Planned Symposia

Planned Symposium 1

Japan-Korea Joint Symposium
– Towards FAOPS2019 –

Mitochondrial homeostasis in cardiovascular function and disease

March 28 (Tue), 9:50 – 11:50, Hall C

1PS01C1-1

Cardiac mitochondrial defects in the sepiapterin reductase KO mouse

Jin Han, Hyoungkyu Kim, Taehee Ko, Nari Kim, Sung-Ryul Lee, Kyungsoo Ko, Byoungdoo Rhee

Dept Physiol, Col Med, CMDC, Inje Univ, Busan, Korea

Tetrahydrobiopterin has been involved in energy metabolism and mitochondria biology without clear underlying mechanism. Using a sepiapterin reductase (Spr) knockout mouse, a model of BH4 deficiency, we found that BH4 regulates transcription of PGC1-α and phosphorylation of AMPK α and β and the expression of their target proteins involved in mitochondria biogenesis (mtTFA, and ERRα), antioxidant (Prx3 and SOD2) and fatty acid utilization (CD36 and CPTI-M) in the hearts. BH4 can binds to Calcium/Calmodulin-Dependent Protein Kinase Kinase 2 (CaMKK2) then activates CaMKIV mediated CREB phosphorylation and AMPK phosphorylation in the heart of model mice. Spr KO mice have shown the development of a lethal cardiomyopathy with mitochondrial dysfunction and exogenous BH4 supplementation successfully rescued those phenotypes. These results provide a novel molecular mechanism of BH4 in the regulation of cardiac energy metabolism nitric oxide synthase independently and suggest that BH4 has therapeutic potential for cardiovascular diseases characterized by mitochondrial dysfunction. (COI: No)

1PS01C1-2

Regulation of cellular protein homeostasis by mitochondrial tRNA modification

Fan-Yan Wei[1], Tsutomu Suzuki[2], Kazuhito Tomizawa[1]

[1]Dept Mol Physiol, Faculty of Life Sci, Kumamoto Univ, Kumamoto, Japan, [2]Dept Chem Biotech, Sch Engineering, Univ Tokyo, Tokyo, Japan

Mitochondrial tRNAs contain a variety of post-transcriptional modifications. A number of pathogenic point mutations impair mt-tRNA modifications, leading to defective translation and mitochondrial dysfunction. We have previously showed that a subset of mitochondrial tRNAs contain taurino-modification in position 34U. Because the absence of the taurine-modification has been observed in some patients having mitochondrial diseases, the taurine-modification has been associated with the development of mitochondrial diseases. To investigate the molecular function of taurino-modification and its relevance to the mitochondrial disease, we characterized Mto1, the enzyme that catalyzes taurine-modification. The constitutive Mto1 knockout mice exhibited a severe growth retardation, which caused the embryonic lethality at early developmental stage. Deficiency of Mto1 resulted in a complete disappearance of taurine-modification in mitochondrial tRNAs, which markedly impaired mitochondrial protein translation. Interestingly, the defective mitochondrial translation triggered a severe cytoplasmic unfold protein response, which led to a decrease of cytoplasmic translation and abnormal calcium signaling. We will present the molecular mechanism by which mitochondrial translation regulates cytoplasmic protein homeostasis. We will also describe experiments showing the cytosolic unfolded protein response is a potential therapeutic target for the treatment of mitochondrial disease. (COI:No)

1PS01C1-3

Physiological functions of mitofusin 2 on cytosolic Ca^{2+} signaling in smooth muscle cells

Hisao Yamamura, Keisuke Kawasaki, Sou Inagaki, Yoshiaki Suzuki, Yuji Imaizumi

Dept Mol Cell Pharmacol, Grad Sch Pharmaceut Sci, Nagoya City Univ, Nagoya, Japan

Calcium signaling microdomain is recognized as a spatiotemporal platform for functional protein assembly and efficient signal transduction between plasmalemmal and subplasmalemmal regions. In this study, we focused on functional coupling between a SR fragment and mitochondrion nearby and its tethering protein, mitofusin 2 (mfn2), for $[Ca^{2+}]_{cyt}$ regulation in highly excitable smooth muscle cells. Depolarization-induced Ca^{2+} influx caused a local $[Ca^{2+}]_{cyt}$ transient (Ca^{2+} hotspot) mediated by Ca^{2+} release from the SR (CICR) at several discrete sites in subplasmalemma areas. The elevated $[Ca^{2+}]_{cyt}$ within localized area triggered Ca^{2+} uptake by neighboring mitochondria. These results suggest that Ca^{2+} hotspots promote ATP production by activation of mitochondrial respiration. The ATP may be provided to the coupling SR. The tight contact between SR and mitochondria in junctional areas is organized by mitofusin proteins. The siRNA knockdown of mfn2 caused a slow cytosolic Ca^{2+} buffering and an attenuated mitochondrial Ca^{2+} uptake following agonist-induced $[Ca^{2+}]_{cyt}$ increase. In addition, mfn2 knockdown reduced resting $[Ca^{2+}]_{cyt}$ level and reduced cell viability. The mRNA expression of mfn2 was upregulated by oxidative stress, and it was blocked by an ROS scavenger. The ROS-induced upregulation of mfn2 promoted the fusion of mitochondria. In conclusion, the contribution of mfn2 to the coupling between SR and mitochondria in local Ca^{2+} microdomain may be essential for the regulation of $[Ca^{2+}]_{cyt}$ in smooth muscle cells. (COI:No)

1PS01C1-4

Mechanism underlying redox regulation of cardiac mitochondria dynamics

Motohiro Nishida[1,2,3,4]

[1]Div Cardiocirc Signal, Okazaki Inst Integr Biosci (Natl Inst Physiol Sci), NINS, Okazaki, Japan, [2]Dept Transl Pharm Sci, Grad Sch Pharm Sci, Kyushu Univ, Fukuoka, Japan, [3]Dept Physiol, SOKENDAI, Okazaki, Japan, [4]JST, PRESTO

Mitochondrial dynamics is precisely controlled by mitochondrial fission and fusion cycle, and hypoxic stress is known to induce mitochondrial fission through activation of dynamin-related GTP-binding protein-1 (Drp1). Although Drp1 activity is reportedly regulated by upstream Ser/Thr-dependent kinases and phosphatase, we noticed that hypoxic stress activated Drp1 without affecting total Drp1 phosphorylation levels in ischemic myocardium, suggesting that Drp1 per se has a machinery to sense hypoxic stress. Exposure of rat neonatal cardiomyocytes to 1% hypoxia for 12 hours significantly increased Drp1 activity as well as mitochondrial hyper-fission. Treatment with electrophile also promoted mitochondrial fission through Drp1 activation. As this Drp1 activation could be reversed by NaHS, a substrate to generate more nucleophilic reactive sulfur species (RSS), such as Cys persulfide and polysulfide. Endogenous Drp1 protein at Cys-624 actually formed Cys persulfide in rat cardiomyocytes, and persulfide level is dramatically reduced by electrophile. Treatment with NaHS for 24 hours completely suppresses MeHg-induced Drp1 activation and cardiac injury. These results strongly suggest that depletion of RSS on Drp1 proteins underlies induction of mitochondrial fission in ischemic myocardium, and irreversible redox-dependent modification of Drp1 will be a risk of chronic heart failure. (COI: No)

Planned Symposium 2

Supported by Miyuki Giken Co., Ltd.

The translatability between basic and clinical studies for the pathophysiology of epilepsy: approach from oscillology

March 28 (Tue), 16:40 – 18:40, Hall A

1PS02A2-1

Overview of current state of epilepsy and unsolved problems

Akio Ikeda

Dept Epilepsy Movement Disorders Physiol, Kyoto Univ Grad Sch Med, Kyoto, Japan

Epilepsy is one of the most common disorders of the central nervous system, and clinically it occurs at any age including elderly. Epileptic neurons produce abnormally hyperexcitable activity and clinically seizures occurred. Interictally, epileptic neurons produce paroxysmal depolarization shifts (PDS) as the fundamental epileptic characteristics, and it could lead to the ictal state once it becomes prolonged and augmented as epileptic outburst. The following matters remains unsolved yet. 1) Transition from interictal- to ictal state has remains unsolved, and functional role of astrocytes has been emphasized. Astrocytes regulate potassium homeostasis by means of Kir4.1 channels, and thus it results in increased excitability in the tripartite synapse. Active behaviors of astrocytes as the functional syncytium could be reflected by DC shifts even in the clinical, wide-band digital EEG (Ikeda et al,1996, 1999; Imamura et al,2011; Kanazawa et al, 2015). The precise interaction between epileptic neurons and astrocytes and the generator and modulator mechanisms of ictal state is mysterious. 2) In the wide-band EEG era, high frequency oscillation (HFO) has been well recorded by both micro- & macro-invasive EEG in clinical situation. However, depending on the range of frequency, i.e., gamma (30-100Hz), ripple (80-250Hz) & fast ripple (>250Hz) (Bragin et al,1999), each generator mechanisms differ from local field potentials to possibly burst of action potentials, has not been full clarified yet. (COI:Properly Declared)

1PS02A2-2

Approach from basic and molecular study - Electrophysiological study of hyperthermia-induced seizures in *Scn1a* mutant rats-

Iori Ohmori[1], Katsuhiro Kobayashi[2]

[1]Dept Special Needs Education, Okayama Univ Grad Sch Edu, Okayama, Japan, [2]Dept Child Neurol, Okayama Univ Grad Sch Med Dent Pharm Sci, Okayama, Japan

Although febrile seizures (FSs) are the most common convulsive syndrome in infants and childhood, the etiology of FSs has remained unclarified. Several missense mutations of the Nav1.1 channel (SCN1A) have been reported in a familial epileptic syndrome. Here, we generated *Scn1a*-targeted rats carrying a missense mutation (N1417H) in the third pore region of the sodium channel by gene-driven ENU (N-ethyl-N-nitrosourea) mutagenesis. Despite their normal appearance under ordinary circumstances, Scn1a mutant rats exhibited remarkably high susceptibility to hyperthermia-induced seizures, which involve generalized tonic-clonic seizures with paroxysmal epileptiform discharges. Whole-cell patch-clamp recordings from HEK cells expressing N1417H mutant channels and from hippocampal GABAergic interneurons of N1417H mutant rats revealed a significant shift of the inactivation curve in the hyperpolarizing direction. We also examined high-frequency oscillations (HFOs) in the ictal cortical EEGs of hyperthermia-induced seizures in the *Scn1a* mutant rats. When generalized tonic-clonic seizures were induced, HFOs with frequencies ranging from 200 to 400 Hz were found to precede spikes during the clonic phases of these seizures in the ictal EEGs. The detection of HFOs from the ictal EEGs of hyperthermia-induced seizures may provide a cue to answering the generation mechanism of febrile seizures. (COI:No)

1PS02A2-3

Epileptogenicity and related network: Approach from clinical neurophysiology

Riki Matsumoto[1], Katsuya Kobayashi[1], Takayuki Kikuchi[2], Kazumichi Yoshida[2], Takeharu Kunieda[3], Akio Ikeda[4]

[1]Dept Neurol, Kyoto Univ Grad Sch Med, Kyoto, Japan, [2]Dept Neurosurgery, Kyoto Univ Grad Sch Med, Kyoto, Japan, [3]Dept Neurosurgery, Ehime Univ Grad Sch Med, Toon, Japan, [4]Dept Epilepsy Movement Disorders Physiol, Kyoto Univ Grad Sch Med, Kyoto, Japan

In order to diagnose and treat epilepsy, it is important to understand the underlying pathophysiology, namely, epileptogenicity and its related network. Patients with intractable partial epilepsy, when the working hypothesis with non-invasive evaluations is insufficient, undergo invasive presurgical evaluations with chronic implantation of subdural electrodes. This opportunity provides us with a valuable opportunity to directly evaluate the cortical excitability, namely, epileptogenicity at and around the epileptic focus. The gold standard method is to decrease the antiepileptic medication and record epileptic seizures with ECoG. It would be clinically ideal if we could assess epileptogenicity and related network in the interictal state. We introduce our studies using direct single pulse stimulation to probe the cortical excitability and seizure and functional networks by recording evoked potentials and induced spectral responses. We focus on the cortical excitability at the epileptic focus and its state dependent dynamic change during sleep, since sleep does change the behavior of epileptic spikes, pathologic high frequency oscillations, and clinical seizures. We also discuss the network specific change with regards to epileptogenicity. (COI:Properly Declared)

1PS02A2-4

Finding mathematical structures in the brain dynamics: theory and its application

Ichiro Tsuda, Takao Namiki

Dept Math, Grad Sch Sci, Hokkaido Univ, Sapporo, Japan

Motivated by the success of interpretations of observed data in terms of nonlinear dynamical systems, we have formulated a mathematical theory for measurements, experiments, data, and trajectories including transient and asymptotic motion. Here, asymptotic motion may be represented by various kinds of attractors such as point attractors, periodic attractors (limit cycles), quasi-periodic attractors, chaotic attractors including higher-order attractors representing complex transition phenomena such as chaotic itinerancy. In this respect, it can be supposed that one will find mathematical structures embedded in the data of brain dynamics, whose structure may represent mental states. Under this assumption, we applied time series analyses on ECoG datasets which were recorded during epileptic seizures of two patients. Each ECoG data was recorded from 30 minutes before the onset of the seizures to 30 minutes after the end of the seizures with 2kHz sampling rate. As studied in Imamura and Ikeda et. al. (2011), DC shift before seizure onset and high frequency oscillation (HFO) around 100Hz during seizures are typically observed. Throughout our analyses we tried to extract mathematical structures which are embedded in the data. On the HFO region of ECoG data, we found that power spectral densities obey either power law distribution of exponent around -2 or the power law with 100Hz peak. We also applied an embedding technique to the data, and found that a type of one-dimensional dynamical systems, like, so called, a circle map, are embedded in the data. (COI:No)

Planned Symposium 3

The impact of mechanobiology in physiology

March 28 (Tue), 16:40 – 18:40, Hall B

1PS03B2-1

Mechanotransduction by cytoskeleton-regulated MS channels at focal adhesions

Masahiro Sokabe

Mechanobiology Lab, Nagoya Univ Grad Sch Med, Nagoya, Japan

Cell mechanosensing is one of the core subjects in mechanobiology, in which mechanosensitive ion channels (MSCs) are the major player. Among MSCs bacterial MscL has been the best studied one owing to its resolved 3 D crystal structure. MscL is activated simply by increased global tension in the plasma membrane, contributing to the cell volume control against hypo-osmotic challenge. By contrast, eukaryotic MSCs seem to be activated mainly by tension in the actin cytoskeleton stress fiber (SF) that anchors focal adhesion (FA). In the case of a Ca^{2+} permeable MSC in endothelial cells, tensile forces conducting along the SF/FA complex activate MSCs in the vicinity of FAs, causing intracellular Ca^{2+} increases. Surprisingly, this system has an ultra-high sensitivity to mechanical force as low as 1 pN. The SF/FA/MSC complex has another important function called active-touch sensing, in which contractile forces in individual SFs pull local regions in the cell substrate via corresponding FAs to activate the MSCs. As generated stress and/or strain in the SF/FA depends on the substrate rigidity, the MSCs can transduce substrate rigidity into local intracellular Ca^{2+}levels. Thus the SF/FA/MSC complex can work as a substrate rigidity sensor that regulates many important cell functions, including survival, proliferation, differentiation and migration of cells. MSCs might have evolved from the simple bacterial ones that directly respond to changes in the global membrane tension, to the elaborated eukaryotic ones that can actively detect local mechanical properties of micro-environments surrounding cells. (COI:No)

