Meeting Symposium

Meeting Symposium 1

Body in the world - coordinates in the brain-

(March 21, 8:30～10:00, Room C)

MS01-1

Transformation of visual motion from retinotopic to spatiotopic coordinates in the cortical areas MT and MST

Inaba, Naoko[1,2] (*[1]Research and Educational Unit of LIMS, C-PIER, Kyoto University, Kyoto, Japan; [2]Dept Integrative Brain Sci, Grad Sch Med, Kyoto University, Kyoto, Japan*)

The retinal image of a visual scene is constantly moving due to our eye movements, yet the visual world around us appears to remain stationary. To understand the neural mechanisms for this perceptual stability, we studied the activities of neurons in two cortical regions, medial superior temporal (MST) area, and its major input, middle temporal (MT) area in awake behaving monkeys. We measured neuronal responses to a large-field textured background that moved briefly at various speeds and directions during smooth pursuit and stationary fixation. The speed-tuning and direction-tuning of neurons were studied to determine if the speed preference and the direction selectivity of each neuron remained the same even during pursuit. We found that most MST neurons were more sensitive to the stimulus motion in space irrespective of the speed and direction of pursuit; *i.e., in spatiotopic coordinates*. On the other hand, most MT neurons selectively responded to the image motion on the retina; *i.e., in retinotopic coordinates*. The result suggests that the MST neurons compensate, at least in part, for retinal image motion resulting from pursuit eye movements. Since the MST area receives visual inputs from the MT area and the extra-retinal information during pursuit is known to be in the MST area, transformation of visual motion from retinotopic to spatiotopic coordinates is likely achieved by neuronal processing between the cortical areas MT and MST.

(COI: No)

MS01-2

Equations of motion for reaching dynamics and coordinate systems in frontoparietal motor system

Tanaka, Hirokazu (*School of Information Science, JAIST*)

How neurons in the primary motor cortex (M1) control arm movements and how a visual input is transformed into motor control signals in the frontoparietal motor network are not yet understood. Here I show that the equations of motion governing reaching simplify when expressed in spatial coordinates, known as Newton-Euler dynamics in robotics. In this fixed reference frame, joint torques are the sums of vector cross products between the spatial positions of limb segments and their spatial accelerations and velocities. The consequences that follow from this model explain many properties of neurons in M1, including directional broad, cosine-like tuning, non-uniformly distributed preferred directions dependent on the workspace, and the rotation of the population vector during arm movements. Remarkably, the torques can be directly computed as a linearly weighted sum of responses from cortical motoneurons, and the muscle tensions can be obtained as rectified linear sums of the joint torques. The model is further extended to the frontoparietal network; the parietal reach area represents a goal-directed hand movement in the retinal coordinates, which in turn is transformed into body-centered coordinates in the premotor areas. The superior parietal lobule including area 5d operates as a forward model computing a prediction of movement from efference copies from the premotor areas. In summary, reaching dynamics expressed in spatial coordinates provides a unifying framework for understanding the functional roles and coordinate systems used in the frontoparietal motor network.

(COI: No)

MS01-3

Representing a target in terms of the background: functions of the background coordinate and its neural correlates

Kitazawa, Shigeru[1,2,3] (*[1]Dept Brain Physiol, Grad Sch Med, Osaka Univ, Osaka, Japan; [2]Dynamic Brain Netw Lab, Grad Sch Frontier Biosci, Osaka Univ, Osaka, Japan; [3]CiNet, NICT, Osaka Univ, Osaka, Japan*)

Our brain represents a target position in terms of our body parts, like in retinal or craniotopic coordinates (egocentric coordinates). In addition, many previous studies reported that a target position can be represented in terms of landmarks in the background (allocentric coordinate). However, it was generally believed that it takes time to represent a target in terms of landmarks, and that the importance of the allocentric representation increases in such un-ecological situations when we are forced to memorize a target position for some period (~5 s). We have recently shown that a target can be represented immediately (within 300 ms) in terms of a frame in the background, and that the background coordinate is utilized to dissociate the effect of target motion from the error resulting from the issued motor control signals. We have further shown by using an fMRI-adaptation technique that neural correlates of the background coordinate is distinct from those of the retinal and craniotopic coordinates, and reside in several regions in the right hemisphere, including the precuneus, the middle occipital gyrus, and the middle temporal gyrus. We finally discuss another possible function of the background coordinate in stabilizing our visual experience.

