPi), one of the output nuclei of the BG, sends GABAergic projections to the thalamocortical neurons and governs the original cortices. Thus, to elucidate roles of the BG in control of voluntary movements, it is essential to understand how BG outputs control thalamocortical activity. In the present study, we identified thalamocortical neurons by electrical stimulation of the forelimb regions of the primary motor cortex and supplementary motor area, and then examined their responses to electrical stimulation of the GPi in macaque monkeys. Single-pulse stimulation induced a biphasic response composed of short-latency inhibition and following excitation. Repetitive stimulation evoked a train of biphasic responses. Local injection of GABA-A receptor antagonist into the vicinity of recorded thalamocortical neurons abolished not only inhibition but also following excitation without changes in spontaneous firing rates, suggesting that the excitation is caused by a post-inhibitory rebound mechanism. Next, we expressed halorhodopsin in the axon terminals of GPi-thalamic projections by injecting an adeno-associated virus vector into the GPi, recorded thalamocortical activity using an optrode during performance of a hand reaching task, and selectively blocked GPi-thalamic inputs by illuminating yellow light in the vicinity of recorded neurons. Inhibitory responses evoked by GPi-stimulation were successfully abolished by the optical stimulation. Task-related firing increase was enhanced by blockade of GPi-thalamic inputs in the half of recorded thalamocortical neurons, but diminished in the other half. These results suggest that GPi-thalamic inputs modulate thalamocortical activity through both GABAergic inhibition and following rebound excitation and contribute to controlling voluntary movements. (COI:No)

## 3P-104

### Interhemispheric inhibition during motor imagery of dominant or non-dominant finger movement in humans: A transcranial magnetic stimulation study

Nan Liang[1,2], Amiri Matsumoto[1], Hajime Ueda[1] (*[1]Dept Hum Health Sci, Grad Sch Med, Kyoto Univ, Japan, [2]Dept Integr Physiol, Grad Sch Biomed Health Sci, Hiroshima Univ, Japan*)

We have previously reported that interhemispheric inhibition (IHI) from the contralateral to ipsilateral motor cortex (M1) increased during voluntary and imagined movements (Liang et al. 2014). By a pure central command of motor imagery, whether the extents to IHI differ between dominant and non-dominant hands remains unclear. By using transcranial magnetic stimulation (TMS) techniques, we examined that the excitability changes in the contralateral and ipsilateral M1 as well as IHI from the contralateral to ipsilateral M1 during motor imagery of dominant or non-dominant finger movement. The right-handed healthy subjects were asked to perform imagery of the index finger abduction with their maximum force in the dominant or non-dominant hand. Single pulse TMS was applied over the contralateral or ipsilateral M1. Then, a conditioning TMS with an intensity ranging from 0.8 to 1.4 times the resting motor threshold (rMT) was applied over the contralateral M1, and the test TMS with an intensity of 1.1 to 1.2 times rMT was applied over the ipsilateral M1. The motor evoked potential (MEP) was recorded from the first dorsal interosseous (FDI) muscles. MEP amplitude significantly increased in both FDI muscles during either dominant or non-dominant imagery. The increased MEP in the left FDI muscle was negatively correlated with that in the right FDI muscle during imagery of the right hand, while there was no correlation in the increased MEPs between the muscles during imagery of the left hand. The conditioned MEP size was positively correlated with that in the contralateral homonymous muscle during right hand imagery, but with no correlation in the case of the left hand imagery. These results suggest that IHI from the contralateral to ipsilateral M1 modulated depending on the contralateral M1 excitability during motor imagery of the dominant hand but not with that of the non-dominant hand. (COI:No)

## 3P-105

### Postural transformation during treadmill walking in Japanese monkeys: kinematic and EMG analysis

Takashi Suzuki[1], Kazunori Morita[1], Masahiko Inase[2], Katsumi Nakajima[1] (*[1]Dept Physiol, Sch. Med, Iwate Med Univ, Yahaba, Japan, [2]Dept Physiol, Facult Med, Kindai Univ, Osaka-Sayama, Japan*)

Japanese monkeys can walk on a treadmill and volitionally transform its trunk posture from horizontal (quadrupedal gait) to vertical (bipedal gait) without interruption of stepping movement. To investigate behavioral processes of the postural transformation during locomotion, we analyzed kinematics and EMG activity of the trunk and limbs in two monkeys. Lateral and back views of the walking animal were videotaped using two high-speed cameras. EMG activity was recorded via chronically implanted Teflon-coated tungsten wires. Postural transformation was initiated by touchdown of either hindlimb (trigger limb, TL) during quadrupedal gait and was accomplished within 1-2 step cycles. In this period, the body axis in the sagittal plane increased from 5° to 70°.

In the frontal plane, the mediolateral hip position during quadrupedal gait located between stance positions of the left and right hindlimbs and the head position between those of forelimbs. Just before the transformation, the hip and head positions shifted toward the TL side (the first postural adjustment). In the following righting-up period, these positions conversely shifted to the opposite TL support side and then moved back to the TL side during subsequent stance phase of the hindlimb contralateral to TL. Antigravity muscles in the hindlimb were active during stance phase and bilateral back muscles were coactive around touchdown of each hindlimb for quadrupedal gait. During righting up, phasic activity of these muscles increased and burst activity of the left and right back muscles occurred alternately. Such mediolateral trunk sway (the second postural adjustment) and activity patterns of antigravity muscles observed during postural transformation continued into subsequent, stable bipedal locomotion. Our results identified two distinct postural adjustments associated with righting-up behavior during locomotion. They contribute to secure dynamic equilibrium during postural transformation and subsequent bipedal locomotion in the same manner as anticipatory postural adjustment precedes voluntary movements to maintain equilibrium. (COI:No)

## 3P-106

### Activation of human spinal locomotor circuitry using transvertebral magnetic stimulation

Kazutake Kawai[1], Toshiki Tazoe[2], Kazuyuki Kanosue[3], Yukio Nishimura[2] (*[1]College of Sports Sciences, Nihon Univ, Japan, [2]Tokyo Metropolitan Institute of Medical Science, Japan, [3]Faculty of Sport Sciences, Waseda Univ, Japan*)

Rhythmic walking-like alternus movements in bilateral legs can be induced by non-invasive transvertebral magnetic stimulation of the human lumbar spinal cord, indicating the existing lumbar locomotor center in mans. This magnetic stimulation would be a novel neurorehabilitation for gait disturbance due to spinal cord injury or stroke. It is important to know the stimulus parameters that activates the spinal locomotor center, however the relationship between stimulus intensity and induced gait has not yet been systematically investigated. The purpose of this study was to find the stimulation intensity necessary to drive the spinal locomotor center effectively, and to verify the mechanism by which the neural elements are recruited. Ten healthy adults' participants were recruited. Transvertebral magnetic stimulation was applied at the lumbar spine level with a stimulation intensity from 10% to 70% maximum of the magnetic stimulator output with an increment of 10%. Most subjects showed no movements in legs at 10% stimulation intensity. Low intensity stimulation tended to induce a hopping-like movement in which both legs moved in phase. When the stimulus intensity was increased, the induced movements changed to a walking-like movement in which the left and right legs moved in anti-phases. In addition, as the stimulus intensity increased, the toe trajectory length of the induced walking-like movement tended to increase. The threshold required to induce walking-like movement was significantly higher than that of hopping-like movement, and 40% to 60% of the magnetic stimulator is required to drive the spinal locomotor center for walking in mans. (COI:No)

## 3P-107

### Chemogenetics to decipher the functional role of the subthalamic nucleus in macaque monkeys

Taku Hasegawa[1], Satomi Chiken[1,2], Kenta Kobayashi[2,3], Atsushi Nambu[1,2,3] (*[1]Div of System Neurophysiol, Natl Inst for Physiol Sci, Okazaki, Japan, [2]Dept of Physiol Sci, SOKENDAI, Okazaki, Japan, [3]Sec of Viral Vector Development, Natl Inst for Physiol Sci, Okazaki, Japan*)

The subthalamic nucleus (STN) is considered to play an essential role in motor control as evidenced by effects of its lesion; the STN lesion induces hemiballismus, involuntary ballistic movements of the limbs, and alleviates the motor symptoms in parkinsonian patients. However, it is not clear how the STN influences the output of the basal ganglia (BG) and controls the movements. The STN sends glutamatergic axons to the internal segment of the globus pallidus (GPi), the output nucleus of the BG, as well as to the external segment of the globus pallidus (GPe), which in turn sends GABAergic axons back to the STN. We suppressed the STN activity with the DREADD (Designer Receptors Exclusively Activated by Designer Drugs) system and analyzed the activity change in the GPi and GPe.

An adeno-associated virus vector expressing the inhibitory DREADD receptor, hM4Di, was infused to the motor area in the STN of three Japanese monkeys (*Macaca fuscata*). After the receptor expression (>3 weeks), abnormal involuntary movements were induced in the contralateral forelimb by the systemic administration of a DREADD ligand.

The single unit activity in the forelimb motor areas of the GPe and GPi was recorded during a reaching task and the following changes were observed after the STN suppression: 1) the decrease in the baseline firing rates in the GPe, 2) the increase of the variability in spike timings both in the GPe and GPi, and 3) the enhanced movement-related activity in the GPe and GPi.

Our results suggest that the STN controls movements not only through changing the firing rates but also through adjusting the spike variability; the STN may play a role to stabilize the firing timings of the GPe and GPi neurons through the excitatory innervation. (COI:No)

## 3P-108

### Detection of motor defects in hindlimbs during locomotion in rats with focal cerebral infarction by 3D kinematical analysis

Tatsuro Kumada[1], Akira Yoshikawa[2], Saho Morishita[3,4], Kazuya Hokamura[5], Masahiko Izumizaki[2], Kazuo Umemura[3] (*[1]Fac Health Med Sci, Tokoha Univ, Japan, [2]Dept Physiol, Showa Univ, Japan, [3]Dept Pharmacol Hamamatsu Univ Sch Med, Japan, [4]Fac Health Promo Sci, Tokoha Univ, Japan, [5]Dept Med Edu, Hamamatsu Univ Sch Med, Japan*)

Precise evaluation of motor outputs is required for unmasking the roles of local neuronal circuits on controlling motor behavior and the long term effects of rehabilitation on motor recovery after cerebral infarction. However, conventional behavior analysis in rodents is suitable for screening of animals with more severe defects, but not sufficient to access these issues. Three-dimensional (3D) kinetic analysis allow us to quantify the motor behavior in spatio-temporal manner and detects even small changes in motor outputs. To elucidate the possibility of this analysis, we developed rats with focal motor cortex infarction by photochemically induced thrombosis (PIT) method, and have evaluated the movement of hindlimbs of these rats during locomotion on treadmill by using the KinemaTracer system (KISSEI COMTEC) with some modifications. Rats with focal motor infarction did not exhibited apparent changes in general gait parameters (gait cycle, stance phase, swing phase, step length and step width), but significant changes in trajectories of several hindlimb joints between pre- and post-1 day operated rats. Interestingly, the deficits were more prominent in distal segments compared to proximal segments. These results suggest that analysis of joint movements by 3D kinetic analysis could evaluate the mild defects in motor behavior in ischemic model in rat. (COI:No)

## 3P-109

### Effects of PPAR gamma agonist on senescence related gene expressions in visceral adipose tissue of aged mice

Masaki Kimura[1, 2], Ryo Shibakawa[1], Yoshihiro Matsumoto[1], Risako Ishii[1], Natsumi Hirano[1], Yoshimasa Saito[1], Hidetsugu Saito[1] (*[1]Dept Pharmacotherapeutics, Fuclt Pharmacy, Keio Univ, Japan, [2]Lab Metabolic Med, Mater Med Res Ins, Univ of Queensland, Australia*)

**Background and Purpose:** Senescence related markers are reported to be increased in white adipose tissue of obese animals. In our previous study, PPAR gamma agonist seemed to ameliorate these senescence related changes. The purpose of this study was to investigate the effects of PPAR gamma agonist (pioglitazone) on senescence related gene expressions in visceral adipose tissue of aged mice.
**Animal and method:** 88-week-old male C57BL/6 mice were assigned to control (OC) or pioglitazone ingestion (OP) group. The OP group took 12-week dietary ingestion of normal diet with 0.02 % (w/w) pioglitazone. At the end of experimental period, abdominal fat tissues were collected for the analysis of adipocyte cellularity and senescence related gene expressions.
**Result and Discussion:** There were no differences in both body weight and total food intake between OC and OP group. Pioglitazone ingestion seemed to make some influences on visceral adipose tissue, those were indicated by the increase in plasma adiponectin concentration and the decrease in fat cell diameter and MEST mRNA expression in epididymal fat. However, we could not find enough changes in senescence related gene expressions in this visceral fat. These results suggested that the further investigation about the age of mice and the volume and duration of pioglitazone ingestion are needed to evaluate the effects of PPAR gamma agonist on visceral adipose tissue of aged mice. (COI:No)

## 3P-110

### Possible Mechanisms of Astrocyte Senescence in a Hepatic Encephalopathy Model

Naoki Adachi[1, 2], Misako Ando[2], Fatma Zahra Sakhri [1], Hideshi Ikemoto[1], Yusuke Ohashi[1], Mana Tsukada[1], Yasunori Takayama[1], Yohei Hirai[2], Masataka Sunagawa[1] (*[1]Dept Physiol, Sch Med, Showa Univ, [2]Dept Biomed Chemi, Sch Sci and Tech, Kwansei Gakuin Univ*)

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome attributed to acute and chronic liver dysfunction. The major symptoms of HE are mild cognitive impairment, stupor and coma as well as motor disturbances such as ataxia and asterixis. Increased blood concentration of ammonia is considered as one of the main causes of the symptoms. Interestingly, recent studies suggest that ammonia-induced premature senescence in astrocytes may contribute to develop the symptoms.
The aim of this study was to clarify the molecular mechanisms underlying the ammonia-induced astrocyte senescence, DNA damage levels, expression/activation of the cell cycle regulators and sirtuin 1 (SIRT1), and senescence-associated secretory phenotype (SASP) using cultured rat cortical astrocytes after ammonia exposure.
Ammonia (5 mM $NH_4Cl$) exposure caused cellular senescence in astrocytes through upregulating cell cycle regulating factors such as p21 and p53. SASP was also observed as increased IL-6 expression. When anti-senescence effects of resveratrol and nicotinamide mononucleotide (NMN) against the astrocyte senescence were determined, NMN showed significant suppression in these senescence-associated markers.
These results suggest a possible involvement of the astrocyte senescence in the pathophysiology of HE, and NMN could have a beneficial effect to ameliorate the neuropsychiatric symptoms in the patients with HE. (COI:No)

## 3P-111

### The effects of anesthetic drug on the rat fetal movement pattern before and after in the non-anesthesia pregnant rat in the ultrasonic tomographic method

Riku Arizono[1, 2], You Ueda[1, 2], Akira Tamaki[1], Akiko Arata[2] (*[1]Hyogo Univ of Health Sci of Rehabilitation, Physical Ther for Int Disorders, Kobe, Japan, [2]Dept. of Physiol, Hyogo Coll of Med, Nishinomiya, Japan*)

Body movement analysis of human fetus has been studying using ultrasonic tomographic imaging. However, that of rat fetus has not been much studying and there was no data of the rat fetal movement. We created the equipment for analysis of rat fetal movement using the ultrasonic tomographic imaging under non-anesthesia, and we studied the comparison of rat fetal movement and human baby movement. In this study, we observed the fetal movement of the total observation time (10 minutes) by using ultrasonic tomographic imaging system in the embryonic day15 (E15), E17, E19, E21 of Wistar rat fetus. We recognized three stages of fetal movements; the body movement, the twitching movement like a reflex and the respiratory movement. All of fetal movement was depressed by anesthetic drug application (GABA: midazolam + a2-adrenaline agonist: medetomidine) + kappa-receptor agonist: butorphanol). We also examined the effects of each anesthetic drug on the fetal movement. Butorphanol significantly depressed the body movement in E21, and midazolam and medetomidine depressed it in E17-19. However, the twitching movement was partially blocked by butorphanol in E19 and it was largely blocked by midazolam and medetomidine in E19-21. These results suggested that 1) we recognized three stages of fetal movements in late pregnant term; 2) the effects of between k-opioid receptor and both GABA and a2-adrenergic receptors might control the different mechanisms in the fetal development. (COI:No)

## 3P-112

### Effects of cigarette smoke extract on endothelial cells-From the viewpoint of DNA damage and cellular senescence

Mari Ishida[1], Chiemi Sakai[1], Keitarou Ueda[1], Masao Yoshizumi[1], Takafumi Ishida[2] (*[1]Dept Cardiovasc Physiol and Med, Hiroshima Univ, Japan, [2]Dept Cardiovasc Med, Fukushima Med Univ, Japan*)