1PS03B2-2

Blood-flow sensing mechanism and their role in vascular physiology and pathology

Kimiko Yamamoto[1], Joji Ando[2]

[1]System Physiol, Grad Sch Med, Univ Tokyo, Japan, [2]Biomed Eng, Dokkyo Med Univ, Japan

Vascular endothelial cells (ECs) sense shear stress and transduce blood flow information into functional responses that play important roles in vascular physiology and pathophysiology. Previously, we found that the shear-stress dependent Ca^{2+} signaling occurs via an ATP-operated channel, P2X4, and that P2X4-mediated shear stress mechanotransduction plays an important role in vascular homeostasis, including in the control of blood pressure, flow-induced vasodilation and blood flow-dependent vascular remodeling. Since P2X4 was activated by ATP released in a shear-stress-dependent manner from cholesterol-rich caveolar domains in the plasma membrane, we recently examined the influence of shear stress on the changes in membrane physical properties, lipid order and fluidity. Two distinct lipid order states coexist in the plasma membranes: a liquid-ordered (Lo) state and a liquid-disordered (Ld) state. The areas of Lo regions decreased immediately after the application of shear stress, and the decrease was most marked in the caveolar domains. Similar changes of the lipid order occurred when shear stress was applied to artificial lipid bilayer membranes of giant unilamellar vesicles, indicating that these responses are physical phenomena. Blockade of these changes in the membrane lipid order by the addition of cholesterol resulted in marked inhibition of the shear stress-induced ATP release. These results indicate that EC plasma membranes directly respond to shear stress by changing their physical properties, which are involved in shear-stress-sensing and control of vascular functions. (COI:No)

1PS03B2-3

Bone homeostasis and mechano-biology

Tomoki Nakashima[1,2,3]

[1]Dept Cell Signaling, Tokyo Medical and Dental Univ, Tokyo, Japan, [2]AMED-CREST, Tokyo, Japan, [3]JST, Tokyo, Japan

The weight-bearing exercises help to build bones and to maintain them strength. Bone is constantly renewed by the balanced action of osteoblastic bone formation and osteoclastic bone resorption both of which mainly occur at the bone surface. This restructuring process called “bone remodeling” is important not only for normal bone mass and strength, but also for mineral homeostasis. Bone remodeling is stringently regulated by communication between bone component cells such as osteoclasts, osteoblasts and osteocytes. An imbalance of this process is often linked to various bone diseases. During bone remodeling, resorption by osteoclasts precedes bone formation by osteoblasts. Based on the osteocyte location within the bone matrix and the cellular morphology, it is proposed that osteocytes potentially contribute to the regulation of bone remodeling in response to mechanical and endocrine stimuli. (COI:No)

1PS03B2-4

Mechanotransduction and transcriptional control of gene expression

Toshihiko Ogura

Dept Dev Neurobiol, IDAC, Tohoku Univ Sendai, Japan

Beating hearts and contracting skeletal muscles generate physical force and then generated force stimulates their cells to maintain homeostasis. In the vascular system, pressure and shear stress stimulate endothelial cells. Physical stimuli are converted into biochemical response in these cells, keeping circulatory conditions constant. Nonetheless, the molecular mechanisms of these signal conversion systems, namely, mechanotransduction, are largely unknown. To explore in detail the mechanism, we developed several new techniques that enable us to manipulate living cells by physical force at an organelle level, focusing on how physical stimuli regulate transcription and gene expression. In this symposium, we introduce our new techniques and a novel mechanotransduction pathway that regulate transcriptional control of gene expression immediately in minutes. (COI:No)

Planned Symposium 4

From genetics to physiology: for understanding molecular functions and disease mechanisms

March 29 (Wed), 8:50 – 10:50, Hall A

2PS04A1-1

Next-Generation Sequencing uncovers molecules essential for human physiology

Hirotomo Saitsu

Dept Biochem, Hamamatsu Univ Sch Med, Hamamatsu, Japan

Recent advances in next-generation sequencing (NGS) technology caused paradigm shift in genetic analysis. Whole exome sequencing (WES), which employs the targeted capture of protein encoding exons and NGS, has enabled the comprehensive examination of mutations in more than 90% of human coding exons. In addition, WES of patients' and their parental samples (trio analysis) can systemically detect de novo mutations in the patients, revealing that de novo mutations are important genetic causes for sporadic human disorders. Intractable infantile epilepsy has been extensively analyzed by WES in the world, and although only three genes were known nine years ago, now 45 genes have been linked to this condition according to the statics of Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim/). Many mutations associated with well characterized human phenotype will be increasingly identified by WES: however, adverse effect of the identified mutations for their molecular functions and disease mechanisms caused by the mutations are largely unsolved. In this presentation, I will show impacts of WES in genetics of rare intractable infantile epilepsy, and highlight importance of elucidating potential disease mechanisms. (COI:No)

2PS04A1-2

Mild functional impairment of neuronal K^+-Cl^- cotransporter KCC2 by biallelic mutations causes migrating focal seizures and severe developmental delay

Tenpei Akita[1], Hirotomo Saitsu[2], Miho Watanabe[1], Naomichi Matsumoto[3], Atsuo Fukuda[1]

[1]Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]Dept Biochem, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [3]Dept Hum Genet, Yokohama City Univ Grad Sch Med, Yokohama, Japan

One of the early-onset epileptic syndromes, epilepsy of infancy with migrating focal seizures (EIMFS), is characterized by migrating polymorphous focal seizures and severe developmental delay. Our whole exome sequencing (WES) recently revealed 3 EIMFS patients with compound heterozygous mutations in *SLC12A5*, encoding the neuronal K^+-Cl^- cotransporter KCC2: a pair of sisters with a mutation causing skipping of exon 3 in the transcript (p.E50_Q93del) and another different mutation (p.A191V); and a boy with p.S323P and p.M415V. The parents of these patients have no symptoms. We also found a girl diagnosed as unclassified intractable epilepsy with different heterozygous mutations through *SLC12A5*-targeted resequencing of a different cohort. Gramicidin-perforated patch-clamp analysis showed strongly suppressed Cl^- extrusion function of E50_Q93del and M415V mutants and mildly impaired function of A191V and S323P mutants. Cell surface expression levels of KCC2 were not greatly affected by these mutations. Heterologous expression of two KCC2 mutants, mimicking the patient status, resulted in an intracellular Cl^- level significantly higher than with wildtype KCC2, but less than without KCC2. Thus the analysis clearly indicates that even mildly impaired neuronal Cl^- extrusion, mediated by two types of KCC2 mutant in an individual, causes EIMFS. (COI:No)

2PS04A1-3

Epilepsy-related ligand-receptor, LGI1 and ADAM22: from disease-causing mutations to molecular functions and therapeutic strategies

Yuko Fukata

Membrane Physiology, NIPS, Okazaki, Japan

More than 30 mutations in *LGI1*, encoding a neuronal secreted protein, cause autosomal dominant lateral temporal lobe epilepsy. Also, LGI1 is a major target of autoantibodies in autoimmune limbic encephalitis, which is characterized by subacute onset of memory impairment and seizures. So far, we have found that secreted LGI1 functions as a ligand for ADAM22 transmembrane protein and this ligand-receptor interaction plays an instructive role in regulating AMPA receptor function. However, the pathogenic mechanisms of *LGI1* mutations remain largely elusive and also unknown is whether *ADAM22* mutations are associated with brain disorders in human. Here, we generated mouse models with *LGI1* mutations and examined how LGI1 function is disrupted in vivo. We found that two types of *LGI1* mutations, compromising intracellular folding/trafficking or ligand activity of LGI1, caused epilepsy. Furthermore, a chemical corrector, restoring folding of mutated LGI1, ameliorated the increased seizure susceptibility of the model mice. Also, by the exome sequencing and whole-genome SNP genotyping for a patient with a severe infantile-onset progressive encephalopathy with intractable seizures, we found compound heterozygous mutations in *ADAM22* that compromise the binding activity of ADAM22 to LGI1. These overall approaches establish a critical role of the LGI1-ADAM22 molecular pathway in the normal brain function and propose a novel strategy to treat human epilepsy. (COI:No)

2PS04A1-4

MIRAGE syndrome: a mystery of *SAMD9* mutations and acquired monosomy 7

Satoshi Narumi

Dept Mol Endocrinol, Natl Res Inst Child Health & Dev, Tokyo, Japan

Congenital adrenal hypoplasia is a rare life-threatening endocrine disorder. To clarify the novel pathogenesis of the disease, I and my colleagues conducted NGS-based investigation on patients without mutation(s) in known causative genes. We analyzed 24 patients, and found that 11 had a germline heterozygous mutation in *SAMD9*, which is a putative tumor suppressor located in chromosome 7. The mutation-carrying patients showed common and characteristic constellation of clinical phenotypes, led us to name as MIRAGE syndrome, which is an acronym for Myelodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes and Enteropathy. We conducted a series of expression experiment to confirm the pathogenicity of identified mutations. Expression of each mutant SAMD9 protein caused profound growth inhibition in HEK293 cells, while expression of wildtype SAMD9 caused mild growth inhibition. Notably, two out of the eleven *SAMD9* mutation-carrying patients developed myelodysplastic syndrome (MDS) accompanied by acquired monosomy 7. Genetic analysis of the MDS cells revealed that the chromosomal deletion resulted in removal of the *SAMD9* mutations. Considering the strong growth-restricting capacity of the mutant SAMD9, fitness of the diseased cells were presumably increased by the removal of the mutation. However, the cost for the "adaptation" was high: loss of entire chromosome 7 and resultant MDS. In the symposium, I will discuss the potential role of functional alterations of tumor suppressors in acquisition of chromosomal abnormalities. (COI:No)

Planned Symposium 5

Joint Symposium with
the Japanese Pharmacological Society

Innovation of optogenetics ~
application to non-excitable cells
and development of the new tools

March 29 (Wed), 8:50 – 10:50, Hall B

2PS05B1-1

Reconstitution of short-term hearing fluctuation by application of the optogenetic approach to non-excitable, non-glial cells in the inner ear

Fumiaki Nin[1], Mitsuo Paulo Sato[2], Taiga Higuchi[1], Kenji F Tanaka[3]

[1]Dept Mol Physiol, Niigata Univ Sch Med, Niigata, Japan, [2]Dept Otorhinolaryngol, Faculty Med, Kindai Univ, Osakasayama, Japan, [3]Dept Neuropsychi, Sch Med, Keio Univ, Tokyo, Japan

Deafness afflicts 15% of world population. A common cause of this serious disease is disorder of the cochlea of the inner ear. In the cochlea, acoustic stimuli are transduced to electrical signals by sensory hair cells and these signals are conveyed to the brain through nervous system. The mechanoelectrical transduction is sensitized by a highly positive potential of an extracellular fluid, endolymph, which hair cells face. Several idiopathic sensorineural deafness types in human show hearing fluctuation over a period of a few hours or even several minutes. These profiles have not been reproduced in animals by conventional methods. Here we utilized the optogenetic approach. The target was the endolymphatic potential correlated with hearing threshold. Genetic engineering of channelrhodopsin-2 with myelin proteolipid protein promoter resulted in stable expression of this channel in a non-excitable, non-glial cell type of an epithelial-like tissue maintaining the endolymph. Illumination to the cochlea induced in a few minutes significant deafness accompanied by reduction of the endolymphatic potential. After cessation of the illumination, the potential and hearing completely recovered over several minutes. These responses were repeatable multiple times. This mouse mimics short-term hearing fluctuation in patients and thereby will contribute to translational medicine of deafness. (COI:No)

2PS05B1-2

Modulatory effects of the changes in membrane potential of oligodendrocytes on axonal conduction and synaptic responses in the hippocampus

Yoshihiko Yamazaki

Dept Physiol, Yamagata Univ Sch Med, Yamagata, Japan

The conduction velocity of action potentials (APs) affects the absolute time it takes to transmit nerve impulses as well as temporal summation at destination synapses. In the rat hippocampus, oligodendrocyte depolarization at the physiological level increases the conduction velocity of APs along myelinated fibers. To examine the effects of the changes in membrane potential of oligodendrocytes on axonal conduction and synaptic responses, we used mice with channelrhodopsin-2 (ChR2) expression restricted to oligodendrocytes. Oligodendrocyte depolarization (by photostimulation on ChR2) induced short- and long-term facilitation on axonal conduction, which was evaluated by the recording of compound APs. On the other hand, oligodendrocyte hyperpolarization in mice expressing archaerhodopsin in oligodendrocytes induced no significant changes in compound APs. We then studied the physiology of facilitation of axonal conduction by investigating the changes in synaptic responses at destination synapses. The projection area of examined axons is divided into three portions and contains two types of principal neurons: regular firing and bursting neurons. We found a significant increase in excitatory synaptic responses in bursting neurons at two out of three portions, but not in regular firing neurons at all portions. These results indicate that oligodendrocyte depolarization contributes to the fine control of axonal conduction and facilitates destination synaptic responses in a region and cell-type specific manner. (COI:No)

2PS05B1-3

The use of photoactivated adenylyl cyclase (PAC) in vitro and in vivo

Ryuta Koyama, Yuji Ikegaya

Lab Chem Pharmacol, Grad Sch Pharmaceut Sci, Univ Tokyo, Japan

In most optogenetic approaches, microbial opsins are expressed in neurons to elicit or inhibit neuronal activity via light-induced generation of transmembrane ionic current, leaving the other intracellular signaling molecules remain to be optogenetically studied. Among the signaling molecules, we optogenecically modulated the intracellular levels of cAMP, a major second messenger, using photoactivated adenylyl cyclase (PAC). PAC, which was originally identified as a sensor for photoavoidance in the flagellate Euglena gracilis, is activated by blue light and rapidly changes its conformation to synthesize cAMP from ATP. We transfected PAC in primary neuronal cultures to examine whether and how intracellular cAMP dynamics regulates axonal elongation and branching. We successfully performed temporal manipulation of intracellular cAMP levels in PAC-transfected neurons and found that short-term elevation of intracellular cAMP induces axonal branching but not elongation, whereas long-term cAMP elevation induces both axonal branching and elongation. Furthermore, using PAC together with siRNA-mediated knockdown, we determined that intracellular cAMP regulates axonal branching and elongation via protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac), respectively. Finally, we have recently generated transgenic mice in which PAC expression can be induced in specific types of neurons or glial cells. Using the PAC-expressing mice, we will be able to spatiotemporally modulate the intracellular cAMP levels in vivo. (COI:No)

2PS05B1-4

Organelle-optogenetics –Intervention of intracellular Ca^{2+} dynamics by light–

Hiroyuki Igarashi[1], Ayaka Yoshikawa[2], Toshifumi Asano[3], Ayumu Konno[4], Shigeo Sakuragi[2], Toru Ishizuka[2], Hirokazu Hirai[4], Hiromu Yawo[1,2]