(COI: No)

MS01-4

Representations of spatial and non-spatial information in the hippocampus

Fujisawa, Shigeyoshi (*RIKEN BSI, Wako, Japan*)

Hippocampal pyramidal cells display place-selective activities in the environmental space, and traveling in the space arises sequential activities of the place cells. Importantly, sequential activities of hippocampal cells are also observed in the representations of information dissociated from 'space', such as the representation of 'time'. We performed large-scale extracellular single-unit recordings from the hippocampal CA1 in rats performing a working memory task, and also found sequentially organized neuronal activities which differentiated between different odor cues during working memory periods. Here we discuss about the roles of hippocampal cell assembly sequences on coordinates of space and time in the brain.

(COI: No)

Meeting Symposium 2

Exercise physiology in advanced aging society: basic and applied aspects

(March 21, 8:30～10:00, Room E)

MS02-1

The stromal cells interaction and formed 3D network following acute muscle trauma

Kobayashi, Masatoshi[1]; Ohta, Keisuke[2]; Nakamura, Kei-ichiro[2]; Sakurai, Tadayoshi[1]
([1]*Nippon Sports Sci. Univ., Tokyo, Japan;* [2]*Kurume Univ., Fukuoka, Japan*)

It has been reported that the mononuclear cells which locate in the interstitial space of damaged muscle tissue might take part in muscle fibers repair. However, the morophology and the strain of these cells are not clear.
In this study, trauma loaded rat skeletal muscles (gastrocnemius) were observed with focused ion beam scanning electron microscope (FIB/SEM) into 600 slice pictures, which were reconstructed into three dimensional images by the program to develop the localization and formation of stromal cells.
As a result, we observed that many cells invaded it in muscle fibers, and three kinds of stromal cells were identified in the interstitial space of the gastrocnemius muscles at the 1st and the 2nd day after muscle damage. The first appeared cells had spindle shaped form. The second cells had many rough endoplasmic reticula (r-ER). The third cells were similar to granulocyte. These 3 types of cells were contacted one another and formed network.
It was suggested that these stromal cells may exchange some information which plays some important roles in regeneration process after muscle damage. These "cell to cell contact" might have an important biomedical meaning in "the transformation of cells", "the cell proliferation" and "the invasion to muscle fibers".

(COI: No)

MS02-2

Exercise-induced brain glycogen decrease and supercompensation

Matsui, Takashi[1, 2]; Kawanaka, Kentaro[2]; Soya, Hideaki[3] ([1]*JSPS Research Fellow-SPD;* [2]*Dept Health and Nutrition, Niigata Univ of Health and Welfare, Niigata, Japan;* [3]*Lab Exerc Biochem, Univ of Tsukuba, Ibaraki, Japan*)

Exercise activates not only skeletal muscles but also brain neurons. Although the brain is fuelled by carbohydrates, how brain carbohydrate metabolism functions and adapts to exercise remains uncertain. Muscle glycogen is broken down and decreases during exercise and is replenished to above basal level with rest after exercise, a process called supercompensation (Nature, 1966). Muscle glycogen supercompensation is an important phenomenon because it is the basis of exercise adaptation in muscle carbohydrate metabolism (the increase in muscle glycogen storage). Recently, lactate borne from brain glycogen stored in astrocytes has been shown as a critical energy source to retain neuronal functions. We here found, for the first time, that prolonged exhaustive exercise induces the decrease and supercompensation in glycogen in several brain loci such as the cortex and hippocampus, as like in muscles. We also confirmed that four weeks of chronic exercise that elevates endurance and cognitive capacity increases the glycogen storage in the cortex and hippocampus. Our results demonstrated the exercise adaptation of brain carbohydrate metabolism in the cortex and hippocampus similar to muscles, which is likely due to accumulative effects of the brain glycogen supercompensation after it decreases with exercise. The exercise adaptation of brain carbohydrate metabolism in the cortex and hippocampus, which control physical and cognitive functions, could contribute to exercise-improved endurance and cognitive capacities.