**Objective:** Smoking exerts a detrimental effect to many organ systems and is responsible for illnesses such as atherosclerosis, cancer and chronic obstructive pulmonary disease. We reported that DNA damage was increased in smokers' blood cells compared to non-smokers. Recently, it has been reported that DNA damage is involved in cellular senescence and the pathogenesis of atherosclerosis. In this study, we investigated whether cigarette smoke extract (CSE) exerts DNA damage and cell senescence in endothelial cells and the physiological influence of DNA damage.
**Methods and Results:** The influence of CSE extracted from tobacco smoke was examined in human umbilical vein endothelial cells (HUVEC). CSE was added to HUVEC and DNA damage formation was quantified by fluorescence immunostaining; DNA single strand breaks (SSBs) with RPA 2 and double-strand breaks (DSBs) with phosphorylated Histone H2AX as indices. SSBs increased at 24 hours after CSE stimulation, and DSBs increased significantly at 72 hours after stimulation. Continuous stimulation with CSE for 7 days resulted in an accumulation of cytosolic DNA and accelerated cellular senescence quantified by senescence-associated $\beta$-galactosidase activity. The mRNA expression of inflammatory cytokines, such as IL-6 and IL-1$\alpha$ was increased and prolonged by continuous CSE stimulation.
**Conclusions:** CSE was shown to induce DNA damage, accumulation of cytosolic DNA, cellular senescence and inflammation of vascular endothelial cells. (COI:No)

## 3P-113

### Coriandrum sativum inhibits migration and invasion of cancer cell through suppressions of MMP-2 and u-PA expression

Honing Huang (*Appl Bio Chem, Grad Sch Agri, Kindai Univ, Japan*)

*Coriandrum sativum*, an annual herb and a member of *Apiaceae* family which is wildly used as a spice worldwide. In our previous study, the effects of *Coriandrum sativum* on cancer cells were investigated by using human hepatocellular carcinoma cell line (HepG2), human colorectal adenocarcinoma cell line (Caco2) and mouse melanoma cell line (B16-F10) *in vitro*. It has been demonstrated that the extract of *Coriandrum sativum* suppressed matrix metalloproteinase 2 (MMP-2) activity and urokinase-type plasminogen activator (u-PA) activity in the conditioned medium and inhibited migration and invasion of cancer cells. Since MMP-2 and u-PA were involved in degradation of extracellular matrix, it was thought that the suppression of MMP-2 and u-PA activities induced the inhibition of migration and invasion of cancer cells. In the present study, we investigated the effects of *Coriandrum sativum* on expressions of MMP-2 or u-PA mRNA and antigen level. Furthermore, the alteration of signal transduction in B16-F10 cells induced by *Coriandrum sativum* was studied. After lyophilization of *Coriandrum sativum* for 24-48 hours, the extract of *Coriandrum sativum* was prepared with methanol. The expression of MMP-2 or u-PA mRNA was quantified by RT-qPCR. MMP-2 or u-PA antigen in the conditioned medium and the alteration of signal transduction in B16-F10 cells were examined by western blot. Compared to control, incubation of B16-F10 cells with the extract of *Coriandrum sativum* significantly suppressed the expression of MMP-2 or u-PA mRNA and decreased MMP-2 or u-PA antigen in the conditioned medium. The extract of *Coriandrum sativum* significantly impaired phosphorylation of Erk and IKB, and translocation of NF-kB into nucleus of B16-F10 cells. Based on these findings, it was confirmed that the extract of *Coriandrum sativum* inhibited migration and invasion of cancer cells through suppression of MMP-2 and u-PA expressions. (COI:No)

## 3P-114

### Eicosapentaenoic acid suppresses Endothelial-to-Mesenchymal Transition of vascular endothelial cells induced by substances secreted by the progress of adipocyte hypertrophy

Tomomi Nakamura[1], Toshinori Yasuzawa[2, 3], Akira Mima[4], Shigeru Ueshima[1, 3, 5] (*[1]Grad Sch Agri, Kindai Univ., [2]Fac Health Sci, Kio Univ., [3]Fac Agri, Kindai Univ., [4]Dept Nephrol, Osaka Med Coll., [5]Antiaging Ctr, Kindai Univ.*)

Recently, obesity increases by the changes of lifestyle. The adipocyte hypertrophy causes obesity, and it is involved in the development of diabetes, dyslipidemia and hypertension. These diseases are thought to advance arteriosclerosis. It is reported that Endothelial-to-Mesenchymal Transition (EndMT) is involved in the development of arteriosclerosis. EndMT is the process in which endothelial cells are transformed into mesenchymal cells, and is induced by TGF-$\beta$, TNF-$\alpha$ or hyperglycemia. On the other hands, it is reported that eicosapentaenoic acid (EPA), which is the n-3 polyunsaturated fatty acids, possesses the vasoprotection ability. In this study, we investigated the effect of substances secreted by the progress of adipocyte hypertrophy on EndMT of vascular endothelial cells (VEC) and further examined the influence of EPA on it. 3T3-L1 preadipocytes were differentiated into adipocytes by changing the medium every third day, and the culture medium was collected at each timing of medium-change. The lipid in adipocyte was confirmed by Oil Red O staining and it was accumulated depending on differentiation. VEC were cultured with Dulbecco's Modified Eagle Medium (DMEM). After reaching to subconfluence, DMEM was changed to 3T3-L1 culture medium. In comparsion with control, VEC cultured with 3T3-L1 culture medium from day 8 of differentiation significantly increased ability of migration and the expression of SM22$\alpha$ (marker protein for mesenchymal cells), decreased the expression of CD31 (marker protein for endothelial cells) and up-regulated Erk/Snail signaling pathway. The addition of EPA into 3T3-L1 culture medium suppressed EndMT and up-regulation of Erk/Snail signaling pathway induced by 3T3-L1 culture medium. Based on these findings, it was suggested that substances secreted into the culture medium by the progress of adipocyte hypertrophy cause EndMT of VEC, and EPA suppresses EndMT through Erk/Snail signaling pathway. (COI:No)

## 3P-115

### Cesium ion suppresses fibroblast migration in an applied electric field

Tomohiro Kawamata, Shingo Tsuji, Daisuke Kobayashi, Akihiro Hazama (*Dept Cell Integrative Physiol, Sch Med, Fukushima Med Univ, Japan*)

Fibroblasts located in the dermis play a key role of the wound healing and the overgrowth of fibroblast causes keloid formation. Endogenous electric fields (EFs), which are generated by epithelial cells, are important to decide the fibroblast movement in the wound site. The previous experiments by our group have shown that cesium ion suppresses cell proliferation temporarily and this finding has a potential for clinical application. However, the effect of cesium ion on the fibroblast movement in an applied EF has not bee reported. In this study, we investigated the difference in fibroblast migration in the presence/absence of cesium ion in an applied EF. The movement of murine NIH/3T3 fibroblast cells was tracked by the computer system with/without 3mM CsCl under 200 mV/mm EF. We observed that cesium ion suppressed cell migration as compared to control medium. Moreover, fibroblasts acquired the migratory ability again after washout of cesium ion. Further studies are needed in order to unveil the detail mechanism under the inhibition of fibroblast migration by cesium ion. (COI:No)

## 3P-116

### Cell death induction of human cancer cells by Mastigias papua fluid components

Kuya Yamamoto[1], Kohki Ide[2], Yuki Ichi[2], Kazunao Hobo[2], Shin Kubota[3], Yuri Mukai[1] ([1]*Dept Electr Bioinform, Sch Sci Tech, Meiji Univ, Japan,* [2]*Dept Electr, Grad Sch Sci Tech, Meiji Univ, Japan,* [3]*Turritopsis Res Inst Reg Biol, Shirahama, Japan*)

Mastigias papua is a metamorphic animal that undergoes a major change in shape during its growth, including a fertilized ovum, planula, polyp, strobila, ephyra, or medusa. In the process of morphological changes, cell death and cell differentiation are occurring in the jellyfish body. Therefore, the possibility is thought that there are components that show the same effect on human cells that are eukaryote as well as jellyfish. This study aims to discover the factors that induce cell death in HeLa cells derived from human cervical cancer and to analyze their functions using the jellyfish extract as a sample.

As the result of the investigation what morphological changes occur when the jellyfish extract was added to HeLa cells, it was found that cell death occurred. In addition, as a result that the cell membrane destruction was examined by trypan blue staining by cytotoxicity test, the cell viability was decreased depending on the total protein concentration in the jellyfish body fluid. Furthermore, the morphological differences in dead cells were observed between high and low concentrations of jellyfish body fluid. Examining the relationship with cell death of HeLa cells by the three types of jellyfish body fluids, extracted from umbrella, umbrella margin and oral arm, the strong activity of inducing cell death was found especially in oral arm extract. In the future, the cell death inducer contained in the extract of the jellyfish oral arm should be identified and clarify the mechanism of cell death. (COI:No)

## 3P-117

### Acute death of tumor cells induced by quinacrine with blue light

Hiromi Hiruma (*Dept Physiol, Kitasato Univ Sch Med, Japan*)

Acridine orange, a weakly basic fluorescent dye, has been used as a photosensitizer for anti-tumor photodynamic therapy. We previously clarified that illumination of blue light on acridine orange-loaded malignant melanoma cells showed intracellular vesicle disruption resulting in cell death. This phenomenon is the underlying principle for acridine orange photodynamic therapy. The present study searched the alternative photosensitizer to acridine orange, which may lead to the extension of choice of photosensitizers. Of weakly basic dyes, only quinacrine but not daunorubicin (daunomycin, an anti-neoplastic agent) showed a similar effect to acridine orange when blue light was illuminated. Other fluorescent dyes Lucifer yellow, Congo red and ruthenium red had no effect. When quinacrine was loaded in osteosarcoma cells, quinacrine remained in intracellular vesicles. During illumination with blue light, the successive disruption of vesicles was observed as a flash of fluorescence, and shortly after that, blebs were formed on the plasma membrane. These cells were died within 5 min. Vesicle disruption and cell death were inhibited by pretreatment with the H+-ATPase inhibitor bafilomycin and by singlet oxygen scavengers, suggesting that vesicle acidification by H+-ATPase is needed for quinacrine accumulation in the vesicles and the generation of singlet oxygen causes vesicle disruption and the following cell death. Therefore, quinacrine, which has already been used for medicine for malaria and prion disease, may be a candidate of photosensitizer for anti-tumor photodynamic therapy. (COI:No)

## 3P-118

### Inflation of ex-vivo Rat Lung in Negative Pressure Chamber Induced ATP Release in Alveoli and Surrounding Blood Capillary

Kishio Furuya[1], Ju Jing Tan[2], Ryszard Grygorczyk[2], Masahiro Sokabe[1] ([1]*MechanoBio, Grad Sch Med, Nagoya Univ, Japan,* [2]*CRCHUM, Univ Montreal, Canada*)

Extracellular ATP and other nucleotides are important autocrine/paracrine mediators that regulate diverse processes critical for physiological and pathological functions in the lung, including mucociliary clearance, surfactant secretion, ventilator induced lung injury (VILI) and chronic obstructive pulmonary disease (COPD). Cellular ATP release is mostly mechanosensitive; however, the impact of physical stimuli on ATP release during breathing has never been tested in intact lungs in real time. In this study, we investigated inflation-induced ATP release in rat lungs *ex vivo* by real-time luciferin-luciferase (LL) bioluminescence imaging coupled with simultaneous infrared tissue imaging under a macro-view microscope. We also developed a negative pressure chamber, which mimics the thoracic cavity, to apply physiological inflation to the lung. With LL solution introduced into air spaces, brief inflation of the lung with –10 to –20 cm $H_2O$) induced a transient ATP release in air-inflated alveoli and it remained spatially restricted to single alveolar sacs or their clusters (70 to 260 $\mu$m). ATP release was stimulus dependent: strong inflation evoked large ATP release that terminated upon alveoli deflation while cyclic inflation produced cyclic ATP release. With LL introduced into blood vessels, inflation induced transient ATP release in blood capillary in wide area of lung surface. The response appeared diffuse but consisted of many small patch-like responses the size of alveolar sacs. Findings suggest that inflation induces ATP release in both alveoli and the surrounding blood capillary network; the functional units of ATP release presumably consist of alveolar sacs or their clusters. (COI:No)

## 3P-119

### STAT6 negatively regulates differentiation and fusion of mouse myoblasts

Mitsutoshi Kurosaka, Yuji Ogura, Kazuhisa Kohda, Toshiya Funabashi (*Department of Physiology, St. Marianna University School of Medicine*)

**Purpose:** Myoblast fusion is an essential for muscle fiber formation to accomplish skeletal muscle development for regeneration and hypertrophy. However, the cellular signaling mechanisms of the myoblast fusion are not well known. It has been suggested that Interleukin-4 (IL-4) plays a role in the process of myoblast fusion. Since IL-4 function is mediated by Signal Transducers and Activator of Transcription 6 (STAT6) in many cell types, we hypothesize that STAT6 is implicated in myoblast fusion as well. The aim of the present study was to clarify the role of STAT6 in the fusion of cultured mouse myoblasts.

**Methods:** Myoblasts were isolated from the lower limb of C57BL/6 mice, and expanded in growth medium. The cells were transfected with short hairpin RNA (shRNA) for STAT6 or scrambled as a control for knockdown of STAT6. In separate experiment, the cells were infected with either adenoviral (Ad)-STAT6 or Ad-empty as a control for overexpression of STAT6. After those treatments, the cells were cultured with differentiation medium for 48 h to induce myoblast fusion. The cells were fixed and immunocytochemically stained to examine the differentiation index (percentage of the number of nuclei in MyHC+ cells to the total number of nuclei), fusion index (percentage of nuclei inside the myotube) and myotube diameter. The cells were also processed to analyze the expression of myogenin and phosphorylation (phospho-) of p70S6K by Western blotting.

**Results:** The differentiation index, fusion index and myotube diameter were significantly increased in STAT6 knockdown cells compared with the control cells. The expression of myogenin and phospho-p70S6K in STAT6 knockdown cells were significantly higher than the control cells. Conversely, the differentiation index, fusion index, myotube diameter and phospho-p70S6K were significantly decreased in STAT6 overexpression cells compared with the control cells.

**Conclusion:** STAT6 is a negative regulator for myoblast differentiation and fusion. (COI:No)

## 3P-120

### Effects of cell wall short-chain carbohydrate on cellular signaling pathway in angiogenesis in melanoma mice model

Bhornprom Yoysungnoen[1], Suthiluk Patumraj[2], Rapepun Wititsuwannakul[3] ([1]*Division of Physiology, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rangsit Campus, Pathumthani 12120, Thailand,* [2]*Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand,* [3]*Department of Biochemistry, Prince of Songkla University, Hat-Yai, Songkla 90110, Thailand*)

Cell wall short-chain carbohydrate (CW-SCC) is a short chain polysaccharide found in the plant cell wall. CW-SCC has been showed an anti-angiogenic property; however, molecular mechanisms of CW-SCC against angiogenesis in malignant melanoma have yet not been reported. Therefore, the purpose of this study was to determine the possible mechanisms of CW-SCC against angiogenesis on VEGF signaling pathway in mice melanoma model. B16F1 melanoma cells were injected into a tail vein of C57BL male mice. One day after injection, either vehicle or CW-SCC (60 mg/kg) was orally administered for 14 consecutive days. After 2 weeks, the metastasized organs (lungs) were dissected out for further histological studies. The microvascular density (MVD) was evaluated by measuring the CD31 expression. Expressions of VEGF, VEGFR-2, p-ERK1/2 and p-Akt were detected by immunohistochemistry. The results showed that MVD in B16F1+vehicle group was markedly increased; however, it was significantly decreased by treatment with CW-SCC. The expressions of VEGF, VEGFR-2, p-ERK1/2 and p-Akt were overexpressed in B16F1+vehicle group. Interestingly, they were attenuated when mice were treated with CW-SCC. In conclusion, CW-SCC exhibited anti-angiogenic activity in the melanoma mice model. It might be mediated by downregulation of VEGF-VEGFR-2 and signaling molecules (p-ERK1/2 and p-AKT) expression. (COI:No)

## 3P-121

### Molecular mechanism of mitochondrial tRNA modification enzyme Mtu1 in reversible infantile mitochondrial disorder

Raja Norazireen Raja Ahmad[1], Fan-Yan Wei[2], Takeshi Chujo[1], Kazuhito Tomizawa[1]
([1]*Department of Molecular Physiology, Faculty of Life Sciences, Kumamoto University,* [2]*Department of Metabolism and Physiology, Institute of Development, Aging and Cancer, Tohoku University*)