[1]Dept Physiol Pharmacol, Grad Sch Med, Tohoku Univ, Sendai, Japan, [2]Dept Dev Biol Neurosci, Grad Sch Life Sci, Tohoku Univ, Sendai, Japan, [3]Dept Cell Biol, Grad Sch Med Den Sci, Tokyo Med and Den Univ, Tokyo, Japan, [4]Dept Neurophysiol Neural Repair, Grad Sch Med, Gunma Univ, Maebashi, Japan

As a ubiquitous second messenger, Ca^{2+} regulates various cellular processes. The Ca^{2+} signal is generated from two major sources; the influx from extracellular space and the release from intracellular Ca^{2+} stores such as endoplasmic/sarcoplasmic reticulum (ER/SR). However, the spatiotemporal manipulation of Ca^{2+} dynamics from individual sources has not been achieved because of its technical difficulty. Here, we established a novel method named "organelle-optogenetics", to specifically target light-responsive cation channel (channelrhodopsin, ChR) to ER/SR, which subsequently induces internal Ca^{2+} release from ER/SR specifically in synchrony with light illumination. We made the ER/SR-targeting ChRs (eg. $CatCh+_{ER}$) and evaluated their physiological functions. 1) The ER/SR-targeting ChRs merged with the ER-marker, KDEL under superresolution microscopy. 2) No membrane current was generated by light under whole-cell clamp. 3) Increase of fluorometric Ca^{2+} was evoked by light in various species of cells expressing $CatCh+_{ER}$ in a manner independent on the extracelluar Ca^{2+}. This response was diminished by the light stimulation in the presence of thapsigargin and bafilomycin. It is suggested that our organelle-optogenetics would be effective to manipulate the release of Ca^{2+} specifically from ER/SR. (COI:No)

2PS05B1-5

Optical control of the genome

Moritoshi Sato

Grad Sch Arts and Sciences, Univ Tokyo, Tokyo, Japan

Complex gene networks are essential for diverse biological phenomena. To understand gene functions in these phenomena, approaches that enable endogenous gene expression to be regulated at will are required. For targeted endogenous gene regulation, a new class of programmable genome targeting technology, known as CRISPR-Cas9 system, has offered powerful tools. Several studies have shown that dCas9 fused with the transcriptional activator domain enables activation of user-defined endogenous genes. However, these RNA-guided programmable transcription factors are unable to offer precise spatiotemporal control of gene expression, despite the fact that many biological phenomena are regulated by highly dynamic patterns of gene expression. We have developed a light-inducible, user-defined, endogenous gene activation system based on CRISPR-Cas9. We demonstrated that this system allows rapid and reversible targeted gene activation by light. In addition, using this system, we have exemplified photoactivation of multiple user-defined endogenous genes in mammalian cells. The present CRISPR-Cas9-based transcription system offers simple and versatile approaches for precise endogenous gene activation in basic biological research and biotechnology applications. Additionally, we have recently developed a new tool, named photoactivatable Cas9 (paCas9), which allows optogenetic control of genome editing in the cell. paCas9 could facilitate improved understanding of complex gene networks and prove useful in biomedical applications. (COI:No)

Planned Symposium 6

Recent advances in cellular metabolism-function coupling

March 29 (Wed), 8:50 – 10:50, Hall C

2PS06C1-1

Metabolic regulation of hematopoietic stem cell function

Keiyo Takubo

Dept Stem Cell Biol, Research Institute, National Center for Grobal Health and Medicine, Tokyo, Japan

Hematopoietic stem cells (HSCs) maintain quiescence by activating specific metabolic pathways, including glycolysis. We do not yet have a clear understanding of how this metabolic activity changes during stress hematopoiesis, such as bone marrow transplantation. We found that p38MAPK is immediately phosphorylated in hematopoietic stem/progenitor cells (HSPCs) after a hematological stress, preceding increased HSPC cycling. Conditional deletion of p38α led to defective recovery from hematological stress and a delay in initiation of HSPC proliferation. Mechanistically, p38α signaling increases expression of inosine-5'-monophosphate dehydrogenase 2 in HSPCs, leading to altered levels of amino acids and purine-related metabolites and changes in cell-cycle progression in vitro and in vivo. Our studies have therefore uncovered a p38α-mediated pathway that alters HSPC metabolism to respond to stress and promote recovery. In this session, I would like to discuss our cutting-edge findings of metabolic regulation of HSCs during acute and chronic stresses including aging. (COI:No)

2PS06C1-2

Manipulation of pluripotent stem cell metabolism for cardiac regenerative medicine

Shugo Tohyama

Dept Cardiol, Keio Univ Sch Med, Tokyo, Japan

Heart regenerative therapy using pluripotent stem cells (PSCs) is a potentially promising strategy for patients with heart disease, but the inability to eliminate residual undifferentiated stem cells in sufficient quantities has been a barrier to realizing this potential. Our previously established non-genetic purification method of differentiated cardiomyocytes using mitochondrial dye is efficient but not suitable to produce large-scale cardiomyocytes due to the usage of FACS. To overcome this problem, we developed a novel method for purifying the bulk of PSC-derived cardiomyocytes by focusing on glucose, glutamine and lactate metabolism in differentiated cardiomyocytes and PSCs. The metabolome analyses unraveled that human PSCs mainly depended on glycolysis and glutamine oxidation for ATP generation. Therefore, under glucose- and glutamine-depleted conditions, human PSCs quickly die due to loss of ATP. In contrast, differentiated cardiomyocytes with mature mitochondria were able to take advantage of lactate oxidation to synthesize ATP under glucose- and glutamine-depleted conditions with lactate. Interestingly, the metabolome analysis using [U-^{13}C]-labeled lactate showed cardiomyocytes could produce glutamate and glutamine from lactate under these conditions. This distinguished metabolic feature of human PSCs allows us to prepare clinical-grade cell sources by eliminating residual undifferentiated stem cells and purifying differentiated cardiomyocytes, which prevents tumor formation in stem cell therapy using human PSCs. (COI:No)

2PS06C1-3

Imaging of intracellular ATP dynamics during apoptosis

Hiromi Imamura

Dept Funct Biol, Grad Sch Bio, Kyoto Univ, Kyoto, Japan

It is generally accepted that live cells contain ATP while dead cells not. However, dynamics and mechanism of intracellular ATP decrease in dying cells are not well understood. Moreover, it is unknown whether there is biological meaning for cells to reduce intracellular ATP level. In this work, we employed a genetically encoded FRET-based ATP biosensor "ATeam" to track dynamics of ATP levels in single apoptotic HeLa cells. It was observed that intracellular ATP levels started to decline after cell shrinkage, which follows the activation of caspase-3, and were almost depleted within 1 hour. We found that either pharmaceutical inhibition or siRNA knockdown of an ATP-releasing anion channel significantly abrogated decrease in intracellular ATP of apoptotic cells, while overexpression of the channel accelerated it. Thus, the release of ATP is the major cause of intracellular ATP depletion in apoptotic cells. We also found that glycolysis has a potential to produce ATP even after the apoptotic program is executed. Interestingly, inhibition of ATP release decreased consumption of glucose. We propose that the intracellular ATP decrease of apoptotic cells is the mechanism to suppress their metabolism for saving the environmental glucose. (COI:No)

2PS06C1-4

Discovery of a GTP sensor with a structural reverse genetic approach

Toshiya Senda[1], Koh Takeuchi[2], Miki Senda[1], Atsuo T Sasaki[3]

[1]SBRC, IMSS, KEK, Tsukuba, Japan, [2]Molprof, AIST, Tokyo, Japan, [3]Dept Internal Medicine, Univ Cincinnati Col Med, Ohio, USA

Since GTP is an energy molecule in cells, reduction of the GTP level reduces the rate of cell growth. It is thus reasonable to consider that the GTP level in cells is monitored and homeostatically regulated. However, no cellular GTP-sensing mechanism has been identified. Here, we show that a type II phosphatidylinositol phosphate kinase, PI5P4Kβ, serves as a GTP sensor [1]. Our proteomics and biochemical study demonstrated that PI5P4Kβ preferentially utilizes GTP for its catalytic reaction. In addition, an enzyme kinetic study suggested that kinetic parameters of PI5P4Kβ are suitable for detecting changes in the cellular GTP level. However, since PI5P4Kβ can utilize not only GTP but also ATP for the enzyme reaction in the cell, a knock out/down experiment is insufficient to analyze the biological function of the GTP-sensing activity of PI5P4Kβ. We therefore took a structural reverse genetic approach [2]. Based on the crystal structures of the PI5P4Kβ-ATP and PI5P4Kβ-GTP complexes, we designed a PI5P4Kβ mutant that lacks GTP-sensing activity without changing the ATP-dependent activity. Biological and metabolomic analyses with the PI5P4Kβ mutant revealed that PI5P4Kβ serves as a GTP-sensor and its GTP-sensing activity is critical for metabolic adaptation and tumorigenesis.[Reference] [1] Sumita et al. Mol. Cell 61, 187 (2016), [2] Takeuchi et al. FEBS J. in press. (COI:No)

Planned Symposium 7

Co-sponsored by the MEXT "Oscillology"

Parkinson's disease: from basic neuroscience to clinical application

March 29 (Wed), 13:30 – 15:30, Hall B

2PS07B2-1

GABA interneurons generate oscillations in the dopamine-depleted striatum

Constance Hammond

INMED, INSERM, Marseille, France

How does the GABAergic network afferent to the spiny projection neurons of the striatum (SPN) reorganize in mice models of Parkinson disease? With the use of patch clamp recording techniques in brain slices from wild type, 6-hydroxydopamine-lesioned or PINK1-/- (a model of the human PARK6 variant) mice, we recorded spontaneous GABAergic currents from SPNs and spontaneous spiking activity of GABAergic interneurons. We report that 50% of dopamine-deprived or 75% of PINK1-/- SPNs of adult mice generated giant spontaneous GABAergic currents either singly or in bursts (at 40-60 Hz), rather than the low-frequency (2-5 Hz), low-amplitude, tonic GABAergic drive common to wild-type SPNs of the same age. These abnormal oscillations were blocked by the co-application of three types of nicotinic antagonists, but were insensitive to glutamatergic antagonists. This suggested GABAergic interneurons generate GABA oscillations under the control of cholinergic interneurons. In support of this, cell-attached recordings revealed that a subpopulation of GABAergic interneurons including low threshold spike (PLTS) but not fast spiking (FS) interneurons generated bursts of spikes in dopamine-deprived conditions. Chronic levodopa or bumetanide treatments or kainic acid lesion of the subthalamic nucleus, all suppressed the giant GABAergic currents of SPNs and replaced them with the control tonic activity. Therefore, a subpopulation of local GABAergic interneurons shifts from tonic to oscillatory mode when dopamine deprived and gives rise to spontaneous repetitive giant GABAergic currents in at least one-half the MSNs. (COI:No)

2PS07B2-2

Multiple signals transmitted by midbrain dopamine neurons

Masayuki Matsumoto

Lab Cogn & Behav Neurosci, Fac Med, Univ Tsukuba, Japan

Dopamine neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) are well known for their strong responses to rewards and cues that predict reward. These neurons have been proposed to transmit a reward value signal called "reward prediction error". However, dopamine is essential not only to reward-related functions such as reinforcement but also to motor and cognitive functions. To investigate whether dopamine neurons transmit signals that are not associated with rewards, we first examined how dopamine neurons respond to non-rewarding, aversive events. We conducted a classical conditioning with a liquid reward and aversive airpuff in monkeys. We found that some dopamine neurons were excited by reward-predicting stimuli and inhibited by airpuff-predicting stimuli, as proposed by the value-coding theory. On the other hand, some other dopamine neurons were excited by both of these stimuli, inconsistent with the theory. The former were observed in a ventromedial part of the SNc and VTA, whereas the latter were located in a dorsolateral part of the SNc. We next examined how dopamine neurons respond to non-rewarding, cognitively demanding stimuli. We recorded dopamine neuron activity in monkeys performing a delayed matching-to-sample task. We found that a subset of dopamine neurons was excited by visual stimuli if the monkey had to store the stimuli in working memory. These neurons were located dorsolaterally in the SNc. Our findings suggest that, although ventromedial dopamine neurons encode reward value signals, dorsolateral ones transmit different signals that are not directly associated with reward information. (COI:No)

2PS07B2-3

Dopamine D1 receptor-mediated transmission maintains information flow through the cortico-striato-entopeduncular direct pathway to release movements

Toshikuni Sasaoka[1,2,3], Asako Sato[2,3], Satomi Chiken[4], Tadashi Okubo[3], Jun Maeshima[3], Satoshi Arai[3], Tomoko Sunayama[2,5], Kanako Oda[1], Seiko Sakai[1], Yoshitaka Maeda[1], Yukihiro Jinbo[1], Satohiro Nakao[1], Toshiya Sato[1,3], Nobuyoshi Fujisawa[1], Atsushi Nambu[4]

[1]Brain Res Inst Niigata Univ, Niigata, [2]Natl Inst Basic Biol, [3]KItasato Univ Sch Med, [4]Natl Inst Physiol Sci, [5]Grad Sch Art Sci, Univ Tokyo, Japan

In the basal ganglia (BG), dopamine plays a pivotal role in motor control, and dopamine deficiency results in severe motor dysfunctions as seen in Parkinson's disease. Dopamine activates striatal direct pathway neurons that directly project to the output nuclei of the BG through D1 receptors (D1Rs), whereas dopamine inhibits striatal indirect pathway neurons that project to the external pallidum (GPe) through D2 receptors. To clarify the exact role of dopaminergic transmission via D1Rs in vivo, we developed novel D1R knockdown mice in which D1Rs can be regulated. Suppression of D1R expression decreased spontaneous motor activity and impaired motor ability in the mice. Neuronal activity in the entopeduncular nucleus (EPN), one of the output nuclei of the rodent BG, was recorded in awake conditions to examine the mechanism of motor deficits. Cortically evoked inhibition in the EPN mediated by the cortico-striato-EPN direct pathway was mostly lost during suppression of D1R expression, whereas spontaneous firing rates and patterns remained unchanged. On the other hand, GPe activity changed little. These results suggest that D1R-mediated dopaminergic transmission maintains the information flow through the direct pathway to appropriately release motor actions. (COI:No)

2PS07B2-4

Oscillatory neurons of the motor thalamus in dystonia

Nagako Murase[1], Masao Matsuhashi[2], Yuji Maeda[3], Hidehiro Hirabayashi[4]

[1]Dept Neurol, NHO Kyoto medical Center, Kyoto, Japan, [2]Research and Educational Unit of Leaders for Integrated Medical System, Kyoto Univ, Kyoto, Japan, [3]Dept Emergency and Critical Care Medicine, Kobe Univ Grad Sch Med, Kobe, Japan, [4]Dept Neurosurgery, National Hospital Organization Nara Medical Center, Nara, Japan

Background & Aim: Ventral oral posterior (Vop) and ventral intermediate (Vim) nuclei in the motor thalamus are often chosen as the targets of stereotactic surgery for upper limb dystonia. Microelectrode recordings during the stereotactic surgery revealed the beta dominant oscillation of Vim in ET and PD at rest (Basha D et al., 2014). In dystonia, the neural oscillation of the lower frequency band is reported in GPi and STN but not in Vim. We analyzed the neural firing activities in the sites which corresponded to the effective contacts of DBS or the sites of ablation. Methods: Microelectrode recordings of Vim and Vop were obtained from three male patients with primary upper limb dystonia without other neurological abnormalities (mean age 37 years) during awake stereotactic surgery. Vim and Vop were the nuclei through which the target was set to the caudal zona incerta. Frequency analysis by interspike intervals and spectral analysis by FFT were done using MATLAB. Results: Frequency of neuronal firing in Vop was lower compared with in Vim, which was compatible with the previous studies. However, in Vim, the rate of beta range activity was 33 (11) % (mean(SD)), whereas that of theta and alpha range was 36 (13) and 18 (5) %. Conclusion: In dystonia, beta oscillation was not dominant in Vim. (COI:No)

Planned Symposium 8

Japan-Taiwan Joint Symposium
– Towards FAOPS2019 –

Ion channels in physiology and patho-physiology

March 29 (Wed), 13:30 – 15:30, Hall C

2PS08C2-1

Neurotransmission of taste mediated by calcium homeostasis modulator ion channels

Akiyuki Taruno[1], Yoshinori Marunaka[1,2]

[1]Dept Mol Cell Physiol, Grad Sch Med, Kyoto Pref Univ Med, Kyoto, Japan, [2]Dept Bio-Ionomics, Grad Sch Med, Kyoto Pref Univ Med, Kyoto, Japan

Taste buds are embedded in the tongue epithelium and make direct contact with the afferent gustatory nerve terminals to transmit taste information to the brain. Although adenosine triphosphate (ATP) has been known as the primary neurotransmitter of taste linking taste cells and the afferent nerves, the molecular mechanism of ATP release from taste cells has remained unknown and long been sought after. We recently identified calcium homeostasis modulator 1 (CALHM1), a novel voltage-gated ATP-permeable channel, as the essential component of the taste cell ATP release machinery. CALHM1 is expressed selectively in sweet-, bitter-, and umami-sensing taste cells and its gene knockout eliminates perception of these taste qualities. However, involvement of unknown regulatory subunit(s) in the CALHM1 channel complex in taste cells was suggested because heterologous CALHM1 currents show much slower gating compared to CALHM1-dependent currents in taste cells. Here we show that CALHM1 and its homologous subunit, CALHM3, are assembled to form heteromeric channels with properties distinct from those of homomeric CALHM1 channel. CALHM1 is interacts with CALHM3. Co-expression of CALHM1 and CALHM3 gives rise to a novel ATP-permeable membrane conductance with a more negative V_{50} value and faster gating than CALHM1 alone. Furthermore, CALHM1 and CALHM3 are expressed and function in the same taste cell population. Collectively, our data identify the heteromeric CALHM1/CALHM3 channel as the bona fide mediator of neurotransmission of tastes. (COI:No)

2PS08C2-2

Concerted trafficking regulation of Kv2.1 and K_{ATP} channels by leptin in pancreatic β-cells

Pei-Chun Chen[1], Yi Wu[2], Show-Ling Shyng[2]

[1]Dept Physiol, Grad Sch Basic Med, Natl Cheng Kung Univ, Tainan, Taiwan, [2]Dept Biochem and Mol Biol, OHSU, USA

In pancreatic β-cells, Kv2.1 channels are the dominant delayed rectifier potassium channels responsible for action potential repolarization. Here, we report that leptin, a hormone secreted by adipocytes known to inhibit insulin secretion, causes a transient increase in surface expression of Kv2.1 channels in rodent and human β-cells. The effect of leptin on Kv2.1 surface expression is mediated by the AMP-activated protein kinase AMPK. Activation of AMPK mimics, whereas inhibition of AMPK occludes the effect of leptin. Inhibition of CaMKKβ, a known upstream kinase of AMPK, also blocks the effect of leptin. In addition, the cAMP-dependent protein kinase PKA is involved in Kv2.1 channel trafficking regulation.Inhibition of PKA prevents leptin or AMPK activators from increasing Kv2.1 channel density,while stimulation of PKA is sufficient to promote Kv2.1 channel surface expression. The increased Kv2.1 surface expression by leptin is dependent on actin depolymerization; and pharmacologically-induced actin depolymerization is sufficient to enhance Kv2.1 surface expression. We show that the leptin-induced increase in surface K_{ATP} channels results in more hyperpolarized membrane potentials than control cells at stimulating glucose concentrations,and the increase in Kv2.1 channels leads to a more rapid repolarization of membrane potential in cells firing action potentials. The study supports a model in which leptin exerts concerted trafficking regulation of K_{ATP} and Kv2.1 channels to coordinately inhibit insulin secretion. (COI:Properly Declared)

2PS08C2-3

Modification of the olfactory transduction channel by small molecules

Hiroko Takeuchi, Takashi Kurahashi

Grad Sch Front Biosci, Osaka Univ, Japan

We empirically know that unpleasant smells are masked by pleasant fragrances. In 2009, it was found certain types of odorants suppress the transduction current in olfactory receptor cells (ORCs). Since there was a big correlation between the channel suppression and human sensory test (R=0.81), the channel suppression by odorants seem to be linked with olfactory masking. In 2013, it was found that 2,4,6-trichloroanisole (TCA) is served extremely strong channel suppression. TCA has been known for the cause of degradation of the quality of foods/beverages. In this study, we investigate the mechanism of transduction channel suppression by TCA and relatives using whole-cell recording from the isolated newt (*Cynops pyrrhogaster*) ORCs. The experiments were performed under the latest ethical guidelines for animal experimentation at Osaka University, based on international experimental animal regulations. In results, EC_{50} of TCA was identified to be 0.19 μM. Other natural suppressor geraniol and artificial CNG channel blocker L-cis diltiazem showed 5.8 μM and 29 μM, respectively. It was surprising to see that the least effective concentration of TCA was 1 aM with the U-tube system. Extremely high efficiency of TCA may be based on high surface to volume ratio of the cilia and dissolution into the ciliary membrane. These results may suggest that CNG channels are suppressed through a partitioning of those substances into the lipid bilayer. The findings not only reveal a mechanism of flavor loss of foods/beverages, but also suggest certain molecular structures as possible olfactory masking agents and powerful channel blockers. (COI:No)

2PS08C2-4

Regulation of adult neurogenesis in the mouse SGZ by Kv1.1 potassium channel

Shi-Bing Yang

Inst Biomed Sci, Academia Sinica, Taipei, Taiwan

The adult neurogenesis is tightly regulated by a balance between cell proliferation and apoptosis to keep the total number of neurons constant in the SGZ. Although the molecular mechanisms that regulate adult neurogenesis have been studied extensively, the role of ion channels and membrane excitabilities in modulating adult neurogenesis remain to be precisely elucidated. Recent studies have found that the mouse lacking functional Kv1.1 channels have an enlarged brain due to excessive adult neurogenesis in the SGZ; however, the mechanisms by which Kv1.1 suppresses adult neurogenesis are still largely unexplored. We found that using mosaic analysis with double markers in adult mouse SGZ, neurons lacking functional Kv1.1 were more abundant than wild-type neurons; in contrast, astrocytes lacking Kv1.1 did not exhibit proliferation advantage. Next, we recorded neuronal progenitor cells in the brain slices, and we found only a subset of neural progenitor cells that were not coupled by gap-junction had a more depolarized potential in the Kv1.1 knockout background. Lastly, we found daily injection of GNF5837, a BDNF antagonist, suppressed the over-population of neuronal progenitor cells in the Kv1.1 knockout mice. Our data suggest that Kv1.1 regulates adult neurogenesis in the SGZ in a cell-autonomous manner, as Kv1.1 hyperpolarizes the neuronal progenitor cells and suppresses BDNF-TrkB signal in the SGZ. (COI:Properly Declared)

Planned Symposium 9

Co-sponsored by the MEXT National Program for Advanced Research Platforms "Imaging Platform"

Mass imaging technical workshop

March 29 (Wed), 13:30 – 15:30, Hall H

2PS09H2-1

Improvements in MALDI imaging used to drive new frontiers of biological hypothesis testing

Easterling L Michael

Bruker Daltonics, Inc. MA, USA

MALDI imaging mass spectrometry (IMS) is a technique that has been developed to successfully detect compounds directly from a surface, such as animal or plant tissue, resulting in a series of spatially-resolved spectra where each mass spectrum is accompanied by relative coordinates. Molecular image maps can then be constructed from these spectra to show the distribution of a compound or compounds across that surface. We will show how recent optimizations to the software, instrumentation, and methods employed for MSI has greatly increased the information content, speed, and robustness of these measurements. Furthermore, intelligent combinations of these advances promote facile derivation of novel chemical intuition and biological significance for a variety of biological systems. (COI: No)

2PS09H2-2

Rapid matrix-free molecular imaging of drugs and metabolites in tissues using desorption electrospray ionization (DESI) mass spectrometry

Thanai Paxton, Futoshi Sato, Maki Terasaki

Analytical and Measuring Instruments Division, Nihon Waters K.K., Tokyo, Japan

Mass spectrometry imaging (MSI) has become an important technique in both biomedical research and DMPK labs for analyzing the distribution of drugs and metabolites. MALDI is the most widely adopted technique for the molecular imaging of biological tissues. Recently however, ambient ionization imaging in the form of DESI-MSI, has become an alternative and growing technology due to the ease-of-use and informative nature of the technique. DESI has larger small metabolite coverage and unlike MALDI, it does not require the application of a chemical matrix to ionize molecules of interest. Also since DESI is not as destructive as MALDI, the same tissue can be imaged multiple times allowing for the development of unique DESI & staining and combined DESI & MALDI imaging workflows. Several examples will be shown to illustrate the power of the technique in drug distribution and disease study such as cancers. (COI:No)

2PS09H2-3

Imaging mass microscopy for high special resolution analysis

Koretsugu Ogata

Analytical & Measuring Instruments Division, Shimadzu Corporation, Kyoto, Japan

Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) is a powerful technique for visualizing the distribution of molecules and drugs in organs. In particular, iMScope TRIO is an "imaging mass microscope" instrument that is equipped with an optical microscopy, an atmospheric pressure ion-source chamber for MALDI and a quadrupole ion trap time-of-flight (QIT-TOF) analyzer. This instrument is capable of narrowing down the laser diameter to minimum of 5 μm for high spatial resolution MS imaging. The iMLayer was developed for the purpose of sample pretreatment (application of matrix) in order to perform MALDI-MS imaging. The deposition method has been adopted as the matrix application method. We developed the protocol for two-stage deposition method. This method solves the problems with the spray method. These problems are include the bigger and uniform matrix particles, lower sensitivity. Using this method, fine, uniform crystals can be created comparatively easily, and biological components can be extracted by supplying a solvent via an airbrush. This technique is essential to achieve a high spatial resolution (5 μm to 10 μm). The number of applications is expected to increase. Examples include pretreatment to increase sensitivity for the detection of drugs with the iMScope TRIO, as well as methods to supply matrix solutions with an airbrush after deposition. In this session we will introduce this method and applications. (COI:No)

2PS09H2-4

Label-free raman imaging for life science application

Tatsuhiko Nakano, Noriaki Nishikawa, Hiroshi Demizu, Ramirez Jennifer

Thermo Fisher Scientific

Raman imaging covers a broad range of material and life science applications and represents an alternative to fluorescence microscopy. Raman scattering has been used for label-free observation of biological molecules since it can detect vibrational frequencies of molecules in living specimens. Raman imaging is attractive because it can be used to monitor the dynamics of living specimens with molecular information which cannot be obtained by conventional microscopy. Thermo Scientific DXR2xi Raman imaging microscope is a high performance spectroscopic imaging system that offers a complete package of integrated hardware and software for data collection and analysis, capable of 600 points/second with high spatial resolution and large data set capabilities to cover the widest range of life science problems. Some of the benefits of Raman spectroscopy include that non-destructive technique and no staining preparation required. These advantages lead to Raman imaging being a promising tool for the next generation of Biological imaging. We will show examples on how the DXR2xi Raman imaging microscope can be used to observe molecules for advancing label-free imaging as a powerful technique for investigation of biological sample. (COI:No)

2PS09H2-5

Imaging mass spectrometry reveals a novel mechanism of abdominal aortic aneurysm development

Hiroki Tanaka[1], Nobuhiro Zaima[2], Naoki Unno[3], Tetsumei Urano[1], Mitsutoshi Setou[4]

[1]Dept Med Physiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]Dept Applied Biol Chem, Grad Sch Agriculture, Kindai Univ, [3]Div Vascular Surgery, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [4]Dept Cell Mol Anat, Hamamatsu Univ Sch Med, Hamamatsu, Japan

Abdominal aortic aneurysm (AAA) is a common fatal disease among elderly people. However, the pathophysiology of AAA is not fully understood. Here, we use imaging mass spectrometry (IMS) to analyse the aneurysmal wall and determine the mechanisms underlying human AAA. In regions of aneurysm, IMS revealed a decreased heme signal indicating hypoperfusion in the aneurysm. We also noticed vasa vasorum (VV) stenosis and excessively accumulation of adipocytes in place of the collagen fibers. Under physiological conditions of aortic wall, the inner layer is nourished by direct diffusion from the luminal blood flow, while the outer adventitia is primarily perfused by the VV. Thereby, the VV hypoperfusion may contributes to inflammation in the aortic tissue, and that the subsequent tissue degeneration leads to the formation of an aneurysm. We further developed an animal model based on the hypothesis, and artificial local arterial wall hypoperfusion with inflammation induced aneurysmal features, including stenosis of the VV and ectopic adipogenesis. Thus, these findings using IMS indicate the existence of a previously unknown mechanism in AAA pathogenesis. (COI:No)

2PS09H2-6

Intracellular imaging of fatty acids by using time-of-flight secondary ion mass spectrometry

Makoto Horikawa[1,2]

[1]Dept Cell Mol Anat, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]International Mass Imaging Center, Hamamatsu Univ Sch Med, Hamamatsu, Japan

Background: Time-of-flight secondary ion mass spectrometry (TOF-SIMS) enables to detect fatty acid species with sub-micrometer order resolution. We have recently succeeded to visualize some fatty acid fragments in a single cell by TOF-SIMS. However, the intracellular distribution of lipid molecules has not been elucidated due to the paucity of effective morphological techniques for the analysis. In this study, we investigated the intracellular distribution of lipid molecules with an appropriate fixation method and TOF-SIMS analysis. Methods: COS-7 cells were cultured with DMSO and 100uM oleate respectively on an indium tin oxide-coated glass slide. Cells were fixed with GA+UA solution containing 2.5% glutaraldehyde, 1% uranyl acetate and 0.1M sucrose, washed with 150uM ammonium acetate and dried with a water-freeze dryer. TOF-SIMS analysis was performed by using a TOF.SIMS5-200P instrument. Results: GA+UA fixation enabled to detect fatty acid fragments of phospholipids both with standard reagents and in a fixed cell. Ultrastructural morphology of cells was also preserved by GA+UA fixation. TOF-SIMS visualize signals corresponding to fatty acid fragments including palmitolate, palmitate, stearate and oleate were detected in the cell body. Oleate treatment raised its signal only in the cell body but not in nuclear. Conclusion: GA+UA fixation method can visualize intracellular distribution of fatty acid fragments in a COS-7 cell with sub-micrometer order resolution. (COI:No)