Mitochondrial transfer RNA (mt-tRNA) contains a variety of chemical modifications that are introduced post-transcriptionally and essential for efficient protein translation as well as energy metabolism in mitochondria. Among 22 subtypes of tRNAs encoded in mammalian mitochondria, $tRNA^{Lys}$, $tRNA^{Gln}$, and $tRNA^{Glu}$ undergo a unique 2-thiouridine modification ($tm^5s^2U$) at its "wobble position" (U34) of the anticodon. The TRMU nuclear gene-encoded enzyme, mitochondrial tRNA-specific 2-thiouridylase 1 (Mtu1) mediated this modification allowing precise cognate codon recognition and ensuring accurate protein translation in mitochondria. It is known that the loss of 2-thiolation modification are associated with the development of reversible infantile liver failure (RILF) disease. As a number of pathogenic mutations were reported to be found in RILF patients with varying degrees of fatality and reversibility, we sought to elucidate the mechanism of Mtu1 role in reversible infantile mitochondria disorder. We first generated Mtu1 KO cells and found that it abolished the 2-thiouridine formation in mt-tRNAs. Loss of Mtu1 enzyme expression also impaired mitochondrial protein translation and complex I and IV of OXPHOS protein expression. Analysis on Mtu1 KO cells with expression of its equivalent pathogenic mutations reveals varying degrees of 2-thiolation modification recovery and expression of OXPHOS complex proteins. It was found that the regulation of Mtu1 enzyme function varies with each pathogenic mutation with some to be more critical to mitochondria function than others. These observations may provide a clue to the reversibility of mitochondrial disease in some patients. (COI:No)

## 3P-122

### Molecular basis of extracellular transport of chemically modified nucleosides in human cells

Sheng-Lan Shi[1], Fan-Yan Wei[2], Takeshi Chujo[1], Kazuhito Tomizawa[1] ([1]*Department of Molecular Physiology, Faculty of Life Sciences, Kumamoto University,* [2]*Department of Metabolism and Physiology, Institute of Development, Aging and Cancer, Tohoku University*)

RNA contains a wide variety of chemical modifications that are critical for maintaining fundamental RNA functions such as structural integrity, intracellular localization, and decoding efficiency. Deficits in RNA modification have been implicated in diverse diseases including type 2 diabetes, myopathy, and live failure. These results demonstrate that RNA modifications are indispensable for homeostatic regulation of human physiology as well as pathogenesis of various diseases.
Interestingly, unlike reversible chemical modifications in protein and DNA, most of RNA modifications are irreversible due to the complexity of RNA modifications and the lack of "de-modifying" genes in genome. Therefore, even RNA is metabolized to single nucleoside, these chemical modifications are stably attached to the nucleoside. The unique property of RNA modifications thus leads to a fundamental question: what is the ultimate fate of these chemically modified nucleosides? Our study suggested modified nucleosides are actively transported to extracellular space and transport of modified nucleosides are mediated by equilibrative nucleoside transporters (ENTs). (COI:No)

## 3P-123

### Exploration of the role of glutamine metabolism in pro-inflammatory reaction of microglia

Teruaki Yamaguchi, Hajime Yano, Junya Tanaka (*Department of Molecular and Cellular Physiology, Ehime University Medical School*)

Excessive pro-inflammatory reaction of microglia exacerbates neurodegenerative diseases including Parkinson's disease. Therefore, controlling the excessive pro-inflammatory reaction can be a new therapeutic target for suppressing the exacerbation of those disease. We found that glutamine metabolism has the potential to play fundamental role in microglial pro-inflammatory reaction. We employed mouse microglia cell line BV2 for *in vitro* experiments. BV2 exposed to a pro-inflammatory stimulus, Lipopolysaccharide (LPS), showed pro-inflammatory reaction including the synthesis of nitric oxide and the increase in mRNA expression of pro-inflammatory cytokines. However, BV2 exposed to LPS with medium containing no glutamine exhibited prominently attenuated pro-inflammatory reaction. In general, the intracellularly incorporated glutamine is primarily converted to glutamic acid by glutaminase (GLS1), and is further supplied to various metabolic pathways. We observed the temporal upregulation of intracellular glutamic acid concentration accompanies to LPS stimuli in the presence of glutamine, while almost no change with low level in the concentration in the absence of glutamine. Moreover, GLS1 inhibitor can suppress the pro-inflammatory reaction of BV2 induced by LPS. In addition, we obtained the result suggest that glutathione, a metabolite of glutamine metabolism, can be involved in pro-inflammatory reaction of BV2. We would like to discuss the role of glutamine metabolism in pro-inflammatory reaction of microglia. (COI:No)

## 3P-124

### Prenatal exposure to bisphenol A induces over-adaptation to predator odor stress in rats

Tetsuya Fujimoto (*Dept Physiol, Osaka Dent Univ, Japan*)

Bisphenol A (BPA) is one of the major environmental endocrine disruptors. We have been studying the effects of predator odor stress on behaviors. This time we examined whether it can be applied to evaluate the effects of BPA. In this study, behavioral tests were conducted at 20 days of age in offspring after fetal exposure to BPA (1.5 mg/kg/day). Spontaneous behaviors (rearing, ambulation, grooming, and freezing) in the straight alley type field (6×44cm, 15cm wall height) during 3 minutes were examined. Next, the same behavioral test was performed in the presence of a predator odor and compared to the non-odor session. There was no significant difference in any parameters between the BPA and control groups in the non-odor. When there was a predator odor, the data were fluctuated compared to the non-odor session, but there was a difference in the fluctuation manner between the BPA and control groups. There is no difference in the fluctuation manner of the rearing and the ambulation in both groups, in which, the scores were decreased by the odor. However, in freezing, the score was increased due to odor in the control group, while there was no change in the BPA group. In grooming, there was no change in the control group but it was increased in the BPA group. In addition, blood stress hormone levels were measured in both no-odor and odor sessions and it was increased due to odor in both the BPA and control groups. In both groups, the hypothalamus-pituitary-adrenal system was activated by odor, but inhibition of immobility and increased grooming were observed in the BPA group. These results suggest that BPA induces a kind of the "over-adaptation" to odor stress. (COI:No)

## 3P-125

### Development of core temperature estimation system using patch-type heat-flux sensors on the chest

Ken Tokizawa[1], Hirofumi Tsuchimoto[2], Toru Shimuta[2] ([1]*Natl Inst Occup Safety Health, Japan,* [2]*Murata Manuf Co, Japan*)

Industrial workers are at risk from heat illness whenever they work for a prolonged time in hot and humid conditions. Wearable technology is now being adopted, but a system that accurately measures core temperature using wearable devices has yet to be reported. We propose a new model based on a dual-heat-flux method that predicts core temperature using data from patch-type sensors on the chest.
We performed experiments that compared our predicted temperatures (Tpre, using a revised algorithm from the dual-heat-flux method), with the actual temperatures in both esophageal (Teso) and rectal (Trec) sites during exercise in three heat conditions. Thirty-two volunteers walked for 60 min at 4–5 km/h at 30℃, 35℃, or 40℃.
In the 40℃ condition, Teso, Trec, and Tpre increased from 37.2 ± 0.2℃, 36.9 ± 0.2℃, and 37.3 ± 0.2℃ to 38.2 ± 0.3℃, 37.9 ± 0.3℃, and 38.0 ± 0.2℃ (mean ± standard deviation), respectively, during exercise. The difference between Tpre and Teso was –0.10 ± 0.15℃ and that between Tpre and Trec was 0.02 ± 0.19℃, using data sampled at 5-min intervals during exercise. In the 35℃ condition, the difference between Tpre and Teso was –0.06 ± 0.17℃ and that between Tpre and Trec was 0.04 ± 0.14℃. In the 30℃ condition, the differences were –0.13 ± 0.24℃ (Tpre–Teso) and 0.06 ± 0.25℃ (Tpre–Trec). Body mass, fat percentage, and sex did not affect the Tpre algorithm, but skin temperature changes during exercise yielded errors.
The error ranges for our system are slightly superior to those in previous studies involving noninvasive core temperature measurements. Our system uses simple wearable devices and can provide real-time, subject-specific, and accurate body core temperature estimates under heat stress conditions. (COI:No)

## 3P-126

### Effect of plastic nanoparticles exposure on cardiovascular regulation: focus on the inflammatory condition in NTS

Linh Pham T.[1, 2], Ko Yamanaka[5], Yasunori Miyamoto[1, 3, 4], Hidefumi Waki[5], Sabine Gouraud[2, 3] ([1]*Grad Sch Humanities and Sciences, Ochanomizu Univ, Tokyo, Japan,* [2]*Program for Leading Graduate School, Ochanomizu University, Otsuka, Bunkyo-ku, Tokyo, Japan,* [3]*Department of Biology, Ochanomizu University, Otsuka, Bunkyo-ku, Tokyo, Japan,* [4]*Institute for Human Life Innovation, Ochanomizu University, Otsuka, Bunkyo-ku, Tokyo, Japan,* [5]*Department of Physiology, Graduate School of Health and Sports Science, Juntendo University, Inzai-city, Chiba, Japan*)

A growing body of evidence shows that plastic nanoparticles (NPs) are omnipresent in the environment and food chain. According to recent studies, NPs can cross biological membranes and blood-brain barrier, alter the gene expression of inflammatory molecules in cells, and trigger neurotoxic effects. How ever, the impact of NPs on mammalian health is not well studied. We recently identified an association between the abnormal expression of pro-inflammatory molecules in the nucleus of solitary tract (NTS), a central site for autonomic cardiovascular regulation, and the blood pressure (BP) levels. In this study, we investigated the effect of NPs on inflammatory condition in the NTS and cardiovascular parameters. 50nm diameter polystyrene nanoparticles (PSNPs) diluted in water (1ng/nl) or vehicle were microinjected into the NTS of male Wistar rats (100nl bilaterally, n=5). The NTS tissues were collected 48 hours after the injection for total RNA extraction and quantitative RT-PCR experiments. In parallel, other sets of male Wistar rats (4 weeks-old, n=6 per group) were exposed to PSNPs diluted in water (1mg/100g body) or vehicle by oral gavage 3 times per week for one month. Weight, BP and heart rate were regularly measured. Interestingly, the transcripts for inflammatory molecules Chemokine (C-C motif) ligand 5 (CCL5)and Tumor Necrosis Factor $\alpha$ (TNF $\alpha$) were found significantly increased in the NTS of rats treated with PSNPs compared to control rats treated with vehicle. Moreover, the BP of rats exposed to PSNPs was found significantly lower than the one of control rats. Our results show that orally ingested PSNPs might affect cardiovascular centers involved in BP regulation. (COI:No)

## 3P-127

### Effects of metabolic cage housing on oxytocin expression in rats

Hirofumi Hashimoto, Yoshiteru Seo (*Dep. Regul. Physiol, Dokkyo Med. Univ.*)

We usually used metabolic cage to house laboratory rats in some behavior studies. Metabolic cages usually have grid flooring. Some previous studies showed that rats have some stress in housing metabolic cages with grid flooring, as a result, their food intake and body weight gain was decrease. We examined the effects of metabolic cage housing on central oxytocin (OXT) on food intake in rats, on fluorescence intensity of OXT-monomeric red fluorescent protein 1 (mRFP1) in OXT- mRFP1 transgenic rats. The food intake had decreased for 4 days and recovered until 7 days after housing metabolic cages. The mRFP1 fluorescence intensity was significantly decreased in the supraoptic nucleus, the paraventricular nucleus, and posterior pituitary after 4 days after housing metabolic cages. These results suggested that central OXT may be involved in anorexia induced by stress in metabolic cages in rats. (COI:No)

## 3P-128

### Relationship between minimum heart rates and body temperature during 2-min resting period of intermittent exercise

Issei Kato[1], Yuta Masuda[1], Shuri Marui[2], Kei Nagashima[2] ([1]*Grad Sch Human Sciences, Waseda Univ., Japan,* [2]*Faculty of Human Sciences, Waseda Univ., Japan*)

**Aim:** Assessment of thermal load during exercise is important in preventing hyperthermic injury. However, the methods have not been well established. The aim of the present study was to test the hypotheses that heart rates(HR) during resting periods of intermittent exercise reflect thermal condition of body in any environmental conditions.
**Methods:** Young healthy male participants(n=12; age, 23.7 ± 2.4; bw, 64.34 ± 8.86 kg) had two trials in hot and humid, and the control environments(HH and CON trials, respectively; HH, ambient temperature(Ta) of 35℃ with 65% relative humidity(Rh); and CON, Ta of 25℃ with 30% Rh). Each participant conducted 5-set 6~8-min graded treadmill exercise(4~10 km/h; 3° slope) with 2-min rest in between. Rectal temperature ($T_{rec}$), skin temperature at 4 sites(chest, upper arm, thigh, and lower leg; $T_{chest}$, $T_{arm}$, $T_{thigh}$ and $T_{leg}$ respectively) and HR were continuously measured. Mean body temperature($T_b$) was calculated as $0.7*T_{rec}+0.3*(0.3*(T_{chest}+T_{thigh})+0.2*(T_{arm}+T_{leg}))$. The minimum value of HR during each resting period was evaluated($HR_{min}$).
**Results:** Both HR and $T_b$ were greater in the HH trial than in the CON trial. $HR_{min}$ was correlated with $T_b$ at the time point in both HH and CON trials($r=0.83$ and $P<0.001$; and $r=0.79$ and $P<0.001$, respectively). Regression equations for the $HR_{min}$ and $T_b$ was similar in both HH and CON trials.
**Conclusion:** In the present study, $HR_{min}$ reflects $T_b$. Moreover, the relationship between the two values was similar in both HH and CON trials. The present study may suggest that, during intermittent exercise, $HR_{min}$ are determined only by the extent of thermal strain, which often differs among the thermoregulatory capacity of individuals. (COI:No)

## 3P-129

### Koreans do not have higher percent body fat than Australians for a given body mass index: a population-based comparison

Duong Duc Pham[1], Seung Ku Lee[2], Chol Shin[2,3], Nan Hee Kim[4], John A. Eisman[5], Jacqueline R. Center[5], Tuan V. Nguyen[5,6,7,8], Chae Hun Leem[1] ([1]*Department of Physiology, Ulsan College of Medicine,* [2]*Institute of Human Genomic Study, Korea University Ansan Hospital ,* [3]*Division of Pulmonary, Sleep, and Critical Care Medicine, Department of Internal Medicine, Korea University,* [4]*Division of Endocrinology, Sleep, Korea University Ansan Hospital,* [5]*Bone and Muscle Research Group & Faculty of Applied Sciences, Ton Duc Thang University, Vietnam,* [6]*University of Technology Sydney (UTS), Sydney, Australia,* [7]*Bone Biology Division, Garvan Institute of Medical Research, Sydney, Australia,* [8]*St Vincent Clinical School, UNSW Australia, Sydney, Australia*)

Many argue that body mass index (BMI) cutoff for obesity should be lower in Asians due to evidence of a higher percent body fat (PBF) at a given BMI compared to the whites. We compared the body composition between 1211 Korean and 1006 Australian men and women aged 60 and higher enrolled in two population-based cohort studies. A whole-body scan using a dual-energy X-ray absorptiometry (LUNAR Prodigy) was used to estimate lean mass, fat mass, and PBF in both studies. Multivariate regression adjusted for age and BMI showed that PBF was equal and lower in Koreans versus Australian in men and women, respectively (mean difference and 95% CI: -0.54 (-1.22 to 0.14) and -2.13 (-2.61 to -1.65) for men and women). Propensity score pair-matching analysis for age and BMI (208 pairs and 423 pairs in men and women, respectively) revealed that Koreans had a compatible and around 2% lower PBF than Australians, in men and women, respectively. No age and BMI interaction effects were found. These findings do not support the need for lowering the threshold of BMI-based obesity definition in Asians, at least for the Korean population. (COI:No)

## 3P-130

### Quantitative electrophysiological monitoring of anti-allergic effects of antihistamines in rat peritoneal mast cells

Itsuro Kazama (*Nursing, Miyagi Univ, Japan*)

Several food constituents, such as catechin in green tea, polyphenol in red wine, nobiletin in orange peel and lactobacilli in yogurt, are known to exert anti-allergic effects. However, these effects have not been examined quantitatively and their precise mechanisms are unknown. Among second-generation antihistamine drugs, olopatadine exerts relatively stronger anti-allergic effects. In addition to antagonizing histamine H1 receptors, olopatadine exerts mast cell stabilizing effects. Exocytotic process in mast cells can be detected electrophysiologically by the changes in membrane capacitance (Cm). Therefore, to quantitatively determine the mast cell stabilizing properties by olopatadine, we examined its effects on the exocytotic process of mast cells. Applying the whole-cell patch-clamp technique in rat peritoneal mast cells, we monitored the drug-induced changes in Cm during exocytosis. By the confocal imaging of the lucifer yellow dye, we also examined the effects of olopatadine on the plasma membrane deformation. Relatively higher concentrations of this drug (100 $\mu$M or 1 mM) almost completely suppressed the GTP- $\gamma$ -S-induced increase in the Cm. In addition, the drug totally washed off the dye trapped within the cell surface. Electron microscopy revealed that olopatadine generated inward bending of the mast cell membrane, which counteracted the plasma membrane deformation in degranulating mast cells. The results provided electrophysiological evidence that anti-allergic drugs, such as olopatadine, exerted mast cell stabilizing properties. This approach would be useful to quantitatively determine the anti-allergic properties of certain drugs or food constituents. (COI:No)