Planned Symposium 10

Co-sponsored by the MEXT "Advanced bioimaging support"

Optical bio-imaging to visualize the hierarchical physiology

March 29 (Wed), 16:50 – 18:50, Hall B

2PS10B3-1

Visualization of immune system in brain

Hiroaki Wake[1,2,3]

[1]Dept Neurophysiol, Grad Sch Med, Univ Kobe, JAPAN, [2]Dept Homeostatic development, NIPS, NINS, JAPAN, [3]PRESTO, JST

Microglia are haematopoietic-cell derived glial cells in the central nervous system (CNS) that function as the only resident immune cells of the CNS. Traditionally, effects of microglia as immune cell in CNS have been thought to be mainly in pathological conditions where they exert neuro-protective or neuro-toxic effects to modify disease progression. However, recent studies have reported that microglial cells play a role in brain homeostasis in the normal physiological state, promoting programmed cell death in both neural development and in adult neurogenesis, and monitoring and phagocytosing synapses. On the other hands, substantial evidence has demonstrated that immune condition can have effects on to the neuronal circuits. However little has been known whether those immune condition could affect on to the function of neuronal circuits. Here we use systemic inflammation model to study the interaction of systemic immune cells and microglia. And we also show the functional regulation of synapses by microglia contacts and the alteration of the synapse response in systemic inflammation model and their affect on the behavior responses. Those data indicate that microglia changes induced by the interaction with systemic immune cells can modulate function of neuronal circuits. (COI:No)

2PS10B3-2

Super resolution imaging of synaptic site to analyze the transmission regulation

Ikuko Yao

Dept Optical Imaging, Hamamatsu Univ Sch Med, Hamamatsu, Japan

Imaging approach is one of a key method to understand a wide variety of physiological phenomenon. Visualizing the fine details of synapses is very helpful to resolve the synaptic issues such as transmission and connectivity, and even network and behavior. Recent super resolution techniques extend the possibility to reveal the molecular features of synapses going over the diffraction limit. With direct stochastic optical reconstruction microscopy (STORM) and structured illumination microscopy (SIM), we imaged synaptic proteins in the brain sections and cultured neuron of neurodegenerative model mice. Consistent with the previous results in electron microscopic analysis, some proteins were upregulated in the synaptic sites. Our results show that super resolution microscopies a powerful tool to investigate synapses. (COI:No)

2PS10B3-3

Mass spectrometry imaging revealed the alteration of the neurotransmitters in brain tissue sections of post-translational modification mutant mouse

Mitsutoshi Setou[1,2]

[1]Dept Cell Biol Anat, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]International Mass Imaging Center, Hamamatsu Univ Sch Med, Hamamatsu, Japan

Various physiological processes are mediated by post translational modifications (PTM). In the brain, PTM can be closely related with neurotransmission via conformational or structural changes. Recent mass spectrometry imaging (MSI) techniques with chemical derivatization enabled us to identify and quantify the distribution of small metabolites including glutamate and GABA. In this study, we verified the glutamate and GABA level and spatial distribution in PTM mutant mouse brain using MSI. High mass accuracy was achieved by Fourier transform ion cyclotron resonance (FT-ICR) MS measurements. Ions of derivatized neurotransmitters were detected and the level was compared between wild type and mutant. Our results by MSI revealed the alteration of the neurotransmitters in brain tissue sections of PTM mutant mouse. (COI:No)

2PS10B3-4

Cruising inside cells

Atsushi Miyawaki

RIKEN BSI, Saitama, Japan

The behavior of biomolecules moving around in cells makes me think of a school of whales wandering in the ocean, captured by the Argus system on the artificial satellite. When bringing a whale back into the sea --- with a transmitter on its dorsal fin, every staff member hopes that it will return safely to a school of its species. There is some concern that a whale fitted with a transmitter may be given the cold shoulder and thus ostracized by other whales. In live cell imaging, a fluorescent probe replaces a transmitter. We label a fluorophore on a specific region of a biomolecule and bring it back into a cell. We then visualize how the biomolecule behaves. Cruising inside cells in a supermicro corps, gliding down in a microtubule like a roller coaster, pushing our ways through a jungle of chromatin while hoisting a flag of nuclear localization signal --- we are reminded to retain a playful and adventurous perspective at all times. What matters is mobilizing all capabilities of science and giving full play to our imagination. We believe that serendipitous findings can arise out of such a sportive mind, a frame of mind that prevails when enjoying whale-watching. (COI:Properly Declared)

Planned Symposium 11

JPS in the next stage;
its future direction

March 29 (Wed), 16:50 – 18:50, Hall E

2PS11E3-1

PLOS ONE as a pioneer of mega journals

Utako Yokoyama, Yoshihiro Ishikawa

Cardiovasc Res Inst, Yokohama City Univ, Yokohama, Japan

PLOS ONE (the Public Library of Science) was launched in 2006 to progress in science and medicine by leading a transformation in research communication. PLOS ONE is the world's first multidisciplinary open access journal and accepts scientifically rigorous research, regardless of novelty, including qualitative researches of basic and clinical science, submissions describing methods, software, and databases, systematic reviews, and studies reporting negative results. It is the policy of PLOS ONE that judgements about importance of any paper are made after publication by the readers, not to delay the publication of work that later proves to be of major significance. To date, PLOS ONE has published more than 135,000 articles since 2006. The number of research papers published PLOS ONE per year increased until 2013 (31,509 articles) and slightly fell after 2014. In 2015, it published 28,107 articles. The top 5 research fields published in PLOS ONE are biochemistry, cell biology, anatomy, organisms, and genetics, followed by physiology. The journal accepts any length of articles including full color throughout and multimedia files. Reuse of articles is subject to a Creative Commons Attribution License, version 2.5. At PLOS ONE, the median review time has grown from 37 days to 125 days over the first ten years of operation, which roughly correspond to the industry-wide averages for biology-related journals. PLOS ONE adopted Article-Level Metrics program and registered readers can leave comments on articles and authors are able to know reactions from readers on the website. (COI:No)

2PS11E3-2

The Journal of Neuroendocrinology as an official journal of International Neuroendocrine Federation

Yoichi Ueta

Dept Physiol, Sch Med, Univ Occup Environ Health, Kitakyushu, Japan

The Journal of Neuroendocrinology as an official journal of International Neuroendocrine Federation (President, Prof. Robert Millar (University of Pretoria, South Africa) since January 2016) has been established since 1989. Founding editor was Prof. Stafford Lightman (Charing Cross and Westminster Medical School, and then University of Bristol) (Editor-in-Chief: 1989-1996), Prof. Gareth Leng (University of Edinburgh) (Editor-in-Chief: 1997-2003), Prof. Julia Buckingham (Imperial College London)(Editor-in-Chief: 2004-2008), Prof. Dave Grattan (University of Otago, New Zealand) (Editor-in-Chief: 2009-2013) and Prof. Julian G. Mercer (University of Aberdeen) (Editor-in-Chief: 2014-present). Firstly, the journal was published bi-monthly (1989-1994), monthly (1995-present), and have become an online journal since 2014. I have been working as one of the editorial board members since 2000. Recent impact factors in 2013, 2014 and 2015 were 3.507, 3.138 and 3.172, respectively. The journal has published the high quality papers in the field of neuroendocrinology by peer reviews working very well since founding. ISI Journal Citation Report Ranking in 2015 shows 53/131 (Endocrinology & Metabolism) and 100/256 (Neuroscience). As a reference, other Endocrine/Neuroendocrine-related journals' impact factors in 2015 are shown as follow: Endocrinology (4.159), Journal of Endocrinology (4.038), Neuroendocrinology (2.583) and American Journal of Physiology-Endocrinology and Metabolism (3.825). (COI:No)

2PS11E3-3

What is the next step for JPS

Yoshihiro Ishikawa

CVRI, Yokohama City Univ Sch Med, Yokohama, Japan

The Journal of Physiological Sciences is the Official and English journal of the Physiological Society of Japan. Its origin goes back to 1927, when the Japanese Journal of Medical Sciences, Biophysics was founded. The Journal was officially established by the Society as the Japanese Journal of Physiology in 1955. It then changed its name to the Journal of Physiological Sciences, with the aim to grow from a domestic to worldwide journal, in 2007. The Journal emphasizes human and vertebrate physiology, but considers comparative papers as well. The Journal has now grown to become the leading journal in Asia. In the FAOPS 2016, the Journal served as the Official Journal of the Meeting. It will also serve so in the FAOPS 2020, which will be held in Japan. The impact factor of the Journal has increased to above 2 in 2016, which indicates that the Journal has been recognized as the leading Journal in the field of Physiology. The Journal is ready to jump up to the next step. With the increasing demand for open access journal, the Journal is going to switch its form of publication from paper based to electronic journal. It also aims to publish all articles in the form of open access so that everyone could have an easy and quick access to our publications. In the symposium, we will discuss the issues related to these future changes with editors from other journals. We appreciate the attendance of young and senior scientists so that we work together to further grow up our historical Journal of the Society. (COI:No)

Planned Symposium 12

Joint Symposium with
Society for Taurine Research

Diverse physiological
actions of taurine

March 29 (Wed), 16:50 – 18:50, Hall H

2PS12H3-1

Taurine ameliorates obesity by regulating adipocyte inflammatory response in mice

Shigeru Murakami

Dept Biosci, Fukui Pref Univ, Fukui, Japan

Taurine is a sulfur-containing amino acid that is not incorporated into protein. Taurine has been shown to play an important role in maintaining cellular physiological homeostasis. The obese state is known to be a chronic inflammatory condition characterized by adipocyte macrophage infiltration and increased production of pro-inflammatory cytokines. We examined the effects of taurine supplementation on obesity with a particular focus in adipose tissues. Obesity was induced by administering a high-fat diet to C57BL/6J mice. Taurine was mixed into the diet and supplemented to mice for 14 weeks. The high-fat diet markedly increased the weights of the body and adipose tissue, which were accompanied by enlarged hypertrophied adipocytes, increased infiltration of M1 macrophages, and elevated production of pro-inflammatory cytokines in adipose tissue. Taurine supplementation suppressed these events. In vitro studies using bone-marrow-derived macrophages revealed that taurine and taurine chloramine, which was physiologically generated from taurine in activated neutrophils, reduced the expression of M1 macrophage markers, increased the expression of M2 macrophage markers, and inhibited the production of pro-inflammatory cytokines. These findings suggest that taurine and taurine chloramine may modulate the polarization of macrophages from inflammatory M1 to an anti-inflammatory M2. This is associated with the amelioration of adipose tissue inflammation and the prevention of obesity and insulin resistance. (COI:No)

2PS12H3-2

Effect of taurine and BCAA intake on muscle damage after exercise

Hajime Ohmori

Faculty Health Sport Sci, Univ Tsukuba, Tsukuba, Japan

Eccentric exercise (ECC) results in delayed onset muscle soreness (DOMS). The pain may prevent beginners from continuing an exercise and athletes from training at higher intensity. Although previous studies have evaluated the effectiveness of branched-chain amino acid (BCAA) intake for attenuating DOMS and muscle damage induced by ECC, their findings have been inconclusive. The aim of this study was to clarify the combined effect of BCAA and taurine (TAU), which has anti-inflammatory and anti-oxidative effects, on DOMS and muscle damage. Thirty-six young untrained male subjects were assigned to 4 groups (placebo + placebo [placebo], BCAA + placebo, placebo + TAU, and BCAA + TAU [combined]) and given a combination of 3.2 g BCAA (or placebo) and 2.0 g TAU (or placebo), 3 times a day, for 2 weeks prior to and 3 days after elbow flexor ECC. DOMS and muscle damage in the biceps brachii were evaluated using the visual analogue scale (VAS), upper arm circumference (CIR), and blood parameters (creatine kinase, lactate dehydrogenase [LDH], aldolase, and 8-hydroxydeoxyguanosine [8-OHdG]). In the combined group, VAS and 8-OHdG 2 days after ECC, CIR 2 and 3 days after ECC and LDH from 1 to 3 days after ECC were significantly lower than those in the placebo group. The areas under the curve from before ECC to 4 days later for CIR, LDH, and aldolase were also significantly lower in the combined group than those in the placebo group. In conclusion, a combination of 3.2 g BCAA and 2.0 g taurine, 3 times a day, for 2 weeks prior to and 3 days after high-intensity ECC may be a useful nutritional strategy for attenuating exercise-induced DOMS and muscle damage. (COI:No)

2PS12H3-3

Maternally-derived taurine regulates the intrinsic properties of the neural progenitors in the mouse developing neocortex

Shiro Tochitani

Res Cent Child Mental Dev, Univ Fukui, Fukui, Japan

Mammalian fetuses and infants can only synthesize limited amounts of taurine. They are dependent on taurine given from their mothers via the placenta or breast milk. Precise regulation of the properties of neural progenitors to produce the diverse cell types in in specific temporal patterns is essential for the histogenesis of central nervous system. We previously obtained evidences that $GABA_A$ receptors participate in this regulatory machinery. Both GABA and taurine are known as endogenous ligands for GABAA receptors. HPLC quantification showed that taurine is dominant in quantity in the developing cortex before E14. We tested whether taurine is involved in the regulation of the cellular properties of neural progenitors. The decrease in the frequency of the Tbr2-positive basal progenitors, the increase in the number of Pax6-positive apical progenitors and the decrease in the thickness of Doublecortin-positive layers were observed in the neocortices of the E13.5 embryos obtained from the dams injected with D-cysteine sulfinic acid (DCSA), an inhibitor of taurine synthesis. The differentiation into Satb2-positive upper-layer neurons was suppressed, while the differentiation into Tbr1-positive deep-layer neurons was enhanced in the E13.5 neocortices exposed to the taurine-deficient condition induced by DCSA. Ca^{2+} imaging studies showed that neural progenitors responded to the applied taurine via picrotoxin-sensitive receptors. These results suggest that maternally-derived taurine regulates the cellular properties of NSCs in the early phase of cortical development. (COI:No)

2PS12H3-4

Taurine depletion reduces postsynaptic $GABA_A$ receptors in layer 2/3 pyramidal neurons of the somatosensory cortex

Yasushi Hosoi[1], Tenpei Akita[2], Miho Watanabe[2], Hiroki Mutoh[2], Hiroaki Miyajima[1], Atsuo Fukuda[2]

[1]First Dept Intern Med, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan

Taurine, an amino sulfonic acid, is abundant in the central nervous system. Taurine can work as an agonist of $GABA_A$ and glycine receptors ($GABA_A$Rs, GlyRs), and intracellular taurine imported by taurine transporter (TauT; SLC6A6) may also activate some kinases, such as WNK1. In this study, we investigated whether fetal and intracellular taurine depletion affects postnatal development of neural circuits and synapses by examining layer 2/3 pyramidal neurons in the somatosensory cortices of TauT knockout (KO) mice at postnatal day 21 ± 2 with the whole-cell patch-clamp technique and immunohistochemistry. Analysis of miniature inhibitory postsynaptic currents indicated a decrease in amplitude in TauT KO mice. On the other hand, tonic currents mediated by extrasynaptic $GABA_A$Rs and GlyRs did not differ between genotypes. Analysis of dose-response to GABA showed no differences in sensitivity of $GABA_A$R between genotypes but smaller $GABA_A$R-mediated currents in TauT KO mice. Furthermore, immunofluorescence intensity of $GABA_A$R gamma2 subunit was decreased in TauT KO mice. These results suggested that the numbers of postsynaptic $GABA_A$Rs were reduced in TauT KO mice. Taken together, our results indicate that taurine depletion may affect trafficking or recycling of $GABA_A$Rs in layer 2/3 pyramidal neurons of the somatosensory cortex. (COI:No)