## 3P-131

### Development of new AMPA receptor positive allosteric modulator using depression model rat

Waki Nakajima, Megumi Hara, Mai Hatano, Tomoyuki Miyazaki, Tetsu Arisawa, Susumu Jitsuki, Takuya Takahashi (*Dept Physiol, Sch Med, Yokohama Univ, Japan*)

AMPA receptor is one of the most important molecules controlling the neuronal activities. Dysfunction of AMPA receptors is believed to underlie some of psychiatric disorders. Recent studies have clarified that AMPA receptors expression is decreased in post-mortem brain of patients with major depressive disorder (Gibbons et al., 2012, Duric et al., 2013). Positive allosteric modulators (PAMs) enhancing AMPA receptors function have been developed toward patients with depression but the effects of PAMs on depression seem to be limited. Here we show that we success in developing a new type of PAMs targeting AMPA receptors improving the depressive phenotype of rodent depression model.
Firstly using LC-MS/MS screening method, we identified the compound, K-1, showing high transitional potency through Blood-Brain Barrier (BBB). Secondly, we synthesized another compound, K-2, showing much higher transitional potency compared to K-1. Thirdly, we also found that K-2 is metabolized immediately in vivo. To improve this character, we newly-synthesized another compound, K-4, hydrolysis-resistant form of K-2. Electrophysiological experiments revealed that these thress compounds increased AMPA receptors current in the CA3-CA1 synapses.
Previously we found the expression of AMPA receptors deceased in rodent depression model, Wistar Kyoto (WKY) rat. To elucidate whether these compounds are effective on improving depressive phenotype of WKY rat, we performed forced swim test (FST) to examine anti-depressive effect of these compounds. Interestingly, K-2 and K-4 reduced immobility time in FST compared to vehicle and K-1. Surprisingly K-4 reduced immobility time in FST compared to K-2.
Taken together, K-4 may be the promising compound as a new type of anti-depressant drugs. (COI:No)

## 3P-132

### The combined efficacy of OTS964 and temozolomide for reducing the size of power-law coded heterogeneous glioma stem cell populations

Michiya Sugimori (*Dept Integrative Neurosci, Grad Sch Med Pharm, Univ Toyama, Japan*)

Glioblastoma resists chemotherapy then recurs as a fatal space-occupying lesion. To improve the prognosis, the issues of chemoresistance and tumor size should be addressed. Glioma stem cell (GSC) populations, a heterogeneous power-law coded population in glioblastoma, are believed to be responsible for the recurrence and progressive expansion of tumors. Thus, we propose a therapeutic strategy of reducing the initial size and controlling the regrowth of GSC populations which directly facilitates initial and long-term control of glioblastoma recurrence. In this study, we administered an anti-glioma/GSC drug temozolomide (TMZ) and OTS964, an inhibitor for T-Lak cell originated protein kinase, in combination (T&O), investigating whether together they efficiently and substantially shrink the initial size of power- law coded GSC populations and slow the long-term re-growth of drug-resistant GSC populations. We employed a detailed quantitative approach using clonal glioma sphere (GS) cultures, measuring sphere survivability and changes to growth during the self- renewal. T&O eliminated self-renewing GS clones and suppressed their growth. We also addressed whether T&O reduced the size of self-renewed GS populations. T&O quickly reduced the size of GS populations via efficient elimination of GS clones. The growth of the surviving T&O-resistant GS populations was continuously disturbed, leading to substantial long-term shrinkage of the self-renewed GS populations. Thus, T&O reduced the initial size of GS populations and suppressed their later regrowth. A combination therapy of TMZ and OTS964 would represent a novel therapeutic paradigm with the potential for long-term control of glioblastoma recurrence via immediate and sustained shrinkage of power-law coded heterogeneous GSC populations. (COI:No)

## 3P-133

### Simultaneous imaging analysis of insulin-responsive liberation and heterotypic fusion in GLUT4 trafficking

Hiroyasu Hatakeyama[1,2,3], Makoto Kanzaki[3] (*[1]Dept Physiol, Kitasato Univ Sch Med, Japan, [2]FRIS, Tohoku Univ, Japan, [3]Grad Sch Biomed Eng, Tohoku Univ, Japan*)

We previously provided critical quantitative information regarding intracellular trafficking of membrane cargo proteins including the insulin-responsive glucose transporter GLUT4 and the transferrin receptor (TfR). For example, statistic comparison of GLUT4 and TfR behaviors with our intracellular trafficking nanometry based on single molecule imaging of quantum dot (QD) fluorescent nanocrystals clearly documented the unique regulatory system for GLUT4. Specifically, we showed insulin-responsive liberation from its static states. We also identified acute heterotypic endomembrane fusion of very small static GLUT4-containing vesicles with a subset of TfR-containing endosomes as being an initial process in the insulin-responsive GLUT4 translocation by employing conventional cell-biological technique. These two distinct principle-based imaging techniques, focused on elucidating the same biological phenomena, raise a simple question regarding the relationship between GLUT4 liberation and heterotypic endomembrane fusion. To directly answer this question, we here performed simultaneous imaging analysis of these two phenomena within a cell. We first labeled myc-GLUT4-mCherry with the QD nanocrystals and the fusion sensor simultaneously, and the labeled molecules were then allowed to reycle back to their stationary compartments, followed by TfR labeling. The broad absorption spectrum and the large Stokes shift of QD allowed us to fully visualize the two distinct fluorescent molecules simultaneously. We observed obvious increases in both fusion sensor intensity and QD movement after several minutes of insulin stimulation and found that the onset of the increases was much more rapid for the fusion sensor than for QD movement. This indicates that heterotypic endomembrane fusion of GLUT4-vesicles with TfR-containing endosomes precedes GLUT4 liberation from its static status in response to insulin. (COI:No)

## 3P-134

### Short-chain fatty acid-evoked transepithelial ion transport in the mice intestine

Shin-Ichiro Karaki[1], Kota Tsukamoto[1], Ikuo Kimura[2] (*[1]Lab Physiol, Dept Env Life Sci, Univ Shizuoka, Japan, [2]Dept Appl Biol Sci, Grad Sch Agri, Tokyo Univ Agri Tech, Japan*)

Short-chain fatty acids (SCFAs) produced by intestinal bacterial fermentation are known not only to be absorbed as nutrients, but also to stimulate large intestinal mucosa inducing fluid secretion and smooth muscle contraction. The effects of SCFAs as stimulants had previously been investigated by utilizing rat distal colon, but there had no direct evidence of the identification of SCFA receptor involving the SCFA-induced fluid secretion. Therefore, in the present study, we attempted to investigate the SCFA-evoked epithelial ion transport in wild type (WT)-, FFA2 (GPR43)-KO and FFA3 (GPR41)-KO mice. In WT mice, mucosa-submucosa tissue preparations of duodenum, jejunum, terminal ileum, cecum, proximal colon, middle colon, distal colon and rectum were mounted on Ussing chambers, and short-circuit current ($I_{sc}$) were measured as an index of net electrogenic transepithelial ion transport. The mucosal addition of propionate ($10^{-3}$ M) to each segment of the intestine evoked a phasic increase in $I_{sc}$, and the effect was evoked the most potent in the cecal preparations. Therefore, we investigated the effect of SCFAs on cecal preparations in detail. As a result, mucosal addition of propionate ($>10^{-5}$ M) concentration-dependently evoked a phasic increase in $I_{sc}$, and achieved the maximum at $3\times10^{-3}$ M, but acetate and butyrate little evoked the response even at $3\times10^{-3}$ M. In addition, the mucosal propionate-evoked response was attenuated by serosal addition of atropine ($10^{-5}$ M), but not by the serosal addition of a neural blockade, tetrodotoxin ($10^{-6}$ M). In FFA2-KO mice cecum, propionate ($10^{-3}$ M) evoked the same response as WT mice, but in FFA3-KO mice, propionate ($10^{-3}$ M) little evoked the response. These results suggest that the propionate-evoked ion transport in the mice cecum are mediated via activation of FFA3 receptors in the apical site of the epithelium and released acetylcholine into the basolateral site. (COI:No)

## 3P-135

### Short-chain fatty acid-evoked transepithelial ion transport in the mice terminal ileum

Kota Tsukamoto[1], Ikuo Kimura[2], Shin-Ichiro Karaki[1] (*[1]Lab Physiol, Dept Env Life Sci, Univ Shizuoka, Japan, [2]Dept appl Biol Sci, Grad Sch Agri, Tokyo Univ Agri Tech, Japan*)

Short-chain fatty acids (SCFAs) defined as 2-6 carbon fatty acids, mainly acetate, propionate, and butyrate, are produced from dietary fiber by bacterial fermentation in the intestine. They not only are absorbed as nutrient, but also stimulate intestinal mucosa inducing a variety of physiological responses. The reflux of SCFAs from cecum to terminal ileum passing through the ileocecal valve may induce a fluid secretion in the terminal ileum, but the SCFA-induced ion transport in small intestine is not fully understood. We therefore investigated the acetate- and propionate-induced ion transport in the mice terminal ileum by the Ussing chamber technique. Transepithelial potential difference was clamped 0mV, and short-circuit current ($I_{sc}$) were continuously measured as an index of net electrogenic transepithelial ion transport. In the mice terminal ileum, mucosal treatment of acetate and propionate concentration-dependently evoked phasic (achieved a peak within 1 min) and broad (achieved a peak within 2 – 10 min) increases in $I_{sc}$. These effects were insensitive for tetrodotoxin, atropine and piroxicam. In the other presentation by us, we showed that the propionate-evoked increase in $I_{sc}$ was completely abolished in the SCFA receptor FFA3 (GPR41)-KO mice cecum, but present study showed that, even in the FFA3-KO mice terminal ileum, propionate and acetate evoked the same increases in $I_{sc}$. Whereas in FFA2 (GPR43)-KO mice, the propionate-evoked phasic increase in $I_{sc}$ was significantly reduced and the acetate-evoked phasic increase in $I_{sc}$ was completely abolished. These results suggest that the effect of SCFAs in the mice terminal ileum was different from colon and further investigation of that should be performed. (COI:No)

## 3P-136

### Tricellular tight junction protein angulin-1 regulates bicellular tight junction permeability and transcellular nutrient absorption mechanisms

Naotaka Ikumi (*Lab of Physiol, Grad Sch of Intg Pharma and Nutri and Sci, Univ of Shizuoka, Shizuoka, Japan*)

The primary function of intestinal epithelia is selective nutrient absorption and concomitantly, blocking noxious substances from entering the internal compartment of living organisms. The intestinal barrier is formed by a polarized monolayer of epithelial cells, which are connected by intercellular junctions. The paracellular barrier is the tight junction (TJ), a complex located in the apicolateral membrane of epithelial cells, which consists of a number of tetraspan TJ proteins, namely from the claudin family, and interconnects the apicolateral membranes of neighboring cells like zippers. In addition to this bicellular TJ structure (bTJ), a tricellular TJ (tTJ) is formed at the meeting point of three cells where three bTJ strands converge. It extends far more basolaterally than the bTJ, forming a vertically orientated triple pair strands structure with a "central tube". This central tube is thought to be a weak point of the paracellular barrier. Angulin-1 is a member of the angulin protein family, which is exclusively expressed at tTJ. The first hint that angulin-1 is involved in barrier function came when, in angulin-1 knockdown studies, a break-down of the barrier occurred. However, whether angulin-1 is actually involved in the intestinal barrier has not been studied. To this end, we investigated the impact of deficiency of angulin-1 on the function of the small intestine by using intestinal-specific angulin-1 deficient (angulin-1 cKO) mice. We measured unidirectional $^{22}Na^+$ and $^3$H-Mannitol fluxes in isolated small intestine by using Ussing chambers. There was no discernable change in $^3$H-Mannitol fluxes. However, transcellular and paracellular $^{22}Na^+$ unidirectional flux were decreased in angulin-1 cKO mice. $Na^+$-dependent nutrient absorption activity was also decrease in angulin-1 cKO mice. Together, these results suggest that the tTJ protein angulin-1 may regulate bTJ ion permeability and transcellular nutrient absorption mechanisms. (COI:No)

## 3P-137

### Role of the right frontal orienting field in visuospatial attention

Daisuke Ishii[1,2], Hironobu Osaki[3], Arito Yozu[1], Kiyoshige Ishibashi[4], Kenta Kawamura[5], Mariko Miyata[3], Yutaka Kohno[1] (*[1]Center for Medical Sciences, IPUHS, Japan, [2]Dept Cogni Behav Physiol, Grad Sch Med, Chiba Univ, Japan, [3]Dept Physiol (Neurophysiol), Sch Med, Tokyo Women's Medical Univ, Japan, [4]Dept Physical Therapy, IPUHS Hospital, Japan, [5]Dept Physical Therapy, IPUHS, Japan*)

Hemispatial neglect is a disorder of higher brain function that occurs after stroke and leads to decreased attention and response to the opposite side of the damaged hemisphere. Because patients with this condition often have motor paralysis and other symptoms owing to the presence of several brain lesions, it is difficult to evaluate the recovery mechanism and training effect of unilateral spatial neglect. Therefore, an effective training approach has not yet been established. In this study, we created a mouse model of unilateral spatial neglect for the development of effective therapies under controlled conditions. Mice (C57/BL6J, 9 weeks old) were intraperitoneally administered rose bengal. The right frontal orienting field (FOF) was irradiated with an LED to produce a focal cerebral infarction. After creating the cerebral infarction, unilateral spatial neglect was evaluated for six consecutive days. Mice in the control group were intraperitoneally administered rose bengal after LED irradiation. The major findings were as follows: (1) Control group did not show unilateral spatial neglect; (2) Mice with cerebral infarction showed symptoms similar to those of unilateral spatial neglect; and (3) The symptoms did not recover 6 days after cerebral infarction. These findings suggest that cerebral infraction of the FOF induces symptoms similar to those of unilateral spatial neglect. (COI:No)

## 3P-138

### Illumination of abnormal neural activities caused by myelin impairment suggests possible contribution to learning deficits

Daisuke Kato[1], Hiroaki Wake[1], Philip Lee[2], Yoshihisa Tachibana[3], Riho Ono[3], Shouta Sugio[3], Yukio Tsuji[3], Yasuyo Tanaka[4], Yasuhiro Tanaka[4], Yoshito Masamizu[4], Riichiro Hira[8], Andrew Moorhouse[9], Nobuaki Tamamaki[6], Kazuhiro Ikenaka[7], Noriyuki Matsukawa[5], Douglas Fields[2], Junichi Nabekura[7], Masanori Matsuzaki[4] (*[1]Department of Anatomy and Molecular Cell Biology, Nagoya University Graduate School of Medicine, [2]National Institutes of Health, [3]Kobe University Graduate School of Medicine, [4]The University of Tokyo, [5]Nagoya City University, [6]Kumamoto University, [7]National Institute for Physiological Sciences, [8]National Institute for Basic Biology, [9]The University of New South Wales*)

Myelin is sheath that forms around axons, regulating the speed of electrical impulses and efficiently transmitting them among the neurons. Myelinated bundles act as cables to connect distant brain regions. Impaired myelin regulation or impairment of myelin itself is frequently associated with deficits in learning and cognition in neurological and psychiatric disorders. However, it has not been revealed what perturbation of neural activity induced by myelin impairment causes learning deficits. Here, we measured neural activity in the motor cortex during motor learning in transgenic mice with a subtle impairment of their myelin. This deficit in myelin impaired motor learning, and was accompanied by a decrease in the amplitude of movement-related activity and an increase in the frequency of spontaneous activity. Thalamocortical axons showed variability in axonal conduction with a large spread in the timing of postsynaptic cortical responses. Repetitive pairing of forelimb movements with optogenetic stimulation of thalamocortical axon terminals restored motor learning. Thus, myelin regulation helps to maintain the synchrony of cortical spike-time arrivals through long-range axons, facilitating the propagation of the information required for learning. Our results revealed the pathological neuronal circuit activity with impaired myelin and suggest the possibility that pairing of non-invasive brain stimulation with relevant behaviors may ameliorate cognitive and behavioral abnormalities in diseases with impaired myelination. (COI:No)

## 3P-139

### VEP in response to simple diagrams by EEG

Yoshiaki Arai (*Dept Eiect & Com, Nagano National Col, Japan*)