Planned Symposium 13

Co-sponsored by the MEXT "Oscillology"

Nonlinear and oscillatory phenomenon in neurophysiology

March 30 (Thu), 8:50 – 10:50, Hall B

3PS13B1-1

Glial regulation of neuronal oscillations

Ko Matsui

Div Interdisciplinary Med Sci, Grad Sch Med, Tohoku Univ, Sendai, Japan

As the membrane potential of astrocytes fluctuates only slightly, it has been considered that astrocytes cannot serve as a medium of much information. However, using recently developed fluorescent probes, dynamic changes in the astrocytic ionic environment have been demonstrated. To reveal the role of astrocytes in brain function, the existence of astrocyte-to-neuron signaling pathway triggered by such astrocytic ionic changes needs to be shown. This study requires an on-demand control of astrocyte activity and, thus, we have created transgenic mice with astrocyte specific expression of light-sensitive membrane protein, channelrhodopsin-2 (ChR2) or archaerhodopsin (ArchT). We noticed that activation of these tools results in acidification and alkalization of the cytosol, respectively, as the main cation that they convey across the plasma membrane is proton. By artificially lowering the intracellular pH buffering capacity, we show that astrocytic ChR2 photoactivation results in more intracellular acidification of astrocytes and enhanced release of glutamate. Using this tool for perturbation of astrocytic function, we show that neuronal oscillatory signals are also affected by changes in the astrocytic state. We also report that optogenetic 'neuronal' stimulation can convert the brain initially to a state prone to hyperexcitability but subsequent stimulation produced a state that is resistant to seizure induction. Such plastic change in the state of the brain could be due, in part, to the plastic changes of astrocyte function. (COI:No)

3PS13B1-2

The roles of ventral striatal Ca^{2+} oscillations in goal-directed behavior

Iku Kimura[1,2], Akiyo Natsubori[3], Hiroshi Nishida[1], Kenji F Tanaka[1]

[1]Dept Neuropsychiatry, Sch Med, Keio Univ, Tokyo, Japan, [2]JSPS Research Fellow, Tokyo, Japan, [3]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

Goal-directed behavior relies on the initiation and sustainment of appropriate actions. It is hypothesized that the ventral striatum mediates goal-directed behavior. However, the cell-based mechanism regulating behavioral components is largely unknown. In this study we focused on two distinct cell populations: dopamine receptor type 1-expressing medium spiny neurons (D1-MSNs) and D2-MSNs, and questioned if either activity encoded the behavioral component. To understand the temporal activity pattern of each cell population during goal-directed behavior, we respectively recorded D1 and D2-MSN activities by the fiberphotometry system that can detect compound intracellular Ca^{2+} dynamics. We found concurrent event-related Ca^{2+} elevations in D1- and D2-MSNs, especially at trial start-related and first lever press-related timings. This oscillatory pattern, called Ca^{2+} oscillation, repeated trial by trial. To examine the roles of Ca^{2+} oscillation in goal-directed behavior, we manipulated their activities via optogenetic inhibition during ongoing behaviors. Optogenetic inhibition of either cell type after trial start prolonged the latency for initiation. The inhibition of D1-, but not D2-, MSNs after initiation also impaired ongoing goal-directed behavior. Taken together, D1- and D2-MSNs exhibited the concurrent Ca^{2+} oscillations, which were necessary to initiate and sustain goal-directed behavior. (COI:No)

3PS13B1-3

Ion channels for the resonant property of neurons

Kouichi Hashimoto[1], Yoshiko Makidono[1], Hisako Nakayama[1], Miwako Yamasaki[2], Taisuke Miyazaki[2], Kazuto Kobayashi[3], Masahiko Watanabe[2], Masanobu Kano[4], Kenji Sakimura[5]

[1]Dept Neurophysiol, Grad Sch Biomed Health Sci, Hiroshima Univ, Japan, [2]Dept Anat, Grad Sch Med, Hokkaido Univ, Japan, [3]Dept Mol Gen, Grad Sch Med, Fukushima Med Univ, Japan, [4]Dept Neurophysiol, Grad Sch Med, Univ Tokyo, Japan, [5]Dept Cell Neurobiol, Brain Res Institute, Niigata Univ, Japan

Some neurons have the ability to enhance output voltage to input current with a preferred frequency, which is called resonance. Resonance is thought to be a basis for membrane potential oscillation. Although ion channels responsible for resonance have been reported, the precise mechanisms by which these channels work remain poorly understood. In this study, we examined ion channels involved in resonance in inferior olivary (IO) neurons. Whole-cell recording was conducted from IO neurons in slices prepared from the mouse medulla. Resonance was reduced but clearly present in Cav3.1 T-type voltage-dependent Ca^{2+} channel knockout (KO) mice. Activation of Cav3.1 channels was strongly membrane potential-dependent, but less frequency-dependent. Remnant resonance in Cav3.1 KO mice was blocked by a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker, ZD7288. Resonance was inhibited by ZD7288 in wild-type mice and impaired in HCN1 KO mice, suggesting that the HCN1 channel was essential for resonance. Waveform of the ZD7288-sensitive current was nearly sinusoidal and strongly frequency-dependent. These results suggested that Cav3.1 and HCN1 channels act as amplifying and resonating conductances, respectively. (COI:No)

3PS13B1-4

Optical imaging of the intrinsic signal revealed a wave-like propagation of the infra-slow oscillation over the rat cortex

Tomokazu Ohshiro, Hajime Mushiake

Dept Physiol, School of Medicine, Tohoku Univ, Sendai, Japan

Distinct rhythmic oscillations of the brain activity at various frequency ranges (e.g., δ: 1 to 4Hz, α: 8 to 15Hz) are recognized, and neural basis of these oscillations have been well characterized. However, the nature and origin of the infra-slow oscillation, a much slower rhythm (about 0.1Hz) are not well understood. Here, we report that an oscillation at this slow range can be detected by optical imaging of the intrinsic signal on the anesthetized rat cortex. The rat was anesthetized with isoflurane, and the skull was thinned by drilling. The cortex was illuminated through the thinned bone with green light, and the reflected light was detected using a CMOS digital camera. The contrast-enhanced image of the cortex showed that the intrinsic signal was distributed unevenly over the cortex with 1 to 2 peak and trough(s), which propagated like waves at 4mm/sec, mostly along the rostral-caudal direction. The intrinsic signal was spontaneously oscillated at about 0.1 Hz. The wave-like spread of the intrinsic signal likely reflects a coordinated vasomotion of the arteries over the cortex, because arteries repeated dilation/constriction at that slow frequency along with the oscillating intrinsic signals. Mechanical whisker stimulation only with the interval of 6 to 8 sec could successfully phase-lock the initiation of the wave propagation, indicating a fixed time-constant of the dilation/constriction. A preliminary result indicated that the carbon dioxide generated locally by neural tissues is involved in the wave generation though an effect on the vasomotion. (COI:No)

3PS13B1-5

Manipulative approaches to nonlinear neural oscillations in the human brain

Keiichi Kitajo[1], Motomu Katsurakawa[1,2]

[1]BSI-Toyota Collaboration Center, RIKEN BSI, Wako, Saitama, Japan, [2]Univ Tokyo Grad Sch Med, Tokyo, Japan

A number of studies indicate that synchronous neural oscillations play significant roles in specific brain functions such as perception and cognition. However, the causal evidence is still missing. We, therefore, investigated the causal relationship between neural oscillations and functional information processing in the human brain using electroencephalography (EEG) combined with transcranial alternating current stimulation (tACS), which can directly modulate rhythmic neural activity in the intact human brain. We delivered tACS over the scalp of healthy human participants and analyzed the changes in task performance and task-related neural oscillations in binocular rivalry and mental arithmetic tasks. Critically, we found improved task performance accompanied by enhanced peak power of EEG activity after delivering tACS in both tasks. The results indicate that there is a causal link between neural oscillations and information processing for conscious perception of visual stimuli in binocular rivalry and mental arithmetic. We speculate that tACS shaped frequency-specific neural circuits associated with information processing in both tasks. The combined tACS-EEG method can potentially show causal roles of nonlinear neural dynamics in various brain functions. (COI:No)

3PS13B1-6

Dopamine and dynamic equilibrium of reinforcement learning

Kenji Morita

Physical and Health Education, Grad Sch Edu, Univ Tokyo, Tokyo, Japan

Dopamine (DA) is crucially involved in both motor and reward processes in the brain. Regarding the reward-related functions of DA, a traditional view was that two temporally distinct patterns of DA, phasic and tonic, represent two different elements of reward processes, namely, reward prediction error (RPE) and motivation or vigor. However, this view has been challenged by recent work. Specifically, a new type of DA signals that gradually ramps towards a reward-associated goal in self-paced behavior has been reported, and suggested to represent a sustained motivation towards the goal (Howe et al., 2013, Nature). We have shown, through computational modeling, that if the learned reward values, stored in synaptic strengths, decay in time, RPE should show a ramping pattern that resembles the observed ramping DA signals, and suggested that the ramping DA might thus be indicative of reinforcement learning (RL) with decay or forgetting (Morita & Kato, 2014, Front Neural Circuits). Moreover, we have recently shown that forgetting in RL can facilitate fast goal-reaching in self-paced behavior, and also incorporation of forgetting into the current neural circuit theory of DA's function in RL can mechanistically explain experimental results that suggest DA's roles in motivation, including the motivational impairments caused by DA depletion (Kato & Morita, 2016, PLOS Comput Biol). Given these results, we propose that when the reward learning system in the brain is active, it might be in a dynamic equilibrium where learning and forgetting are balanced. (COI:No)

3PS13B1-7

Increased LFP θ power in primate motor areas reflects memorization of movement

Ryosuke Hosaka[1], Hidenori Watanabe[2], Toshi Nakajima[2], Hajime Mushiake[2]

[1]Dept Applied Math, Fukuoka Univ, [2]Dept Physiol, Tohoku Univ Sch Med, Sendai, Japan

In our life, preferential works are often interrupted by boring ones and then we come back to the original works.To deal with both works, we keep the original in mind, complete the interruption, and resume the original.However, it is unclear how brain function contributes to keep and retrieve the original works.Here we shows that the θ frequency band (< 6Hz) of LFPs in motor cortex of two monkeys reflects keeping of the original works during the interruption ones. The monkeys were demanded blocked interruption task, and each block consisted of four trials.On visually guided (VIS) trials, the movement was instructed by a visual cue and the monkeys performed and memorized the movement.On memory-guided (MEM) trials, the same movement as VIS trial was performed without the visual guidance.On interruption trials, the same or different movement as VIS trial was instructed by visual cue and was performed.On retrieval trials, the monkeys performed the same movement as VIS trial again. Power of θ band increased in MEM trials and kept increased during interruption trials.The increase of θ power was significantly greater in interruption than VIS trials in the pre-SMA ($p<0.05$, t-test).On retrieval trials, θ power was recovered to the baseline in one monkey.LFP θ power might contribute to movement memorization during interruption and subsequent retrieval.Our experiment was performed under the Guidelines for Institutional Animal Care and Use published by Tohoku Univ. (COI:No)

Planned Symposium 14

Japan-China Joint Symposium
– Towards FAOPS2019 –

Progress in computational physiology

March 30 (Thu), 8:50 – 10:50, Hall C

3PS14C1-1

Simulation study of multiscale Ca^{2+} signals

Jianwei Shuai

Dept Physics, Xiamen Univ, China

The calcium ion (Ca^{2+}) acts as a ubiquitous cellular messenger to regulate a wide variety of cellular processes, such as gene expression, neural synapse function, and morphology. The main mechanism modulating cytosolic Ca^{2+} concentration ([Ca^{2+}]) in non-excitable cells is the Ca^{2+} release through the inositol 1,4,5-trisphosphate receptor (IP_3R) channel localized on the membrane of the endoplasmic reticulum (ER). The Ca^{2+} fluorescence experiments indicate that IP_3Rs are non-uniformly distributed on the ER membrane. In many cell types, the functioning channels are grouped into channel clusters within a domain of hundreds of nanometers. The IP_3R clusters are separated by a few micrometers. As a result of this spatial heterogeneity, Ca^{2+} signals display a multiscale spatio-temporal organization, which grows from the opening of a single IP_3R (i.e. blips), into concerted activation of a few IP_3Rs within a cluster to generate slightly larger Ca^{2+} elevations (i.e. puffs), and ultimately ignites global waves via cluster-cluster interactions in response to different [IP_3]. Different simulation models have been suggested for the discussion of dynamics of blips, puffs and global waves. With the models, the stochasticity of blips, the initiation and termination of puffs, and the periodicity of global waves have been investigated. The integration dynamics of stochastic local Ca^{2+} signals into periodic oscillations of cell-wide release is discussed with the simulation. (COI:No)

3PS14C1-2

Experimental and model analysis of transmitter release mechanisms at small presynaptic terminals in the CNS

Shinya Kawaguchi

Grad Sch Brain Sci, Doshisha Univ, Kyoto, Japan

Neuronal transmission relies on the very rapid neurotransmitter release at small synapses in the central nervous system. The small size and rapid kinetics of the presynaptic terminals make it difficult to study the presynaptic mechanisms of transmitter release. Direct patch-clamp recordings from exceptionally large presynaptic terminals, such as that in calyx of Held and a hippocampal mossy fiber bouton, have clarified how presynaptic transmitter release is controlled. However, it is unknown whether the same mechanisms are operating also at conventional synapses with a size of 1 μm. To address this issue, we have attempted to directly record from a small presynaptic structure of the axon of cerebellar granule cells, a parallel fiber bouton, using dissociated culture of cerebellar neurons. Fluorescent labeling of a cultured granule cell enabled us to target a patch pipette to a small axonal varicosity (1 μm). We recorded presynaptic Ca^{2+} currents through voltage-gated Ca^{2+} channels, membrane capacitance changes, and postsynaptic currents. From these electrophysiological data, the number and type of Ca^{2+} channels in a single presynaptic bouton, the size of readily releasable pool of synaptic vesicles, and the regulatory mechanisms for synaptic transmission were examined. These experimental data enabled us to develop a computational model of presynaptic transmitter release regulation in a small bouton. In this symposium, I would like to demonstrate the comprehensive view of presynaptic function in the central nervous system obtained by the combined application of experiments and modeling. (COI:No)

3PS14C1-3

A simulation study on Ca^{2+} regulation of energy metabolism in cardiac mitochondria

Satoshi Matsuoka[1], Ayako Takeuchi[1], Ryuta Saito[2], Yukiko Himeno[3]

[1]Dept Integ Sys Physiol Fac Med Sci Univ Fukui, Fukui, Japan, [2]Adv Drug Res Lab Mitsubishi Tanabe Pharma Co. Saitama, Japan, [3]Dept Bioinfo, Col Life Sci, Ritsumeikan Univ, Shiga, Japan