Our first visual brain cortex consist of column architecture and reflect correct image which suited the retina. And at the study of using fMRI, it is proved the original image is reconstructed by the activity of retina. But at the study of useing electroencephalography(EEG), that is impossible now. If it would be possible, application would spread.
In this experiment, the purpose was to think the relations between an shape of visual evoked potential(VEP) and original simple image which retina was stimulated by. 13 parsons of voluntary healthy aduits of 20 years old level cooperated.
9 subjects data that measured comparatively at low noise were analyzed. Each subjects watched simple images a circle, a triangle, a square, a cross, a star shape and an nothing for stimulation. The obtained data was calculated 20 times of addition average for the response form of VEP.
As a results of that the response of VEP stimulated by any images were bigger amplitude than VEP stimulated by nothing image. Furthermore, differences were seen at form and size of the electric potential and might be correspondence of the image with the original image by checking correration together. (COI:No)

## 3P-140

### Parahippocampus volume changes provide an early indication of declining of olfactory ability and cognitive function in elderly subjects

Yuri Masaoka[1], Satomi Kubota[2], Haruko Sugiyama[3], Masaki Yoshida[4], Akira Yoshikawa[1], Nobuyoshi Koiwa[5], Motoyasu Honma[1], Ryuta Kinnno[2], Natsuko Iizuka[1,2], Masahiro Ida[6], Kenjiro Ono[2], Masahiko Izumizaki[1] (*[1]Dept Physiol, Showa Univ School of Med, Japan, [2]Dept of Neurol, Showa Univ School of Med, Japan, [3]Sensory Sci Res, Kao Corporation, [4]Dept of Ophthalmol, Jikei Univ, Japan, [5]Dept of Health Sci, Univ Human Art and Sci, [6]National Hospital Organization Mito Medical Center*)

The aim of this study was to investigate the relationship between olfactory recognition and morphological changes in olfactory brain regions including the amygdala, hippocampus, rectus, parahippocampus, orbitofrontal cortex and medial frontal cortex in 27 elderly subjects. The specific aim of the study was to determine which brain areas are associated with the initial decline of olfaction in elderly subjects, which occurs before the onset of dementia. All subjects underwent magnetic resonance imaging to measure anatomical brain volume, and subjects were assessed using tests of olfactory test and Montreal Cognitive Assessment (MoCA). The main finding of this study was that the decrease of olfactory ability was associated with small left parahippocampal. In addition, the group of subjects with lower left parahippocampal volume showed lower cognitive scores. The parahippocampus has an important role in context processing from the hippocampus and amygdala, relaying memory retrieval and emotional reaction to the conscious awareness of the context organized in the orbitofrontal cortex. It is possible that volume changes in the parahippocampus due to pathological changes contribute to the decline of olfactory recognition. (COI:No)

## 3P-141

### The effects of acute isometric handgrip exercise on cognitive function

Takuro Washio[1], Kazuya Suzuki[1], Shotaro Saito[1], Kazuki Tamiya[1], Hironori Watanabe[1], Soichi Ando[2], Shigehiko Ogoh[1] (*[1]Toyo University, [2]The University of Electro-Communications*)

There is strong epidemiology evidence for a positive association between hypertension or age-related decline in cerebral blood flow (CBF) and cognitive impairment. However, the relationship between exercise mediated change in hemodynamics and cognitive function remains unclear. A recent study reported that isometric handgrip exercise (IHG) training reduces resting arterial blood pressure (ABP) and improves cognitive function in treated hypertensive patients, indicating that IHG-induced reduction in resting ABP may be associated with improvement in cognitive function. We hypothesized that an acute bout of IHG improves cognitive function, and this improvement is associated with CBF and ABP responses. Fourteen healthy subjects performed a cognitive task (go/no go task) before and immediately after exercise protocol, which consisted of four sets of 2-min unilateral IHG at 25% of maximum voluntary contraction. Mean arterial blood pressure (MAP) and middle cerebral artery blood velocity (MCAv) were measured continuously throughout the experiment. MAP and MCAv significantly increased during IHG ($P<0.05$) and returned to the resting baseline level after the exercise protocol. The exercise protocol did not change the number of error trials in the go/no-go task but decreased its reaction time (RT) ($P = 0.02$), indicating that an acute bout of IHG improved cognitive function. Interestingly, a difference in RT between before and after exercise protocol was correlated with change in MAP during IHG ($r = 0.59$, $P = 0.03$), but not that of MCAv and recovery of the hemodynamic response. These results indicate that the recovery of the hemodynamic response is not involved in enhanced cognitive function. In contrast, since abnormal exercise pressor response affects cognitive function after IHG, ABP monitoring during IHG may be important to ensure that blood pressure does not become excessively elevated and to effectively improve cognitive function as well. (COI:No)

## 3P-142

### Anterior cingulate cortex regulates the expression of observational fear

Kensaku Nomoto[1], Madoka Nakamura[1], Hanako Murayama[1], Kazunari Nagai[1], Mayuko Miyakoda[1], Keisuke Hayashi[1], Miki Sugihara[1], Kazutaka Mogi[1], Tsuyoshi Koide[2], Takefumi Kikusui[1] (*[1]Companion Animal Research Lab, Sch Vet, Azabu Univ, Japan, [2]National Institute of Genetics, Japan*)

Observational fear is the ability to exhibit fear-related behavior such as freezing and escaping when observing fear of a conspecific. Although previous studies have shown that the anterior cingulate cortex (ACC) plays an important role in observational fear, the underlying neural mechanisms are not well understood. To address this issue, we performed behavioral, neurophysiological, and chemogenetic experiments while animals performed the observational fear paradigm, in which one mouse (termed as an observer) observed fear-related behavior exhibited by a conspecific receiving footshocks (termed as a demonstrator).
First, we examined observational fear behavior in three mouse strains: MSM, C57BL/6J, and the hybrid between MSM and C57BL/6J (MSMB6F1). Because MSMB6F1 strain showed adequate observational fear and moderate irritability compared to MSM and C57BL/6J strains, we used MSMB6F1 mice in the following experiments.
Next, we recorded the neural activity of the ACC neurons during observational fear by using fiber photometry. We found that the ACC neurons of the observer were activated when the demonstrator received footshocks. We did not see significant activation associated with freezing behavior of the observer. This suggests that ACC activities are involved in the perception of fear of a conspecific, but not in the execution of freezing behavior.
Finally, we examined the necessity of ACC for the expression of observational fear. The ACC neurons were inactivated in either non-specific or projection-specific manners by using the DREADD system. Our preliminary results showed that chemogenetic inhibition of the ACC-periaqueductal gray (PAG) pathway decreased freezing behavior of the observer, suggesting that this pathway plays an essential role in the expression of observational fear.
In conclusion, our results indicate that the ACC is necessary for converting perception of fear of a conspecific to the expression of observational fear, possibly through its projection to the PAG. (COI:No)

## 3P-143

### The effects of muscimol injections into the nucleus accumbens on the waiting behavior and brain slice analysis of the network activity

Naoko Okada[1], Masaki Okubo[1], Yoko Machida[1], Yutaka Komura[2], Takashi Tominaga[3], Riichi Kajiwara[1] (*[1]Grad Sch of Sci and Tech, Meiji Univ, Japan, [2]Grad Sch of Human and Environmental Studies, [3]Lab for Neural Circuit Systems, Inst of Neurosci, Tokushima Bunri Univ*)

The nucleus accumbens (NAc), an essential structure for guiding action selection and cost/benefit decision making, possesses many GABAergic medium spiny neurons (MSNs). The MSNs are innervated by the dopaminergic neurons in the reward system as well as the excitatory neurons in the corticolimbic circuitry. Their activity is modulated by the fast-spiking GABAergic (and cholinergic) interneurons in the NAc. Thus, the GABAergic transmission in the NAc should affect various actions such as impulsive-control behavior.
In the present study, we designed the two-choice task using a modified M-shaped-maze for investigating the waiting behavior for delayed reward in rats and investigated the involvement of the GABAergic transmission in the NAc. In the center lane of M-shaped-maze, the rat has to wait for the LED-cue turned on before going into the correct lane where a sound stimulus is presented. The waiting period, i.e., "delay for the reward" was presented randomly (0, 5, 10, 15, 20, 25 seconds). As the delay increased, the error rate of waiting behavior for future reward was also increased and seemed to correlate with the delay period used at a previous trial. The intra-NAc infusion of a $GABA_A$ receptor agonist fluorophore-conjugated muscimol (FCM) affected the cue-selection behavior required waiting a period.
To confirm how the NAc neurons are activated and affected by drugs acting on GABA receptors, we performed local field potential recordings and voltage-sensitive dye imaging in horizontal brain slices containing bilateral NAc. We recorded the neural responses following the electrical stimulation to the anterior commissure or the white matter at the rostral/dorsal border of the NAc. The synaptic responses recorded from NAc-core show paired-pulse facilitation, and the response was enhanced by $GABA_A$ receptor antagonist gabazine (1μM). While, focal application of the FCM diminished the response in the NAc. (COI:No)

## 3P-144

### A possible coding for experience: super bursts, ripple-like events, and synaptic diversity

Junko Ishikawa, Takuto Tomokage, Dai Mitsushima (*Dept Neurosci, Grad Sch, Yamaguchi Univ, Japan*)

The hippocampal CA1 is necessary to maintain experienced episodic memory in many species including humans. To monitor the temporal dynamics of processing, male rats were recorded multiple-unit firings of CA1 neurons in habituated home cage and experienced either 4 episodes for 10 min: restraint stress, social interaction with female or male, or observation of a novel object. Before the experience, the neurons mostly exhibited sporadic firings with some synchronized (≈ 50 ms) ripple-like firing events. After the onset of episode exposure, the restraint or social interaction with other rats induced spontaneous high-frequent firings (super bursts) intermittently, while the object observation induced the events inconsistently. Then, minutes after the initiation of episode, CA1 neurons frequently exhibited ripple-like firings with less-firing silent periods. The number of ripple-like events depended on the experienced episode and correlated with the total duration of super bursts. Experience clearly diversified multiple features of individual ripple-like events with experience-specific manner, sustained for more than 40 min in the home cage.
Ex vivo patch clamp analysis further revealed the experience-promoted synaptic plasticity. Compared with inexperience controls, the episodes with female, male, and restraint cell-dependently increased AMPA- or GABAA receptor-mediated postsynaptic currents, while the contact with novel object increased GABAergic currents only. Since multivariate ANOVA in multi-dimensional virtual space revealed experience-specific super bursts, and following ripple-like events and the synaptic plasticity, we hypothesized that the experience-specific super bursts and following synaptic plasticity are responsible to create the experience-specific ripple-like events and memory. It is possible to decipher encrypted experience by the deep learning of the orchestrated ripple-like firings and synaptic plasticity in multiple CA1 pyramidal neurons. (COI:No)

## 3P-145

### AI analysis for episode-specific ripple-like firings of hippocampal CA1 neurons

Ryo Sato, Junko Ishikawa, Takuto Tomokage, Dai Mitsushima (*Dept Neurosci, Grad Sch Med, Yamaguchi Univ, Japan*)

Although the multiple CA1 neurons frequently forms synchronized (≈ 50 ms) ripple-like firing events, the role of the events is still unclear. Here we recorded multiple-unit firings of CA1 neurons from freely-moving male rats in habituated home cage, and they experienced either of 4 episodes for 10 min: restraint stress, social interaction with female or male, or observation of a novel object. We subsequently recorded the firings for more than 30 min in their home cage. The episodic experiences not only increased the number of events, but also changed the 4 features of ripple-like events such as duration, amplitude, arc length, and number of peaks. Moreover, multivariate analysis of variance in the 4-dimensional virtual space revealed significant episode-specificity of individual ripple-like events.

Using artificial intelligence (AI) system, we further analyzed thousands-pair of Euclidean distance among the individual ripple-like events to measure the similarity. The diversity of ripple-like events was low before the experiences, but most ripple-like events exhibited relatively long distance from other ripple-like events. Although the diversity of the events was high in 20 to 30 min after the experience of restraint stress, many pairs of the events exhibited close Euclidean distance showing multiple clusters of similar ripple-like events. The diversified ripple-like events and the clustered similarity might contain definitive information to represent experienced episode. (COI:No)

## 3P-146

### Alpha oscillations are related to accumulation of relevant information in the parietal cortex

Yuki Suda, Takanori Uka (*Dept Integrative Physiol, Grad Sch Med, Univ Yamanashi, Japan*)

Flexible decision making is accomplished by the integration of appropriate information depending on context. Although the spiking activity recorded in area LIP of the parietal cortex is known to be related to the accumulation process for relevant information, little is known concerning how the oscillatory alpha-band activity, known to be related to discrimination performance, relates to the accumulation process. To address this issue, we investigated firing rate and alpha oscillations of LIP neurons while monkeys performed a switching task.

We trained two macaque monkeys to flexibly switch between discriminating motion direction (upward vs downward) or stereoscopic depth (near vs far) in a random dot stereogram. Task difficulty was varied by manipulating the motion coherence and the binocular correlation of the visual stimulus. While the monkeys performed this task, we recorded local field potential (LFP) and single unit activity from isolated LIP neurons. We calculated the event-related spectrum perturbations (ERSP) from LFP, and computed the time course of the alpha-band (8-14Hz) activity.

We confirmed the typical strength-dependent increase in firing rates for both motion coherence and binocular correlation following visual stimulus onset, and the convergence of firing rates just before the saccade. On the other hand, the alpha-band activity gradually decreased after stimulus onset irrespective of stimulus direction, and the decrement slope was dependent on the absolute value of stimulus strength for relevant but not irrelevant features. These activities converged with respect to each choice around the time of saccade, suggesting that alpha oscillations are related to the evidence accumulation process in addition to spiking activity for flexible decision formation. (COI:No)

## 3P-147

### Effect of the genotype of elongation of very long chain fatty acids protein 5 (ELOVL5) on the psychological state of Japanese elders

Tamami Ueda (*Nutr Phyol, Grad Phar Sci. Josai Univ, Japan*)

The number of patients suffering from mood disorder, a psychiatric condition, is increasing. Systemic inflammation is now recognized as a major etiological factor for this condition. C-reactive protein (CRP), a marker of inflammation, could also serve as a marker for mood disorder. The expression of elongation of very long chain fatty acids proteins 5 (ELOVL5), enzymes known to be involved in the synthesis of polyunsaturated fatty acids (PUFAs), has been reported to reduce the risk of the disorder. Thus, the present study investigated the correlation between the Self-rating Depression Scale (SDS), apathy scale, or CRP and PUFA levels in the red blood cells (RBCs). Furthermore, effect of the rs2397142 (CC versus CG+GG) allele of ELOVL5 on enzyme activity, fatty acid (FA) synthesis, and psychiatric stabilization were also investigated. In total, 853 samples from the Shimane chore study were assigned included in this study. FA profiles of RBC membranes were analyzed by gas chromatography. Genomic DNA was extracted from leukocytes using a standard phenol/chloroform method. SNPs in ELOVL5 was genotyped using the TaqMan assay. The level of emotional disturbance in the subjects was assessed using the SDS, apathy scale, and CRP were used. When the SDS score was higher than 40, the subject was considered to have a tendency for emotional disturbance. If the apathy scale was higher than 16, the subject was considered to have a tendency of low motivation. CRP level was found to correlate with SDS score significantly. Although there was no significant difference in the SDS score between the CC and CG+GG genotype groups, the CRP levels tended to be higher in the latter. PUFA levels did not differ significantly based on the rs2397142 genotype.

Collectively, the findings suggest that ELOVL5 rs2397142 exerts a very limited effect on the psychological state of Japanese elders. (COI:No)

## 3P-148

### Physiological function of Neuromedin B in beige adipocyte

Ryoko Higa[1], Ikuko Morisaki[2], Kenshiro Shikano[1], Toshikatsu Hanada[2], Reiko Hanada[1] ([1]*Dept Neurophysiol, Fac Med, Oita Univ, Japan,* [2]*Dept Cell Biol, Fac Med, Oita Univ, Japan*)

Beige adipocytes are thermogenic adipocytes distinct from classical brown adipocytes. It has a key role in regulation of systematic energy homeostasis in mammals. Beige adipocytes were reported increasing the energy expenditure and improving metabolic abnormalities such as obesity and insulin resistance. To reveal the molecular mechanism of beige adipocytes contributes to understand in the detail of energy metabolism. However, it is not fully understood. Then, to assess differentiation and activation of beige adipocytes, we generated white and beige adipocyte cell lines derived from the inguinal subcutaneous white adipose tissues of a single C57BL/6J mouse. Comparing with the characteristics between white and beige adipocytes revealed that beige adipocytes are less adipogenesis than white adipocytes. To elucidate which molecules could regulate beige adipocytes adipogenesis, we performed shRNA library screening. Then, it was found Neuromedin B (NMB) as a candidate gene that suppresses beige adipocyte differentiation. NMB is one of bombesin like peptides and known to be involved in regulation of energy homeostasis. However, there is no report on its relationship with beige adipocytes. In this study, we generated NMB gene-deficient mice using the CRISPR-Cas9 system in order to investigate the function of NMB in beige adipocytes. Here, we will present some data of NMB gene-deficient mice and their control mice in analysis of energy metabolism and beige adipogenesis. (COI:No)

## 3P-149

### Menstrual cycle influence on breast skin temperature measured during sleep

Shuri Marui, Kaho Ito, Kei Nagashima (*Body Temp. Fluid Lab., Fac. Human Sciences Waseda Univ., Japan*)

**Background:** Body temperature at a rest (i.e., basal body temperature, BBT) is fluctuated in about a month by changes in menstrual cycle in women of reproductive age. It is important to determine the site of measuring the BBT more easily compared to the sublingual temperature. The purpose of this study was to investigate whether breast skin temperature could be used in healthy women to detect the menstrual cycle.