The cardiac energy metabolites such as ATP, ADP and NADH are kept relatively constant during physiological cardiac workload transition. Ca^{2+} has been thought as a regulator of the constancy, though its details are not yet clarified. To elucidate the roles of Ca^{2+} in the metabolite constancy, we constructed and analyzed a detailed mathematical model of cardiac mitochondria based on experimental data. The model well reproduced the experimental data on Ca^{2+}- and inorganic phosphate-dependencies of oxygen consumption, NADH level, and mitochondrial membrane potential, under the *in vitro* condition of isolated mitochondria with malate/glutamate used as substrates. When the model was incorporated into a simple cardiac cell model and malate/glutamate were used as substrates, the metabolite constancy during workload transition could be maintained at physiological cytosolic Ca^{2+} concentration, but not at Ca^{2+} below physiological range, and NADH level was Ca^{2+}-dependent. However, the Ca^{2+}-dependency of NADH almost disappeared and energy metabolites became more stable under the *in vivo* condition that malate, glutamate, pyruvate, citrate and 2-oxoglutarate were used as substrates. It was revealed that mitochondrial substrates have significant influence on metabolite constancy during cardiac workload transition, and Ca^{2+} has only a minor role under physiological conditions on the metabolite constancy. (COI:No)

3PS14C1-4 (AP4)

Physiological and pathophysiological modeling studies of excitation conduction in the heart: prediction from changes in the subcellular Na^+ channel distribution

Kunichika Tsumoto, Yoshihisa Kurachi

Div Pharma, Grad Sch Med, Osaka Univ, Osaka, Japan

The contractile function in the heart results from the accurate propagation of action potentials (APs) in cardiomyocytes. The voltage-gated sodium (Na^+) channels, playing key roles in AP initiation and propagation, alter the distribution within a myocyte in congenital and acquired heart diseases. We hypothesize that an alteration in subcellular Na^+ channel distribution may lead to the development of lethal arrhythmias. To test this hypothesis, we have proposed physiologically relevant *in silico* ventricular myofiber 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 have investigated the relationship between the altered subcellular Na^+ channel distribution and arrhythmogenicity through a computer simulation of AP propagation in the myofibers. 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 distributions. (COI:No)

Planned Symposium 15

Novel roles of GABA in the hypothalamic function

March 30 (Thu), 8:50 – 10:50, Hall D

3PS15D1-1

Chronological embryonic development of GABAergic networks in the hypothalamic nuclei involved in feeding behavior

Chitoshi Takayama, Chigusa Shimizu

Dept Mol Anat, Sch Med, Univ Ryukyus, Okinawa, Japan

The hypothalamus is the control center for energy expenditure and food intake. The neurotransmitter,gamma-amino butyric acid (GABA), plays important roles in energy balance and feeding behavior in the hypothalamus. In the present study, to reveal the time course of GABAergic network maturation in the hypothalamus, we performed immunohistochemistry for glutamic acid decarboxylase (GAD), a marker of GABAergic terminals, vesicular GABA transporter (VGAT), a marker of inhibitory terminals, and K^+-Cl^- cotransporter2 (KCC2), a marker of GABAergic inhibition. GABAergic terminals, detected as GAD- and VGAT-positive dots, increased in number during development. Localization of the KCC2 was almost concomitant with GABAergic terminal formation except in paraventricular nucleus (PVN), suggesting that GABAergic synapses formation may be the start of GABAergic inhibition in the three nuclei. The GABAergic networks matured in the following chronological order. The lateral hypothalamus matured first, and the PVN followed, with their maturation finishing before birth. These are involved in increasing food intake. However, density of the GABAergic terminals was still low in the ventromedial hypothalamus (VMH) on the day of birth and were few in the arcuate nucleus (Arc), suggesting that the VMH, involved in suppressing food intake, and the Arc, the control center, were still immature at birth. Overall, the present study showed that GABAergic networks in the "feeding center" matured before birth, while those in the VMH, the "satiety center" and higher control center, matured after birth. (COI:No)

3PS15D1-2

Activation of GABAergic neurons in dorsomedial hypothalamus promotes food intake

Shigetomo Suyama[1], Zesemdorj Otgonuul[1], Hiroshi Onodera[2], Toshihiko Yada[1]

[1]Div IntegrativePhysiol, Dept Physiol, Jichi Med Univ sch Med, Tochigi, Japan, [2]Photon Sci Cen Univ Tokyo, Dept Electric Engineering, Univ Tokyo, Tokyo, Japan

Dorsomedial hypothalamus (DMH) has been known as orexigenic center, since its lesion decreases food intake and body weight. However, principal orexigenic neuron in DMH remains unknown. In the present study, we used optogenetics and demonstrated that the GABAergic neuron serves as a principal orexigenic neuron in DMH. To manipulate activity of GABAergic neurons in DMH, the mice expressing ChRFR(C167A), channelrhodopsin variant, specifically in DMH-GABAergic neuron were produced. Light exposure to the DMH increased food intake. The DMH GABAergic neurons projected to the paraventricular nucleus (PVN) of hypothalamus where anorexigenic neurons are located. Light exposure to the PVH increased inhibitory postsynaptic current on PVH neurons and increased food intake. The GABAergic neurons in DMH were depolarized by lowering extracellular glucose and hyperpolarized by leptin administration. These results indicate that DMH-GABAergic neurons are activated in hunger state and promotes food intake via inhibition of PVN anorexigenic neurons. DMH-GABAergic neurons serve as the principal orexigenic neuron.There is no conflict of interest to declare. (COI:No)

3PS15D1-3

The role of excitatory action of GABA in the regulation of reproduction

Miho Watanabe[1], Junichi Nabekura[2,3], Atsuo Fukuda[1]

[1]Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]Dept Homeostatic Develop, Natl Inst Physiol Sci, Okazaki, Japan, [3]Dept Physiol Sci, SOKENDAI, Hayama, Japan

Gonadotropin-releasing hormone (GnRH) neurons form the final common pathway for the central regulation of reproduction. γ-amino butyric acid (GABA) has long been implicated as one of the major players in the regulation of GnRH neurons. Although GABA is typically an inhibitory neurotransmitter in the mature adult central nervous system, we previously reported that most mature GnRH neurons show the unusual characteristic of being excited by GABA. GABA acts excitatory when $[Cl^-]_i$ is high, due to the low expression of the K^+-Cl^- cotransporter (KCC2), which excludes Cl^- from neurons and maintains GABAergic synaptic inhibition. To investigate the functional role of the excitatory action of GABA in GnRH neurons *in vivo*, we generated new transgenic mice (GnRH-tTA; KCC2-tetO) in which KCC2 can be induced restricted in GnRH neurons using tetracycline controlled gene expression system. Using this mice, GABA action to GnRH neurons could be modulated *in vivo* restricted in GnRH neurons reversibly in specific time point. GnRH-tTA; KCC2-tetO mice failed to exhibit estrous cyclicity, ovulation and pregnancy. Ovarian histology revealed an abundance of small follicles. Furthermore, GnRH-tTA; KCC2-tetO mice showed advanced puberty onset. These results suggest that the excitatory action of GABA on GnRH neurons has an important role in the female reproduction. (COI:No)

3PS15D1-4

A novel role of GABA in the release of CRH in the hypothalamic-pituitary pathway

Keisuke Kakizawa[1], Atsuo Fukuda[2]

[1]Second Dept Med, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan

Corticotropin releasing hormone (CRH), which is synthesized in the paraventricular nucleus (PVN) of the hypothalamus, plays an important role in the endocrine stress response. In non-stressful conditions, γ-aminobutyric acid (GABA)-ergic inputs exert an inhibitory action on CRH neurons in the PVN. This GABAergic inhibition requires relatively low intracellular Cl^- concentrations ($[Cl^-]_i$), which are maintained by K^+-Cl^- cotransporter (KCC2). In our latest study, by using heterozygous GAD67-GFP knock-in mice which exhibited decreased GABA content, we demonstrated that the release of CRH was impaired. The $GABA_A$ receptor ($GABA_AR$) and Na^+-K^+-$2Cl^-$ cotransporter (NKCC1), but not KCC2, were expressed in the terminals of the CRH neurons at the median eminence (ME). In contrast, CRH neuronal somata were enriched with KCC2 but not with NKCC1. Thus, $[Cl^-]_i$ may be increased at the terminals of CRH neurons compared with concentrations in the cell body. Moreover, vesicular GABA transporter-containing GABAergic terminals projecting from the arcuate nucleus (ARC) identified by retrograde labeling, were present in close proximity to CRH-positive nerve terminals. Furthermore, in the CRH-GCaMP3 mice, a $GABA_AR$ agonist increased the $[Ca^{2+}]_i$ levels in the CRH neuron terminals but decreased the $[Ca^{2+}]_i$ levels in their somata. Additionally, the increases in $[Ca^{2+}]_i$ were prevented by an NKCC1 inhibitor. Here, we propose a novel mechanism by which the excitatory action of GABA promote CRH release from axon terminals with NKCC1-driven high $[Cl^-]_i$ in the ME. (COI:No)

3PS15D1-5

Roles of GABA in the circadian clock in the mouse suprachiasmatic nucleus

Daisuke Ono[1], Ken-ichi Honma[2], Yuchio Yanagawa[3], Sato Honma[2]

[1]Neurosci Res, RIEM, Nagoya Univ, Nagoya, Japan, [2]Res & Educ Center Brain Sci, Hokkaido Univ, Grad Sch Med, Sapporo, Japan, [3]Dep of Genet & Behav Neurosci, Gunma Univ Grad Sch Med, Maebashi, Japan

In mammals, the suprachiasmatic nucleus (SCN) in the hypothalamus plays a critical role in the expression of circadian rhythms. In individual SCN cells, circadian rhythms with intrinsic period are generated by a transcription/translation negative feedback loop involving several clock genes and their protein products. Circadian rhythms of individual cells are diverse in the period and amplitude. However, they are integrated by the SCN neural network to show a coherent circadian rhythm in the output signals such as spontaneous firing, and finally in behavior, hormones and autonomic nervous activities. The SCN contains a number of neurotransmitters which play important roles in organizing the neural network. Among them, GABA is expressed in almost all SCN neurons. Although several studies proposed possible functions of GABA, roles of GABA in the SCN circadian clock are still largely unknown. In the present study, we genetically disrupted the GABA release by knocking out the vesicular GABA transporter (Vgat) as well as the GABA synthesis by knocking out the glutamic acid decarboxylase (GAD) 65 and GAD67, and measured molecular circadian oscillation together with its output signals in the cultured SCN. Simultaneous multifunctional measurement (bioluminescence imaging of PER2::LUC, spontaneous firing with Multi-electrode array dish, and intracellular calcium with GCaMP6) revealed that GABA is involved in the stabilization of circadian outputs from the SCN. (COI:No)

Planned Symposium 16

Joint Symposium with
the Japanese Association of Anatomists

Biology in 'functional recovery'

March 30 (Thu), 8:50 – 10:50, Hall E

3PS16E1-1

The morphological and functional biology of nerve regeneration

Hiroshi Kiyama

Dept Functional Anatomy & Neurosci, Nagoya Univ, Grad Sch Med, Nagoya, Japan

Peripheral nervous system has a higher potential in functional recovery after traumatic and inflammatory nerve injury. Understanding of the molecular mechanisms underlying this higher potential could be useful for therapeutic strategies to repair injured CNS. During last two decades we have identified hundreds of molecules whose expressions were crucial for proper nerve regeneration after nerve injury. In this symposium I briefly introduce molecular composition and their expression regulation, which allow proper survival and nerve regeneration, and then more precisely address recent topics on the neuron non-autonomous repair mechanism: neuron and non-neuronal cell interactions. For the proper regeneration, involvements of microglia in CNS and Schwann cells in PNS are vital. Our recent data demonstrated variety of lipid mediators such as fatty acids and lysophospholipids play crucial roles as determinants of microglial phenotypes, and these lipids therefore could be potent targets to manipulate microglial morphology and function. As for the neuron-Schwann cell mediator, a neuron specific metallopeptidase DINE/ECEL1 (damage induced neuronal endopeptidase) is addressed. We recently found that the enzymatic activity of DINE is associated with axon-Schwann cell interaction and critical for proper nerve branching and subsequent NMJ formation in target muscles. Although we have not revealed overall molecular mechanism underlying nerve regeneration yet, the accumulated data are providing us with several therapeutic targets to promote CNS repair as well as biological interests. (COI:No)

3PS16E1-2

Requirement of exocrine tissue formation for proper endocrine development in murine pancreas

Yoshiya Kawaguchi

Center for iPS Cell Research and Application, Kyoto Univ Kyoto, Japan

The mature pancreas is composed of two functional components: exocrine and endocrine tissue. Both tissue types originate during embryonic organogenesis from a common pool of multipotent pancreatic progenitors located within the pancreatic buds, however, the interdependence of these two cell types during their formation is not well understood. In this study, we generated mutant mice, in which the exocrine tissue is hypoplastic, in order to reveal a possible requirement for exocrine pancreas tissue in endocrine development and/or function. Since previous studies showed an indispensable role for Pdx1 in pancreas organogenesis, we used Elastase-Cre-mediated recombination to inactivate Pdx1 in the pancreatic exocrine lineage during embryonic stages. Along with exocrine defects, including impaired acinar cell maturation, the mutant mice exhibited substantial endocrine defects, including disturbed tip/trunk patterning of the developing ductal structure, a reduced number of Ngn3-expressing endocrine precursors, and ultimately fewer beta-cells. Notably, postnatal expansion of the endocrine cell content was extremely poor, and the mutant mice exhibited impaired glucose homeostasis. These findings suggest the existence of an unknown but essential factor(s) in the adjacent exocrine tissue that regulates proper formation of endocrine precursors and the expansion and function of endocrine tissues during embryonic and postnatal stages. (COI:No)

3PS16E1-3

Protein kinase Cγ plays a crucial role in motor function in mature cerebellum

Hirokazu Hirai, Nobutaka Takahashi, Masashi Watanabe, Ayumu Konno

Dept Neurophysiol & Neural Repair, Gunma Univ Grad Sch Med, Gunma, Japan

Protein kinase C (PKC), a family of serine- and threonine-specific protein kinases, plays a variety of regulatory roles in physiological functions. Classical PKCs (PKCα, PKCβ and PKCγ) are activated by calcium and second messenger diacylglycerol, in which PKCγ is expressed exclusively in neurons of the brain and spinal cord. Cerebellar Purkinje cell (PC) expresses PKCα and PKCγ. PKCγ-deficient mice that lack PKCγ in the whole brain and spinal cord have been shown to exhibit normal cerebellar long-term depression (LTD), but impaied motor coordination and deficient pruning of climbing fibers (CFs) from developing PCs, suggesting the critical roles of PKCγ in the brain development and the possible contribution of the developmental abnormalities for the motor deficit. However, the physiological significance of PKCγ in the mature animals remained unknown. Here we show that matured PCs express 50-times higher amount of PKCγ than that of PKCα. Adeno-associated virus (AAV) vector-mediated rescue of PKCγ specifically in PCs of the matured PKCγ-deficient mice significantly improved the behavioral performance in a virus titer-dependent manner, while the multiple CF innervation of PCs remained unaltered. These results provide strong evidence that high amount of PKCγ expressed in PCs critically regulates motor function in adult mice, which is independent of multiple CF innervation of PCs and cerebellar LTD. (COI:No)