**Methods:** Data were collected from ten healthy young women. The subjects were nonsmokers, were not taking oral contraception, and had regular menstrual cycles. The lower left breast skin temperature was measured every 15 minutes during sleep using a temperature sensor attached to clothing and sublingual temperature was measured in the morning using a 10-second digital thermometer. Both the breast skin and sublingual temperatures were measured every day throughout three periods.

**Results:** The average temperature of the lower left breast until two hours before waking up ($T_{chest}$) was lower than the sublingual temperature among all subjects. $T_{chest}$ was significantly associated with the sublingual temperature among the subjects maintaining regular sleep-wake cycles. $T_{chest}$ showed a biphasic pattern similar to the sublingual temperature.

**Conclusion:** The menstrual cycle affects the breast skin temperature during sleep. It was suggested that the BBT could be estimated from the temperature of the lower left breast. (COI:No)

## 3P-150

### Characterization of cold intolerance and daily torpor in the house musk shrew (*Suncus murinus*)

Yasutake Shimizu[1, 2, 3], Kanako Nomura[1], Hiroki Shimaoka[2], Yuuki Horii[2], Kazuhiro Horii[2], Takahiko Shiina[1, 2] ([1]*Lab Vet Physiol, Fac Appl Biol Sci, Gifu Univ, Japan,* [2]*Dept Basic Vet Sci, Lab Physiol, Unit Grad Sch Vet Sci, Gifu Univ, Gifu, Japan,* [3]*G-CHAIN, Gifu Univ, Japan*)

The house musk shrew (*Suncus murinus*: suncus) is cold intolerant because the animal is induced to hypothermia and immobilized in the cold environment. However, precise mechanism of cold intolerance in the suncus is still unclear. Therefore, the aim of the present study was to characterize cold intolerance in the suncus. Both sexes of suncus were used in this study. The suncus were bred in the cold room at 4℃, and then they were induced to hypothermia and immobilized within 3 days. This result shows that the animals are cold intolerant. For inducing habituation to cold environment, temperature in the breeding room was decreased gradually. After habituation to cold environment, the suncus kept body temperature in the cold room at 4℃ for longer time. In addition, the size of brawn adipose tissue was increased. These results indicate that cold intolerance of the suncus might be derived from insufficiency of thermogenesis. On the other hand, we also examined property of daily torpor in the suncus. So, we will report the results. (COI:No)

## 3P-151

### Cold-induced activation of BAT thermogenesis increases circulating miR-122 level possibly through the secretion from muscle

Jussiaea Valente Bariuan, Yuko Okamatsu-Ogura, Ayumi Tsubota, Kazuhiro Kimura
(*Laboratory of Biochemistry, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan*)

Brown adipose tissue (BAT) is responsible for non-shivering thermogenesis and required for body temperature maintenance in cold environments. Since BAT enhances energy expenditure, it is considered as an anti-obesity target. BAT activity in human is negatively associated with circulating microRNA-122 (cir-miR-122) level, which is suggested to ordinarily come from the liver. To elucidate the relation of BAT function with cir-miR-122, we compared miR-122 levels between C57BL6/J mice exposed to cold environment (10 ± 2℃) for 4 hours and placed at normal room temperature (23 ± 2℃). Cold exposure significantly increased the expressions of *Uncoupling protein 1* (*Ucp1*), a key mitochondrial protein for thermogenesis, indicating the activation of BAT. Cir-miR-122 level was significantly increased after cold exposure, but not at room temperature. Cold exposure caused no change in miR-122 and its precursor levels in the liver. In contrast, cold exposure significantly decreased miR-122 expression in the muscle, but not in BAT, suggesting that increased cir-miR-122 level was due to the enhancement of its secretion from the muscle. To examine whether BAT thermogenesis was a prerequisite for increased cir-miR-122 and decreased miR-122 expression in the muscle, effect of cold exposure was examined in *UCP1*-KO mice, lacking BAT thermogenic function. While the expressions of thermogenesis-related genes in BAT, except for that of *Ucp1*, was significantly increased after cold exposure, no significant changes were observed in cir-miR-122 and muscle miR-122 expression in UCP1-KO mice. These results suggest that cold-induced activation of BAT thermogenesis increased cir-miR-122 through the secretion of miR-122 from muscle, although further study is required to find the missing link between BAT thermogenesis and miR secretion from the muscle. (COI:No)

## 3P-152

### Decreased thermal sweating of central sudomotor mechanism in tropical Africans compared to temperate Koreans

JeongBeom Lee[1], Lee Hye-Jin1, JeongHo Kim[1,2], Tae-Hwan Park[2], Young-Ki Min[1]
([1]*Department of Physiology, College of Medicine, Soonchunhyang University, 31, Suncheonhyang 6-gil, Dongnam-gu, Cheonan-si, 31151, Republic of Korea,* [2]*A student at the College of Medicine, Soonchunhyang University, 31, Suncheonhyang 6-gil, Dongnam-gu, Cheonan-si, 31151, Republic of Korea*)

Tropical natives sweat less and preserve more body fluid than temperate natives, tolerating heat stress. However, the mechanisms involved in such sweating reduction has not been fully elucidated. We examined sudomotor responses of tropical natives (Africans, n=36 males) and temperate natives (Koreans, n=41 males) subjects during hot water (43℃) leg immersion (central sudomotor response). Correlations between mean body temperature, basal metabolic rate (BMR) and sweat rate were also examined.
All procedures were done in an automated climate chamber. Local skin temperatures and BMR were measured and mean body temperature was calculated. Sweating activities which include evaporative loss rate, sweat onset time, sweat rate, sweat volume and whole body sweat loss volume were examined.
In the heat load test, Africans showed lower mean body and local skin temperatures than Koreans before and after heating. Before and after heating, BMR declined significantly in Africans, while that of Koreans declined less. Local sweat onset time increased more in Africans than in Koreans. Local evaporative loss rate, local sweat volume, local sweat rate, and whole body sweat loss volume reduced in Africans than in Koreans. There were positive associations of mean body temperature and resting BMR with mean sweat rate.
In conclusion, we observed the larger reduction of sudomotor activity in tropical Africans than in temperate Koreans, which was associated with their lower mean body temperature and lower BMR. (COI:No)

## 3P-153

### Evaluation of the quantitative sudomotor axon reflex test in healthy human

Hye-Jin Lee[1], Jeong-Beom Lee[1], Tae-Hwan Park[2], Young-Ki Min[1]([1]*Department of Physiology, College of Medicine, Soonchunhyang University, 31, Suncheonhyang 6-gil, Dongnam-gu, Cheonan-si, 31151, Republic of Korea,* [2]*A student at the College of Medicine, Soonchunhyang University, 31, Suncheonhyang 6-gil, Dongnam-gu, Cheonan-si, 31151, Republic of Korea*)

The purpose of this study was to quantitatively assess the difference in sudomotor function between healthy males and females by measuring skin surface area and activated sweat gland density (ASGD). The quantitative sudomotor axon reflex test (QSART), a method for evaluating autonomic nervous system activity, was used for quantification. In QSART, the sweat glands are activated directly or indirectly by the subcutaneous application of neurotransmitters, such as 10% acetylcholine, through iontophoresis (2 mA * 5 min). This series of mechanisms is called the sudomotor axon reflex. Blue-black pigmented spots were counted in 0.5 cm × 0.5 cm areas under a microscope in triplicate, and average sweat gland density (count/cm2) was calculated. After recording age, height, weight and several measurements of the forearm, QSART was performed on 201 (116 males, 85 females) healthy subjects aged 21 to 78 years to measure ASGD. The result of independent sample t-test showed higher ASGD in women. The body surface area and the surface area of the forearms were higher in men but the number of activated sweat glands was not significantly different according to sex. The activated sweat gland counts of the body and forearms were analyzed through linear regression by age for males and females. The results demonstrate that an attenuation of sudomotor function occurs with aging in both sexes. Moreover, the findings showed a progressive increase in onset time and a decrease in sweat rates, SGD and SGO with increasing age in both sexes. (COI:No)

## 3P-154OU

### Physiological function of GRP in energy metabolism regulation mechanisms

Teppei Ueda, Ryoko Higa, Kenshiro Shikano, Reiko Hanada (*Department of Neurophysiology, Faculty of Medicine, Oita University.*)

Metabolic syndrome involves a large number of diseases and is a risk factor for obesity-related diseases. The number of those patients are increasing but there is no effective drug. In recent years, beige adipocytes were discovered as a therapeutic target because of their ability to produce heat and enhance energy metabolism like brown adipocytes. More recently, they have been reported to improve glucose tolerance. However, there are still many unclear the mechanism about differentiation and activation of beige adipocytes. To reveal the mechanism of beige adipocytes differentiation and activation, we established white and beige preadipocyte cell lines from subcutaneous adipose tissue of mouse and compared mRNA expression levels by RNA-seq. Then, we found a remarkable increasing of gastrin-releasing peptide (GRP) in mature beige adipocyte activated by Forskolin. GRP, a bioactive peptide, is known to be involved in systemic metabolism and central nervous system control. GRP receptor-deficient mice decreased insulin secretion during elevated blood glucose periods. However, the relationship between beige adipocytes and GRP is unknown. In this study, we generated GRP gene knockout mice (GRP KO) using the CRISPR-Cas9 system to investigate the function of GRP in beige adipocyte. We will present the data of GRP KO mice and wild type mice in analysis of body weight gain, food intake, energy metabolism, locomotor activity and glucose tolerance under a normal diet or a high fat diet. (COI:No)

## 3P-155

### Pathophysiological role of VEGF receptor 3 on tumorigenesis of prostate cancer

Aya Yamamura[1], Nayeem Md Junayed [1], Hiroyuki Muramatsu[2], Susumu Suzuki[3], Masato Watanabe[2], Kogenta Nakamura[2], Kazuhiro Yoshikawa[3], Motohiko Sato[1]
([1]*Dept Physiol, Aichi Med Univ, Japan,* [2]*Department of Urology, Aichi Medical University,* [3]*Division of Research Support, Research Creation Support Center, Aichi Medical University*)

Prostate cancer is one of the most common and lethal malignancies in men worldwide. Although the androgen receptors (ARs) contribute to the progression of prostate cancer, other hormones may be also involved in it. Vascular endothelial growth factors (VEGFs) play a critical role in several cancer events including angiogenesis and tumorigenesis. However, the pathophysiological role of VEGF signaling in prostate cancer remains to be elucidated. In the present study, the involvement of VEGF receptors and their downstream pathways on cell proliferation, migration, and tumor development was examined using human primary prostate epithelial cell line (PrEC) and prostate cancer cell lines (LN-CaP and PC-3). Among three VEGF receptors, the protein expression of VEGFR3 in PC-3 cells was much higher than other prostate cells. PC-3 cells is a metastatic and highly migratory prostate cancer cells and the cell growth is androgen-independent. In PC-3 cells, VEGF-C facilitated the phosphorylation of VEGFR3 in a time-dependent manner and the stimulatory effect was mostly blocked by a selective inhibitor of VEGFR3, MAZ-51. MAZ-51 also inhibited the phosphorylation of Akt. Importantly, the treatment with MAZ-51 dose-dependently reduced the viability, proliferation, and migration of PC-3 cells. Furthermore, MAZ-51 blocked the tumor growth of PC-3 cells implanted into nude mice. These results strongly suggest that the activity of VEGFR3 involved in cell proliferation, migration, and development of prostate cancer. Therefore, VEGFR3 may be a novel therapeutic target for prostate cancer. (COI:No)

## 3P-156

### Roles of oxidative stress induced extracellular vesicle derived from mesenchymal stem cell

Hisaki Hayashi[1], Takuya Matsui[1], Junayed Nayeem M.[1], Rie Takahashi[1], Aya Yamamura[1], Mitsushi Ikemoto[2], Motohiko Sato[1]([1]*Dept Physiol, Sch Med, Aichi Med Univ, Japan,* [2]*AIST*)

To date, mesenchymal stem cells (MSC) including bone marrow derived stem cells have been intensively investigated as a cell–based therapy and regenerative medicine. Extracellular vesicle, exosome, derived from MSC has been reported to have anti-oxidant potential, however, roles of exosome in age related macular degeneration, induced by oxidative stress in eyes, has not been reported yet. Purpose of this study is to analyze the effect of bone marrow MSC derived exosome on recovery from oxidative stress induced cellular damage in eyes. First, exosome from bone marrow derived MSC was isolated by combination of filtration and sucrose gradient ultracentrifuge method, and the quality was evaluated. Particle size analyzer showed average diameter of exosome was 91.0 nm. Immunocytochemistry and flow cytometry indicated 100% of cultured endothelial cells incorporated exosome labeled with ExoGrow fluorescent labeling reagent. Transwell assay demonstrated that exosome stimulated cell migration activity in cultured endothelial cells. These results indicated exosome isolated here was biochemically intact and physiologically active. Since it is known that various stress stimulation change exosome profile of MSC, therefore, exosomal protein expression profile and its change induced by moderate reactive oxidative species are being analyzed with LC-MS/MS system, and will be discussed. (COI:No)

## 3P-157

### The Effect of Gum Arabic on the Diabetogenic Effect of Streptozotocin in Mice

Ammar Boudaka, Zakariya Al-Amri, Ismail Al-Qanobi, Nawaf Al-Mushifri, Hajar BaOmar, Intisar Al-Lawati (*Department of Physiology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman*)

**Background:** Diabetes mellitus is becoming increasingly common worldwide. Plant-based therapies have been shown to have anti-diabetic properties. Gum Arabic (GA) is a prebiotic agent that possesses antioxidant and anti-inflammatory properties. The effect of GA on the diabetogenic effect of streptozotocin (STZ) is rather unknown. Therefore, the purpose of the current study is to study whether GA plays any prophylactic or therapeutic roles in a mouse model of streptozotocin-induced hyperglycemia.
**Methods:** Male C57Bl/6 mice (8 weeks) were randomly divided into five groups: control (received plane water), GA (in drinking water), STZ-injected, GA-STZ (GA for 2 weeks before STZ injection) and STZ-GA (GA after STZ injection) groups. Body weight and fasting blood sugar were measured in all groups. Then hyperglycemia was induced by a single intraperitoneal injection of STZ into the respective groups. Post-STZ injection, body weight and fasting blood sugar were recorded weekly for 4 weeks. At the end of the experiment, plasma insulin was determined and pancreatic tissue sections were stained histomorphometric analysis.
**Results:** STZ and STZ-GA mice exhibited a significant weight loss and hyperglycemia over the course of 4 weeks compared to the control, GA and GA-STZ groups. Plasma insulin levels were significantly lower in STZ and STZ-GA compared with the other groups. Light microscopic assessment revealed an extensive loss of pancreatic islets in STZ and STZ-GA mice and dramatic decrease in the size of the remaining islets. Immunofluorescence studies showed a decrease in the number of insulin-positive beta cells in STZ and STZ-GA mice while the glucagon-positive alpha cells accounted for a major proportion of residual islet cell mass.
**Conclusion:** GA seems to play a prophylactic role against the streptozotocin-damaging effect on beta cells, which makes this dietary fiber as a novel pharmaceutical target with prophylactic potential in diabetes mellitus.
**Keywords:** diabetes, STZ, hyperglycemia, insulin, Gum Arabic. (COI:No)

## 3P-158

### Roles of LOXL2 in exosomal fraction on lymph node metastasis of head and neck squamous cell carcinoma

Hajime Yano, Tomoyoshi Sanada[2], Islam Afsana[1], Reina Tanimoto[1], Teppei Ueda[2], Naohito Hato[2], Junya Tanaka[1] ([1]*Department of Molecular and Cellular Physiology, Graduate School of Ehime University Medical School,* [2]*Department of Otorhinolaryngology, Head and Neck Surgery, Ehime University Medical School*)

The secretory enzyme lysyl oxidase like 2 (LOXL2) is assumed to contribute to tumor progression through participation in cellular events including remodeling extracellular matrix and epithelial-mesenchymal transition. In the previous study, we identified elevated gene expression of LOXL2 in human head and neck squamous cell carcinoma (HNSCC) lymph node metastases. Here we assessed the significance of LOXL2 in the metastasis and of liquid biopsies for detecting HNSCCs and their risk of the metastasis.
LOXL2 protein expression was assessed in human tongue HNSCC tissues by immunohistochemistry as well as in the serum from three patients by immunoblotting. Serum samples were further fractionated in exosomes and supernatant by ultracentrifugation, which were subjected to immunoblot and *in vitro* LOX activity analyses. Then, exosomal LOXL2 levels from 38 serum samples from HNSCC patients and seven healthy volunteers were measured using polymer sedimentation exosome preparation and subjected to statistical analysis.
Immunoblot analyses revealed that LOXL2 was present in serum exosomal fractions from three volunteers without anamnesis as well as three patients. Mean LOXL2 expression was approximately 3-fold higher in patients. Immunohistochemical LOXL2 staining was detected in HNSCC cells in addition to non-cancerous lipid tissues and some muscles in a human tongue HNSCC sample. Further measurements of exosomal LOXL2 by ELISA showed higher LOXL2 levels in patients.
Elevated serum exosomal LOXL2 levels can be an indicator of HNSCC. A follow-up clinical study will be required to determine the clinical utility of using LOXL2 to diagnose HNSCC and/or determine the risk of metastasis. (COI:No)

## 3P-159OU

### tRNA processing defects link to p53-dependent neurodegenerative disease

Masanori Inoue[1, 2], Kazumasa Hada[1], Hiroshi Shiraishi[1], Tohru Ishitani[3], Masaki Matsumoto[4], Kenji Ihara[2], Toshikatsu Hanada[1] ([1]*Department of Cell Biology, Oita University Faculty of Medicine,* [2]*Department of Pediatrics, Oita University Faculty of Medicine,* [3]*Integrated Signaling Systems, Department of Molecular Medicine, Institute for Molecular & Cellular Regulation, Gunma University,* [4]*Department of Molecular and Cellular Biology and Division of Proteomics, Medical Institute of Bioregulation, Kyushu University*)

Fragments of transfer RNA (tRNA), derived either from pre-tRNA or mature tRNA, have been discovered to play an essential role in the pathogenesis of various disorders such as neurodegenerative disease. CLP1 is an RNA kinase involved in tRNA biogenesis, and mutations in its encoding gene are responsible for pontocerebellar hypoplasia type-10. Mutation of the CLP1 gene results in the accumulation of tRNA fragments of several different kinds. These tRNA fragments are expected to be associated with the disease pathogenesis. However, it is still unclear which of the tRNA fragments arising from the CLP1 gene mutation has the greatest impact on the onset of neuronal disease. We found that 5' tRNA fragments derived from tyrosine pre-tRNA (5' Tyr-tRFs) caused p53-dependent neuronal cell death predominantly more than other types of tRNA fragment. Human neuroblastoma cells transfected with 5' Tyr-tRFs were susceptible to cell death and the neuronal cell death induced by 5' Tyr-tRFs was prevented by p53 gene deletion. As an in vivo model, 5' Tyr-tRFs caused severe developmental abnormalities and induced neuronal cell death through p53 activation. These results provide a new conceptual framework in neurodegenerative diseases for how the accumulation of 5' Tyr-tRFs can affect neurons, and might help explain fundamental molecular principles of neurodegenerative diseases. (COI:No)

## 3P-160OU

### Creation of neurodegenerative disease model and elucidation of pathogenesis due to abnormal RNA metabolism

Hiroyuki Yatsuka[1, 3], Kazumasa Hada[1], Ryouhei Umeda[2], Kenichi Kimoto[3], Reiko Hanada[2], Toshiaki Kubota[3], Toshikatsu Hanada[1] ([1]*Department of Cell Biology, Oita University Faculty of Medicine,* [2]*Department of Neurophysiology, Oita University Faculty of Medicine,* [3]*Department of Ophthalmology, Oita University Faculty of Medicine*)

The RNA exosome is an evolutionarily conserved ribonuclease complex that is critical for both the processing and degradation of a variety of RNAs. Exosome component 2 (EXOSC2) is a component of the exosome complex. Recently, homozygous or compound heterozygous mutations in the EXOSC2 gene was identified in German families. The patients show neuronal disorders, such as retinitis pigmentosa, progressive hearing loss, and mild intellectual disability. Mutations.However, the relevance of the EXOSC2 gene mutation in the neurological disease was entirely unknown. In this study, we aim to elucidate the pathophysiological mechanism of RNAexosome related diseases. To investigate the in vivo function of EXOSC2, we generated an exosc2 knockout zebrafish model by CRISPR/Cas9 system. The exosc2 knockout zebrafish showed an abnormal phenotype in neuronal development, meaning that the phenotypic resemblance between human patients and zebrafish mutants. Furthermore, we showed senescence in exosc2 knockout zebrafish by histochemical assay for SA-$\beta$-gal activity. Considering the properties of RNA exosomes, which contribute to degrading various types of RNA, it is conceivable that the accumulation of abnormal RNA molecules in cells is the trigger for neuronal diseases and senescence. We are now under the investigation of global RNA analysis using the zebrafish model. (COI:No)

## 3P-161OU

### Establishment and Functional Analysis of Pontocerebellar Hypoplasia Type 10 mouse model

Ikuko Morisaki[1], Yuji Arai[2], Hiroshi Shiraishi[1], Reiko Hanada[3], Takashi Kobayashi[4], Toshikatsu Hanada[1] ([1]*Department of Cell Biology, Oita University Faculty of Medicine,* [2]*National Cerebral and Cardiovascular Center,* [3]*Department of Neurophysiology, Oita University Faculty of Medicine,* [4]*Department of Infectious Disease Control, Oita University Faculty of Medicine*)

Pontocerebellar Hypoplasia is one of the hereditary neurodegenerative diseases, and the disease has been reported up to 10 types so far. Our previous report found that CLP1 works as RNA kinase which phosphorylate 5' hydroxyl group RNA functions. Recently, it has been reported that Pontocerebellar Hypoplasia type 10 is caused by the CLP1 mutation (R140H). CLP1 is one of the components of transfer RNA (tRNA) splicing endonuclease complex (TSEN complex) which removes intron from the pre-tRNA. We established and analyzed Clp1 kinase-dead mouse model. We found the accumulation of abnormal mature tRNA fragments induced apoptosis of neuron cell death. The region of R140H is different from the region of kinase domain, so we established R140H model mouse using CRISPR/Cas9 system. R140H model mouse displays milder neuron paralysis than kinase-dead model mouse. Future investigation will aim to perform phenotypic and molecular analysis of the influence of abnormal mature tRNA fragments in motor neurons. (COI:No)

## 3P-162OU

### Deficiency of VRK1 causes microcephaly and alters social interactions in zebrafish

Masahito Shide[1], Ryohei Umeda[1], Kazumasa Hada[2], Kenshiro Shikano[1], Ryoko Higa[1], Hirotaro Urushibata[2], Hiroshi Shiraishi[2], Toshikatsu Hanada[2], Reiko Hanada[1] ([1]*Dept Neurophysiol, Fac Med, Oita Univ, Japan,* [2]*Dept Cell Biol, Fac Med, Oita Univ, Japan*)

Vaccinia-related kinase 1 (VRK1) is one of a serine/threonine kinase. It has been reported that VRK1 gene mutation in human causes pontocerebellar hypoplasia and cerebral dysplasia. However, the mechanisms of brain diseases caused by dysfunction of VRK1 are unclear. In this study, to investigate the physiological functions of VRK1 and its involvement in pontocerebellar hypoplasia, we measured body and brain sizes and performed a series of behavioral analyses using VRK1 knockout zebrafish (VRK1 KO). We used CRISPR/Cas9 system to generate VRK1 KO. We established VRK1 KO which has 5 bp deletion causing a frameshift mutation. Morphological analysis showed that both body lengths and weights in VRK1 KO were smaller than control zebrafish (CNT). In addition, microcephaly was observed in the VRK1 KO compared to CNT. We measured the diameter of forebrain, midbrain, and cerebellum using HE stains in the brain slices, and those of VRK1 KO was shorter than CNT in each brain region. The novel tank diving test showed the decrease of locomotor activity and anxiety-like behavior in VRK1 KO. The social interaction test suggested that VRK1 KO has a strong interest in novel zebrafish. Taken together, we showed that VRK1 KO became microcephaly with growth retardation and increased interest in novel zebrafish. (COI:No)

## 3P-163

### The effects of overweight on a2-plasmin inhibitor / plasmin complex after strenuous exercise

Kihachiro Fukada[1], Hidehiko Kushi[2], Terue Takashina[1] (*[1]Institute of Humanities and Social Sciences, Nihon University, Tokyo, Japan, [2]College of Humanities and Social Sciences, Nihon University, Tokyo, Japan*)

**Introduction:** Some studies have reported an increase in fibrinolytic activity after acute strenuous exercise. Conversely, fibrinolytic activity is inhibited in overweight person. In this study, our aim was to evaluate whether being overweight affects fibrinolytic activity after acute strenuous exercise. Being overweight was defined using body mass index (BMI) as a measure of the degree of obesity.
**Subjects and Methods:** Twelve healthy young men aged 19 to 23 years old who engaged in daily exercise participated in this study. Seven of these men were categorized in the BMI<25 group, and five were in the BMI>25 group. Venous blood samples were collected from the subjects pre- and post-performance of the Cooper test. This test involved running as far as possible within a 12-minute period. a2-plasmin inhibitor / plasmin complex (PIC, as a marker of fibrinolytic activity) levels were measured using the collected blood samples.
**Results:** The PIC levels increased significantly in the BMI<25 group (pre: 0.5 ± 0.02 ug/mL, post: 1.9 ± 0.3 ug/mL, $P<0.05$), but these were not significantly increased in the BMI>25 group (pre: 0.5 ± 0.08 ug/mL, post: 1.0 ± 0.1 ug/mL, $p>0.05$).
**Conclusions:** Using BMI as an index for evaluation, this study showed that fibrinolytic activity is inhibited in overweight young men after acute strenuous exercise. (COI:No)

## 3P-164

### Continuous inspiratory resistive breathing increases motor cortex inhibition in a lower limb muscle

Yuma Hara[1], Linlang Quan[1], Kei Hatano[1], Yoshinori Ohtsuka[2], Takahiro Yunoki[3] (*[1]Grad Sch Educ, Hokkaido Univ, Japan, [2]Dept Sports and Human Studies, Sapporo Int Univ, Japan, [3]Fac Educ, Hokkaido Univ, Japan*)

We investigated the effect of inspiratory muscle fatigue on central fatigue and corticospinal excitability in a leg muscle. Seven healthy male subjects performed a 30-min inspiratory resistive breathing (IRB) task (breathing frequency: 50 breaths/min, tidal volume: 2.0 times the resting value, inspiratory resistance: 40cm$H_2O$). Vastus lateralis (VL) responses to transcranial magnetic stimulation (TMS) of the motor cortex [motor evoked potential (MEP) and cortical silent period (CSP)] and electrical stimulation of the femoral nerve [maximal M-wave (Mmax)] were recorded during 5-s isometric knee extension at the intensity of 20% of maximal voluntary contraction (MVC). Voluntary activation (VA) of the VL was assessed via TMS during 5-s MVC. The VL responses, maximal inspiratory pressure (PImax), and respiratory effort sensation were measured 5 min before the start of the IRB task (baseline) and between 10 and 15 min (middle phase) and between 25 and 30 min (end phase) after the start of the IRB task. In addition, blood lactate concentration was measured before (baseline) and after (end phase) the IRB task. Although there was no significant difference in PImax among the three measurement phases, respiratory effort sensation and blood lactate concentration increased significantly from baseline to the end phase of the IRB task ($P<0.05$). The CSP was significantly longer in the end phase than in baseline and the middle phase ($P<0.05$), while MEP, Mmax, and VA remained unchanged from baseline to the end of the IRB task. These results suggest that development of inspiratory muscle fatigue or increased respiratory effort sensation increases intracortical inhibition in the leg motor cortex. (COI:No)

## 3P-165

### The effects of estrogen on muscle hypertrophy and muscular water homeostasis in skeletal muscle following resistance exercise training

Yung-Li Hung[1], Minenori Ishido[2], Shuichi Machida[3] (*[1]Inst. of Health and Sports & Med., Juntendo Univ., Chiba, Japan, [2]Div. of Human Sci., Faculty of Engineering, Osaka Inst. of Tech., Osaka, Japan, [3]Dept. of Health and Sports Sci., Juntendo Univ., Chiba, Japan*)

Compared to older men, older women exhibit more frailty and loss of skeletal muscle mass. It is thought that the lack of estrogen after menopause may crucially contribute to reduced muscle mass and strength in older women. The increase of extracellular water in the skeletal muscle is associated with reduced estrogen levels. The extracellular water to intracellular water ratio is increased and associated with muscle weakness in older women. Estrogen is considered to regulate muscular water; however, the mechanism by which estrogen regulates muscular water is unknown. Water channel aquaporin-4 (AQP4) and ion channel Na-K-Cl cotransporter 1 (NKCC1) regulate water homeostasis in the skeletal muscle, thus regulating its physiologic functions, such as hypertrophy and atrophy. We investigated the effects of estrogen on AQP4 and NKCC1 in the skeletal muscle by using estrogen-deficient animals following resistance exercise training. Female adult rats (10 weeks old) were divided into the following 6 groups: sham sedentary, sham climbing training, ovariectomy sedentary, ovariectomy climbing training, ovariectomy plus estrogen treatment sedentary, and ovariectomy plus estrogen treatment climbing training groups. The estrogen deficiency is caused by the ovariectomy. After 8 weeks of climbing training, the weight of the flexor halluces longus (FHL) muscles were significantly increased in the sham climbing training group but not in in the ovariectomy climbing training group. On the contrary, ovariectomy plus estrogen treatment resulted in exercise-induced muscle hypertrophy. AQP4 and NKCC1 protein expressions tended to be decreased after ovariectomy, and the estrogen treatment reversed this decrease of AQP4 and NKCC1 in ovariectomized animals. Nevertheless, these differences regarding protein expression among the groups were not significant. Therefore, these data suggest that estrogen regulated exercise-induced muscle hypertrophy but did not affect the expressions of AQP4 and NKCC1 in the skeletal muscle. (COI:No)

## 3P-166

### Decrease in the expiratory duration induced by the photostimulation of inhibitory neurons within the lateral solitary nucleus during inspiration

Noriyuki Hama[1], Shigefumi Yokota[2], Masashi Fujitani[1,2], Yasumasa Okada[3], Naohiro Koshiya[4], Hidehiko Koizumi[4] (*[1]Department of Neural and Muscular Physiology, Shimane University School of Medicine, Shimane, Japan, [2]Dept of Anatomy, Shimane Univ. Sch of Medicine, Japan, [3]Clinical Research Center, Murayama Medical Center, Japan, [4]Cellular and Systems Neurobiology Section, NINDS, NIH, USA*)

Respiratory rhythm is generated by the central pattern generator (CPG) that is composed of interneurons within the ventral respiratory column and modulated by a variety of visceral information, such as blood gases and lung volume. The information from lung stretch receptor inputs to the respiratory CPG via GABA/glycinergic neurons within the lateral solitary nucleus (lateral NTS). This inhibitory input is thought to cause the transition from inspiration to expiration. However, it remains unknown how these inhibitory neurons affect the respiratory rhythm. Using optogenetic approaches, we previously showed that the photostimulation of lateral NTS inhibitory neurons during the inspiratory phase terminated the ongoing inspiration and decreased the duration of following expiration, but the photostimulation during the expiratory phase could not perturb the respiratory pattern. In this study, to examine the role of lateral NTS inhibitory neurons in the determination of respiratory phases, we applied the photostimulation at the different timing within the inspiratory phase (0, 100 or 200 ms after the onset of inspiration). The photostimulation applied at 0 or 100 ms after the onset decreased the duration of ongoing inspiratory and following expiratory phases. In contrast, the photostimulation applied 200 ms after the onset could not perturb the ongoing inspiration but decrease the duration of the following expiration. These results suggested that the decrease in expiratory duration induced by activation of inhibitory lateral NTS neurons was not caused by the termination of inspiratory phase and inhibitory lateral NTS controls the expiratory duration. (COI:No)

## 3P-167

### The role of monocarboxylate transporters in maintaining respiratory neuron activity

Sayumi Kotani, Tien Lin Shih, Hiroshi Onimaru, Masahiko Izumizaki (*Department of Physiology, Showa University School of Medicine, Tokyo*)