3PS16E1-4

Development of the therapeutic strategy to regulate reorganization of the injured central nervous system

Toshihide Yamashita[1]

[1]Dept Mol Neurosci, Grad Sch Med, Osaka Univ, Osaka, Japan, [2]Grad Sch Front Biosci, Osaka Univ, Osaka, Japan

Initial behavioral deficits resulting from brain injury are frequently followed by spontaneous recovery of function, although this recovery is quite limited. Synaptic plasticity in pre-existing pathways and the formation of new circuits through collateral sprouting of lesioned and unlesioned fibers are important aspects of the spontaneous recovery process. Although reorganization of the neural network is considered to contribute to this recovery, behavioral plasticity is not fully understood. Furthermore, the molecular mechanism of this phenomenon is poorly understood. We have explored the mechanism of this behavioral plasticity, and more importantly, we have obtained evidence to show that immune modulation, inflammation-induced neovessels, and some types of microglia enhance plasticity and survival of neurons by secreting trophic factors. Disorders of the central nervous system, such as cerebrovascular diseases, cerebrospinal trauma, and encephalomyelitis, often cause spatiotemporal changes in the nervous system and in various biological systems, such as the immune system and vascular system. These immune cells, neovessels, and microglia may prove to be drug targets for the treatment of CNS injuries, CNS inflammation, and neurodegenerative diseases. I will talk about our recent findings that uncover the molecular mechanism of formation and restoration of neuronal network in the CNS. (COI:No)

Planned Symposium 17

Joint Symposium with
the Japan Neuroendocrine Society

Developments of neuropeptide neuron research with new technologies

March 30 (Thu), 14:30 – 16:30, Hall B

3PS17B2-1

A new in vitro model using mouse iPS cells to study endoplasmic reticulum stress in vasopressin neurons

Hiroshi Arima, Kazuki Mitsumoto, Daisuke Hagiwara, Hidetaka Suga

Dept Endocrinology and Diabetes, Nagoya Univ Grad Sch Med, Nagoya, Japan

Familial neurohypophysial diabetes insipidus (FNDI) is an autosomal dominant disease characterized by progressive polyuria due to progressive decreases in arginine vasopressin (AVP) release. We made FNDI model mice in which a mutation causing FNDI was introduced in the neurophysin II (NPII) coding region of the mouse AVP gene locus. FNDI mice manifested progressive polyuria as do patients with FNDI. Our data also showed that mutant proteins are accumulated in a sub-compartment of the endoplasmic reticulum (ER) of AVP neurons. By forming such a structure called ER-associated compartment (ERAC), AVP neurons are likely to reduce ER stress. However, the detailed mechanisms by which ERAC is formed remains to be clarified. To establish in vitro model to work on ERAC formation, we have employed iPS cells from FNDI mice, which were differentiated into AVP neurons. Preliminary data showed that the differentiated AVP neurons express the mutant NPII. Our in vitro model is thus promising to elucidate new molecules or pathways which reduce ER stress. (COI:No)

3PS17B2-2

Interaction between ghrelin and GLP-1 regulates feeding through vagal afferent system

Hideyuki Sakoda[1], Zaved Tm Waise[1], Farhana Naznin[1], Tadashi Okada[1], Koji Toshinai[2], Masamitsu Nakazato[1,3]

[1]Dept Internal Medicine, Univ, Miyazaki, Miyazaki, Japan, [2]Shigakkan University, Obu, Japan, [3]AMED-CREST, Tokyo, Japan

Several gastrointestinal hormones transmit signals involved in the feeding regulation to the brain via the vagal afferent nerve. We investigate the way of conveying energy information from the gut to brain via the autonomic nerve. The orexigenic peptide ghrelin from the stomach and the anorectic peptide glucagon like peptide-1 (GLP-1) from the intestine are sensed by peripheral vagal afferent endings, and play an important role in the regulation of feeding. Both ghrelin receptor and GLP-1 receptor are co-localized in the nodose ganglion neurons that receive gut-derived feeding signals. In rodents, the injection of ghrelin after GLP-1 administration did not increase food intake, and the injection of GLP-1 after ghrelin administration did not suppress it. In nodose ganglion neurons, ghrelin and GLP-1 mediated calcium response was blocked by preadministration of GLP-1 and ghrelin, respectively. The electrophysiological study confirmed that firstly administered peptide canceled vagal afferent electrical activity induced by the secondly injected peptide. These findings highlight the importance of the vagal afferent system in mediating the interaction between ghrelin and GLP-1 to maintain short-term energy homeostasis. (COI:No)

3PS17B2-3

Orexin acts on Locus Coeruleus to enhance and sustain emotional behavior

Takeshi Sakurai[1,2], Shingo Soya[1,2], Toolu Takahashi[2], Takashi Maejima[2]

[1]WPI-IIIS, Univ Tsukuba, Ibaraki, Japan, [2]Dept Mol Neurosci, Kanazawa Univ, Japan

Orexin A and orexin B are a pair of neuropeptides, localized exclusively in the lateral hypothalamus. A series of studies suggested that orexin-deficiency causes narcolepsy in humans and other mammalian species, highlighting roles of this hypothalamic neuropeptide in the maintenance of wakefulness. Salient emotional information activates orexin neurons in the lateral hypothalamus (LH-OX neurons), leading to increases in arousal and autonomic function. However, how this circuit alters animals' behavior remains unknown. We found that noradrenergic neurons in the locus coeruleus (LC-NA neurons) which project to the lateral amygdala (LA) receive direct presynaptic input from LH-OX neurons. Pharmacogenetic or optogenetic silencing of this circuit inhibited sustained expression of fear responses, as did acute blockade of the orexin receptor-1 (OX1R) by an antagonist administered just before the test session. In contrast, optogenetic stimulation of the LH-OX→LC-NA circuit after conditioning induced freezing behavior in a similar but distinct context. Upregulating orexinergic tone by fasting also enhanced freezing behavior. These findings demonstrate that the LH→LC→LA pathway plays an important role in the regulation of fear-related behavior in response to environmental stimuli, and dysfunction of this circuit may underlie inappropriate generalization of fear. These results suggested that activation of the LC-NA neurons by orexin neurons induces fear generalization acting on the LA. These mechanisms might be involved in the pathophysiology of PTSD and/or insomnia. (COI:No)

3PS17B2-4

Transgenic approaches to regulate the neuronal activity in vasopressin neuron

Mitsuhiro Yoshimura, Takashi Maruyama, Yoichi Ueta

Dept Physiol, Sch Med, UOEH, Kitakyushu, Japan

Rapid developments in optogenetic or chemogenetic approaches give us new insight in the field of neuroscience. Although there are several ambitious studies to express light-sensitive protein, channelrhodopsin 2 (ChR2), or specific drug-sensitive protein, designer receptors exclusively activated by designer drug (DREADD), by viral transfection techniques have been performed on oxytocin neurons and their axon terminals, to our knowledge, there is no successful study on arginine vasopressin (AVP) neurons using these techniques. We generated a transgenic rat that expresses the AVP-ChR2-enhanced green fluorescent protein (eGFP) fusion gene in the magnocellular neurosecretory cells (MNCs) of the hypothalamus. The eGFP fluorescence which indicates the expression of the ChR2 gene was observed in the supraoptic nucleus (SON) and the magnocellular division of the paraventricular nucleus (PVN). The intensities of eGFP fluorescence in those nuclei and posterior pituitary showed marked increased after chronic salt loading (2% NaCl to drink for 5 days). Confocal laser scanning microscopic observation revealed that ChR2-eGFP was localized mainly in the membrane of MNCs. Whole-cell patch-clamp recordings were performed from a single MNC isolated from the SON of the transgenic rats and blue light evoked action potentials repetitively in a current clamp mode. We have just generated transgenic rat which expresses DREADD under the downstream of the AVP promoter. In this presentation, some of the data using this AVP-DREADD transgenic rat will also be introduced. (COI:No)

3PS17B2-5

Transgene expression and site-specific ablation in oxytocin system by making use of transgenic animals and virus vectors

Masahide Yoshida, Yuki Takayanagi, Ayumu Inutsuka, Tatsushi Onaka

Dept Physiol, Jichi Med Univ, Tochigi, Japan

A variety of physiological stimuli, including social stimuli, stress and food intake, have been shown to activate oxytocin-synthesizing neurons. Oxytocin receptors are widely distributed within the brain. Precise functional heterogeneity among oxytocin systems remains to be clarified. Tools for manipulation of neural activities of oxytocin-synthesizing neurons or oxytocin receptor-expressing neurons are critical for identifying roles of each oxytocin pathway. In order to control selective neural activity, transgenic animals have been produced. Animal lines expressing DNA recombinases, such as a Cre or Flippase, in combination with DNA recombinases-dependent expression of proteins by using virus vectors have been developed for achieving transgene expression in specific neurons. Diphtheria toxin /diphtheria toxin receptor (DTR) system has also been developed for conditional site-specific ablation of oxytocin or vasopressin-synthesizing neurons. We used BAC clone transgenic rat strains expressing a Flippase or DTR under the control of the oxytocin gene promoter, and knock-in mouse strains expressing a Cre under the control of the endogenous regulatory region of the gene encoding oxytocin or the oxytocin receptor. We will show examples of studies with transgenic animals and virus vectors in oxytocin research. (COI:No)

Planned Symposium 18

Associates of Young Researchers of Physiology

Modulation of body systems based on various sensory inputs – from the perspectives of basic research and clinical application –

March 30 (Thu), 14:30 – 16:30, Hall C

3PS18C2-1

An Infant cooperation to maternal carrying: comparative analyses in humans and mice

Sachine Yoshida[1,2], Ryuko Ohnishi[3,4], Yousuke Tsuneoka[1], Satoko Oda[1], Masaru Kuroda[1], Hiromasa Funato[1], Kumi O Kuroda[3]

[1]Dept Anatomy, Sch Med, Toho Univ, Tokyo, Japan, [2]JST, PRESTO, Kawaguchi, Japan, [3]RIKNE, BSI, Wako, Japan, [4]Fac Edu, Univ Ryukyus, Okinawa, Japan

Mother-infant bonding is the earliest and most important relationship of mammalian infants. To promote this bond, infants have innate behaviors to seek maternal proximity. However, the neurophysiological mechanisms underlying these infant behaviors remain largely unknown. Here we show a novel set of infant cooperative responses during maternal carrying using comparative analyses in humans and mice. Infants carried by a walking mother immediately stopped voluntary movement and crying and exhibited a rapid decrease in the heart rate, compared with those held by a sitting mother. Mouse pups also showed similar calming responses as defined by immobility and diminished ultrasonic vocalizations and heart rate. Further mouse experiments revealed that somatosensory and proprioceptive input signaling were required for induction, and parasympathetic and cerebellar functions mediate cardiac and motor output, respectively. Lesion analyses raised the possibility that the anterior cingulate cortex is involved in the regulatory mechanism of the developmental alteration in the calming response after the maternal separation. The loss of the calming response hindered maternal rescue of pups, suggesting a functional significance for this response. These findings collectively indicate that the infant calming response is a coordinated set of central, motor, and autonomic regulations and is a conserved component of mammalian mother-infant interactions. (COI:No)

3PS18C2-2

Spatiotemporal interactions in perception and motor action

Tsuyoshi Kuroda

Fac Inform, Shizuoka Univ, Hamamatsu, Japan

People often utilize spatial representations for addressing time which is an abstract concept; for example, a horizontal axis is used to represent time when a time course of events is explained. The neural mechanisms processing time and those processing space may be identical or overlapped. Indeed, time and space interact with each other in perception. The kappa effect is an illusion where the empty duration between two stimuli is perceived as longer when the spatial distance between them is made physically longer. I will present two of my studies involved with this spatiotemporal illusion. Most previous studies demonstrated the kappa effect with three stimuli marking two neighboring empty intervals, and the classical psychophysical model, in principle, predicts no spatial effects when only two stimuli are presented on each trial. I replicated the kappa effect with only two visual stimuli, whereas the effect was reduced when each stimulus was much deviated from the fovea (Kuroda et al., 2016, Multisens Res). The finding is consistent with the Bayesian model predicting the effects of spatial acuity. I also tested if the spatiotemporal interactions as in the kappa effect also occur in motor action (Kuroda & Miyazaki, 2016, Sci Rep). A spatial factor during motor action was manipulated by letting participants use an identical hand or different hands for two button pushes reproducing the standard duration between two tactile stimuli. The different-hand condition yielded a shorter reproduced duration than the identical-hand condition when the standard was 1000 ms or longer. Implications of the results were discussed in terms of the pacemaker-counter model. (COI:No)

3PS18C2-3

Sensory processing in schizophrenia and autism spectrum disorder

Yoshiro Nakagawa

Dept Occupational therapy, Chubu Univ, Aichi, Japan

People with schizophrenia and autism spectrum disorder have difficulty in sensory processing. This impairments can affect a wide range of daily life. There are two approaches of understanding sensory processing impairments. First, sensory processing impairments have been investigated by reducing them to brain dysfunction. The neuroimaging techniques such as MEG and EEG have made it possible to study on those impairments. Second, sensory processing impairments have been regarded as an altered relation between subject and objects in psychopathology. The relation structures the distribution of sensation as we perceive objects with our senses. Schizophrenia can maintain this vulnerable relation prior to the onset. This established relation is collapsed at the onset in schizophrenia. While autism spectrum disorder may construct another relation between self and external environment, which differs from typical development. I would mainly discuss about similarities and differences of sensory processing between schizophrenia and autism spectrum disorder from a clinical point of view. (COI:No)

3PS18C2-4

Unusual sensory features in autism spectrum disorders and occupational therapy

Kanae Matsushima

Grad Sch Med, Kyoto Univ, Kyoto, Japan

Unusual sensory features such as hyper- or hypo-reactivity to sensory input, e.g., atypical responses to specific sounds or textures, are common in children with autism spectrum disorders (ASD), and are related to social behaviors and daily lives. Little is known about the neurophysiological mechanisms underlying these unusual sensory features. Recently, autonomic nervous system (ANS) activity, particularly parasympathetic nervous system activity, has gained interest and has been used to measure atypical emotional sensory responses in ASD. Several studies have reported that children with ASD have significantly lower resting-state vagus nerve activity than typically developing children. These results may reflect an inability of children with ASD to modulate their responses to stressors, including sensory stimuli. We found that children with ASD who displayed more severe visual/auditory hyper-reactivity in their daily lives have lower high frequency (HF) component in heart rate variability (HRV) (Matsushima et al., 2016). The HF-HRV reflects parasympathetic cardiac control related to respiratory sinus arrhythmia. Future studies will further investigate the association between sensory features and the ANS in children with ASD. Child-centered interventions, including occupational therapy, which are designed to enhance intrinsic motivation, interest in the environment, and playful intent, have provided children with ASD with an ability to self-regulate their own behaviors and feelings in their daily lives. These interventions may affect vagus nerve activity and treat sensory processing problems in children with ASD. (COI:No)