Monocarboxylate transporter (MCT) plays an important role in providing metabolic support to neurons by functioning as the principal transporters for lactate in the nervous system; lactate that is produced by astrocytes is transported to neurons through the so-called astrocyte-neuron lactate shuttle. It is unknown how the lactate shuttle system contributes to maintaining respiratory neuron activity. Alpha-cyano-4-hydroxycinnamate (4-CIN) is known as a MCT-2 inhibitor. In the present study, we examined the effects of 4-CIN on respiratory activity in the brainstem-spinal cord preparation from newborn rat (P0-P3). Bath application of 4-CIN (5 mM) depressed the respiratory rhythm accompanied by a decrease of the C4 amplitude. After washout, the respiratory rhythm and C4 amplitude recovered. The respiratory activity depressed by 4-CIN was partially recovered by bath-application of L-lactate (1 mM) or an ATP-sensitive potassium channel blocker, glibenclamide. Local application of 4-CIN to spinal cord or the medulla decreased C4 amplitude or the rhythm, respectively. Burst activities on preinspiratory and inspiratory neurons in the medulla were also depressed. In the presence of TTX, furthermore, some respiratory neurons were hyperpolarized during 4-CIN application. In some respiratory neurons in which miniature-like EPSPs or IPSPs were detected in the presence of TTX, these synaptic potentials were inhibited by 4-CIN with partial recovery after washout. We concluded that 4-CIN might depress transmitter releases via action on the presynaptic terminal as well as the postsynaptic membrane of respiratory neurons. Our results suggest that the lactate shuttle is important in maintaining respiratory neuron activity. (COI:Properly Declared)

## 3P-168

### Early postnatal development of inspiratory neuron-type in the pre-Bötzinger complex of mice

Yoshihiko Oke[1], Fumikazu Miwakeichi[2,3], Yoshitaka Oku[1], Johannes Hirrlinger[4,5], Swen Hulsmann[6,7] (*[1]Dept Physiol, Hyogo Col of Med, Hyogo, Japan, [2]Dept Stats Modeling, Inst Stats Math, Tokyo, Japan, [3]Dept Stats Sci, Sch of Multidisciplinary Sci, Grad Univ Adv Stud, Tokyo, Japan, [4]Carl-Ludwig-Inst for Physiol, Univ of Leipzig, Leipzig, Germany, [5]Dept of Neurogene, Max Planck Inst of Exp Med, Germany., [6]Clinic for Anesthesiol, Gettingen Univ, Gettingen, Germany, [7]DFG Res CNMPB, Gettingen, Germany*)

Spontaneous inspiratory rhythm is generated in the pre-Bötzinger complex (preBötC), one of the kernel of the respiratory center in the brainstem. As components of the neuronal network for rhythm generation, characteristics of single inspiratory neuron and functions of cell-types in the preBötC have been well investigated. Next, the neuronal network structure of inspiratory neurons should be addressed to understand the mechanism of rhythm generation. However, even ratios of inspiratory neuron-types to the total number of inspiratory neurons in the network remain unrevealed. The ratios might change after birth. From late embryonic stage to birth, the ratio of glycinergic and/or GABAergic neurons to the total number of inhibitory neurons were shown to change in the ventrolateral medulla (Rahman et al., 2015). Furthermore, $GABA_A$-mediated modulation of respiratory rhythm was changed after P3 (Ritter and Zhang, 2000). Here, we estimated the ratios of inspiratory neuron-types to the total number of inspiratory neurons in the preBötC during early postnatal days using double-transgenic mice expressing EGFP in glycine transporter 2 -positive neurons and tdTomato in glutamic acid decarboxylase 65 -positive neurons. We prepared rhythmic slices including preBötC from the mice between postnatal day 1-10 and set the bin width to 2 days for the analysis. Rhythmic bursting activities and neuronal activities could be recorded using local field potential (LFP) and calcium imaging, respectively. Inspiratory neurons were detected as ROIs of radius 4 pixels (3.9 μm) in which the neuronal calcium signal had high maximum cross-correlation coefficients to the LFP by screening and were double-checked by visually confirming the calcium signal. Then, we classified inspiratory neurons into four types based on the expression of the fluorescence proteins. Finally, we evaluated early postnatal development of inspiratory neuron-types in the preBötC by comparison of the ratios at respective bins. (COI:No)

## 3P-169

### Quantitative assessment of liver fibrosis using SHG

Yuri Hayashi[1], Hiroki Takanari[2], Eiji Hase[2], Takeo Minamikawa[2], Takeshi Yasui[2], Mayuko Shimizu[3], Koichi Tuneyama[3] (*[1]Sch Med, Tokushima Univ, Japan, [2]pLED, Tokushima Univ, Japan, [3]Dept Patho Lab Med, Tokushima Univ, Japan*)

**Introduction:** Tissue fibrosis relates to various pathological conditions and impairs physiological function of the organs. Assessment of tissue fibrosis is mainly dependent on tissue staining. However, it is difficult to get detailed information of fibrosis. It is known that the light with twice frequency of irradiated light is generated when collagen is irradiated with an ultrashort pulse laser, which is called second harmonic generation (SHG). In the present study, we propose to assess fibrosis in detail by a novel optical method, SHG.
**Methods & Results:** Liver tissue were obtained from mice with steatohepatitis induced by high-fat feeding (SH) and mice with liver cirrhosis induced by intraperitoneal injection of carbon tetrachloride (LC), and sliced to 5-μm thickness. We have customized SHG microscope equipped with a femtosecond pulse laser with a wavelength of 810 nm. The laser power was adjusted to 20 mW on the stage, and Galvano mirror was set to scan a 162μm square range on the x-y plane. The samples were exposed to the laser for 10 seconds and 256 pixels square images were obtained. The intensity of SHG signal and fiber orientation based on SHG images were analyzed by Image J software. There was no significant difference in the integrated intensity of SHG between SH and LC. However, the fibrosis pattern was different between the two models; linear fibrosis in SH, and dotted fibrosis in LC. The dispersion of fiber orientation was significantly large in LC (27.6¬¬ ± 4.5, n=6) when compared to SH (12.8 ± 3.1, n=7, p=0.018 vs. LC), suggesting that fibers are randomly constructed in LC.
**Conclusion:** We succeeded in detecting liver fibrosis using SHG in different pathological liver disease models. The SHG microscopy could be a powerful tool for qualitative and quantitative assessment of fibrotic tissue. (COI:No)

## 3P-170

### Toward molecule-specific formation of neuron-microelectrode junctions

Samyoung Kim[1], Sm. Ahasanul Hamid[1], Mieko Imayasu[1], Tomoyuki Yoshida [2], Hidekazu Tsutsui [1] (*[1]JAIST, Japan, [2]Toyama University, Japan*)

The microelectrode techniques have long enabled us to investigate the physiological properties of electrically active cells and networks. Although these techniques generally allow high temporal resolution recordings, they intrinsically lack cell-type specificity. Target cells must be identified by means of other indirect criteria such as marker gene expression and cellular morphology, which is not well-compatible with parallel recordings from highly heterogeneous cellular networks.
By combining the robust bioactivities of synapse organizing molecules with microfabrication techniques, we have set out to establish a means enabling the molecule-specific formation of neuron-microelectrode junctions. Following contact of neurons with a microelectrode immobilized with natural synapse organizing molecule, presynaptic terminals were successfully induced onto the electrode. Also, we have generated some sets of prototype engineered synapse organizing molecules that do not cross-react with each other. It is expected that such molecular tools are exploited to develop the micro- or nano-electrode techniques which permit selective recording from genetically specified cells. (COI:No)

## 3P-171

### Summer training camps decreases cell-mediated immunity

Terue Takashina[1], Hidehiko Kushi[2], Kihachiro Fukada[1] (*[1]Institute of Humanities and Social Sciences, Nihon University, Tokyo, Japan, [2]College of Humanities and Sciences, Nihon University, Tokyo, Japan*)

**Introduction:** The prevalence of upper respiratory infection has been reported to increase due to excessive exercise. Although the effects of short-term exercise load on immune function have been studied, a few reports have evaluated the effects of prolonged exercise load. Therefore, we focused on a rugby club training camp, in order to examine the effects of prolonged high-intensity exercise load on cell-mediated immunity.
**Methods:** The subjects were 10 male university students who were members of rugby football clubs (Age : 19.4 ± 0.2 year, Height : 177.0 ± 2.0 cm, Weight : 90.6 ± 2.8 kg). Blood samples were collected from the subjects before and after a 25-day training camp. The count of T helper 1 (Th1) and natural killer cell (NK cell), natural killer cell activity (NK activity), noradrenaline were measured.
**Results:** Th1 showed a significantly low value after the camp in comparison with before the camp (pre : 20.5 ± 1.0 %, post : 16.8 ± 0.8 %, p <0.01). NK cell (pre : 22.1 ± 1.9 %, post : 16.1 ± 1.2 %, p <0.01) and NK activity (pre : 52.7 ± 3.3 %, post : 40.8 ± 2.8 %, p <0.01) showed a significantly low value after the camp in comparison with before the camp. Noradrenaline showed no significant difference (pre : 393.5 ± 36.5 pg/mL, post : 482.4 ± 38.3 pg/mL, p = 0.057).
**Conclutions:** The results of this study demonstrate that summer training camps decreases cell-mediated immunity. (COI:No)

## 3P-172

### Assessment of dermatitis using Raman spectroscopy

Meiko Kanazawa[1], Satoru Hashimoto[2], Takanori Inoue[3], Hiroki Takanari[4] (*[1]Sch Med, Tokushima Univ, Japan, [2]RIEM, Nagoya Univ, Japan, [3]Sch Sci Tech, Oita Univ, Japan, [4]pLED, Tokushima Univ, Japan*)

**Background:** Diagnosis of dermatitis mainly depends on subjectivejudgments such as doctor's visual inspection and patient's symptoms, and it is difficult to make an objective assessment. When the materialis irradiated by a laser, Raman scattered light with a differentwavelength from the laser is generated. Physical properties can beknown by analyzing the wavelength transition from the laser to theRaman scattered light. We propose to apply Raman spectroscopy tothe evaluation of dermatitis.
**Methods:** Dermatitis model mice in which ear skin was inflamed byPMA application were prepared, and ear tissues were collected 6, 12, and 24 hours after PMA application. The tissue without PMAapplication was used as normal control. The tissue was placed on thestage of Raman microscope, and Raman scattered light generated bya 785 nm laser was recorded. The Raman spectra were comparedbetween normal and inflamed skin. The analysis was conductedmainly on the Raman spectra newly generated in inflamed tissue.Pathological specimens with HE staining were prepared after Ramanspectroscopy, scored for vasodilation and edema, and compared withthe score based on Raman spectra.
**Results:** In the inflamed skin, a new bimodal spectrum was frequentlyobserved at around 1,600 cm-1 that completely matched with Ramanspectrum of the blood, suggesting the spectrum reflected vasodilationin the tissue. A broad spectrum was also observed at around 3,000cm-1 in inflammation model that was consistent with Raman spectrumof water, suggesting the signal reflected interstitial edema. Thesespectra increased by time after PMA application and reached maximum at 12 hours. Such changes in Raman spectra over timecoincided with changes in histological vasodilation and edema.
**Conclusion:** We succeeded in detecting Raman spectra, which maydue to vasodilation and edema caused by inflammation, and thespectral and histological changes over time were consistent. (COI:No)

## 3P-173OU

### RNA kinase links prostate cancer progression

Hiroyuki Fujinami[1, 2], Hiroshi Shiraishi[1], Toshitaka Shin[2], Hiromitu Mimata[2], Toshikatsu Hanada[1] (*[1]Department of Cell Biology, Faculty of Medicine, Oita University, [2]Department of Urology, Faculty of Medicine, Oita University*)

In recent years, the relationship between abnormal RNA metabolism and various diseases such as cancer and neurodegenerative diseases is becoming clear. In mammals, CLP1 and NOL9 are known as RNA kinases that directly phosphorylate RNA. CLP1 is involved in transfer RNA (tRNA) maturation, and NOL9 is involved in ribosomal RNA (rRNA) maturation, both of which have been shown to be involved in RNA metabolism. CLP1 has been reported to be a gene fusion with cancer-related gene (AF10) in acute monoblastic leukemia. On the other hand, NOL9 forms a complex with LAS1L. LAS1L has been shown to be more highly expressed in castration resistant prostate cancer compared to normal prostate cancer. However, the molecular mechanism of RNA kinase in the development and progression of cancer has not been clarified yet. Therefore, we analyzed the involvement of RNA kinase in cancer development and progression using a prostate cancer model. As a result, PC3 had higher RNA kinase activity than LNCaP, and also higher cell growth, colony formation activity. Overexpression of CLP1 in LNCaP enhanced RNA kinase activity and cell proliferation. CLP1 reduced 5' tyrosine tRNA fragments accumulation in LNCaP. These results suggest that the RNA kinase activity of CLP1 is associated with cancer cell proliferation ability. (COI:No)

## 3P-174OU

### Physiological $^{18}$F-FDG uptake in the anal canal in adults: evaluation with PET/CT

Yankel Sena, Shunro Matsumoto, Christopher Silman, Kenichiro Otsuka, Takashige Kiyota (*Departments of Radiology, Oita University Faculty of Medicine, Oita, Japan*)

**Objectives:** To determine the physiologic uptake of $^{18}$F-fluorodeoxyglucose (FDG) in the anal canal in adults using positron emission tomography fused with computed tomography (PET/CT).
**Materials and methods:** We conducted a retrospective study for $^{18}$F-FDG PET/CT imaging on patients without symptoms and pathology in the anal region from January 2015 to August 2019. After excluding one patient with Crohn's disease, one patient with hemorrhoid and two patients with history of rectal cancer, a final total of 201 patients were included in this study. FDG uptake were quantitatively and qualitatively evaluated on both early and delayed phases. Quantitative method was calculated using maximum standardized uptake value (SUVmax) and was compared to liver SUVmax and distal rectum SUVmax. Qualitative method was performed with comparison to the background uptake such as perianal surrounding muscles.
**Results:** The mean of anal canal SUVmax on the early phase was 2.26 (range 1.00-6.30) and that on the delayed phase was 2.52 (range 1.00-8.80). Early and delayed anal to liver SUVmax ratio was 0.74 (range 0.24-2.25) and 0.81 (range 0.23-2.32). Furthermore, early and delayed anal to rectal SUVmax ratio was 0.87 (range 0.30-1.89) and 0.90 (range 0.30-1.27), respectively. The qualitative method found 22 (10.9%) patients with positive FDG uptake.
**Conclusions:** The physiologic 18-FDG uptake in the anal canal in adults can be occasionally high in a visual manner. Measuring the anal canal SUVmax and comparing it to rectal uptake might help to differentiate between normal and pathological uptakes. (COI:No)

## 3P-175OU

### TRAF6 in T cells exacerbates the severity of experimental autoimmune encephalomyelitis by up-regulating CCR6 expression

Naganori Kamiyama, Benjawan Saechue, Astri Dewayani, Thanyakorn Chalalai, Shinya Hidano, Nozomi Sachi, Sotaro Ozaka, Shinpei Ariki, Yasuhiro Soga, Mizuki Goto, Takashi Kobayashi (*Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan*)

Multiple sclerosis (MS) is a progressive neurological disease that affects the central nervous system (CNS). Although many studies have implicated Th17 cells and their downstream pathways in the pathogenesis of CNS autoimmunity, the pathogenic mechanism is not yet clear. Experimental autoimmune encephalomyelitis (EAE) is the most commonly used for the rodent model of MS. It has been reported that IL-17 produced by Th17 cells is crucial for the development of both EAE and MS.

TRAF6 transmits the intracellular signal from TLR, thereby regulates innate immune responses. It has been reported that T cell-specific TRAF6-deficient (CD4-Cre; $TRAF6^{fl/fl}$) mice resulted in the increased number of Th17 cell suggesting TRAF6 function in the adaptive immunity. Thus, we sought to better understand TRAF6 function in the development of EAE. We found that CD4-Cre; $TRAF6^{fl/fl}$ mice were highly resistant to EAE due to the down-regulation of chemokine receptor CCR6 on Th17 cells. Recently, it has been revealed that CCL20, the only ligand of CCR6, produced by astrocytes regulates Th17 cell trafficking into the brain via CCR6 /CCL20 pathway in EAE mice. We confirmed that adoptively transferred TRAF6-deficient T cells did not induce EAE. The number of infiltrated Th17 cells and the pathological impact of demyelination in the brain were significantly reduced in the mutant mice suggesting that the migration ability of TRAF6-deficient Th17 cells into the CNS was impaired. In vitro experiments showed that TRAF6 regulated the promoter activity of CCR6 gene. Taken together, CCR6 in Th17 cells up-regulates by TRAF6 leading to the enhanced migration of auto reactive T cells into the CNS in EAE mice. (COI:No)

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