(COI: No)

MS02-3

Dietary supplementation with ubiquinol-10 decelerates senescence and age-related hearing loss in SAMP1 mice via the activations of sirtuins and their downstream molecules

Sawashita, Jinko[1, 2]; Tian, Geng[2]; Higuchi, Keiichi[1, 2] ([1]*Dept Biol Sci Intractable Neurol Dis, IBS-ICCER, Shinshu Univ, Matsumoto, Japan;* [2]*Dept Aging Biol, Inst Pathogenesis Preventive Med, Shinshu Univ Graduate Sch Med, Matsumoto, Japan*)

We have revealed previously that supplementation with reduced form of coenzyme Q_{10} (ubiquinol-10, QH_2) significantly delayed senescence in Senescence-Accelerated Mouse Prone-1 (SAMP1) mice (1, 2). In our recent study, we found that QH_2 can also delay progression of age-related hearing loss in SAMP1 mice (3). Here, we report that dietary QH_2 supplementation prevents age-related decreases in the expression of sirtuins, which results in the activation of PGC-1 α , a major factor that controls mitochondrial biogenesis and respiration, as well as SOD2 and IDH2, which are mitochondrial antioxidant enzymes. QH_2 supplementation also increases mitochondrial complex I activity and numbers of mitochondria, and decreases levels of oxidative markers. Furthermore, QH_2 increases cAMP levels by activating adenylate cyclase and repressing phosphodiesterase that, in turn, activated CREB and AMPK in HepG2 cells. These results suggest that QH_2 supplementation may enhance mitochondrial activity by increasing levels of sirtuins and their downstream molecules, and is followed by protection against the progression of aging and symptoms of age-related diseases (3).
1, Yan J *et al.* (2006) Exp Gerontol 41:130-140
2, Schmelzer C *et al.* (2010) Mol Nutr Food Res 54:805-815
3, Tian G *et al.* (2014) Antioxid Redox Signal 20:2606-2620

(COI: No)

MS02-4

Effects of dairy products intake on thigh muscle strength and *NFκB2* gene methylation during walking training in middle-aged and older women

Masuki, Shizue[1, 3]; Taniguchi, Shun-ichiro[2, 3]; Nose, Hiroshi[1, 3] ([1]*Sports Med Sci;* [2]*Mol Oncol, Shinshu Univ Grad Sch Med, Matsumoto, Japan;* [3]*IBS, Shinshu Univ, Matsumoto, Japan*)

Muscle atrophy with aging is the fundamental causes for lifestyle-related diseases. As for the mechanisms, muscle atrophy may induce release of cytokines from the muscle or other organs, causing chronic systemic inflammation. In this study, we assessed whether post-exercise dairy products intake (PEDPI) during 5-month interval walking training (IWT) enhanced the increase in thigh muscle strength and ameliorated the susceptibility to inflammation in middle-aged and older women. Subjects (n=37, 54-74 yr) were randomly divided into 3 groups: IWT alone (CNT, n=12), IWT + PEDPI of low dose (LD, n=12; 4g protein, 3g carbohydrate, and 3g fat) or 3 times higher dose (HD, n=13). They were instructed to repeat $\geq$5 sets of fast and slow walking for 3 min each at $\geq$70% and 40% peak aerobic capacity for walking, respectively, per day $\geq$4 days/wk. We determined thigh muscle strength and promoter methylation in *NFκB2* gene, a well-known transcriptional regulator of inflammation, by PyroSequencing before and after IWT. After IWT, thigh muscle strength significantly increased in HD ($P<0.05$) while not in CNT or LD despite similar training achievement among groups ($P>0.5$). Moreover, an increase in *NFκB2* methylation after IWT was 43±9% in HD, greater than 9±9% in LD ($P<0.05$) and -10±9% in CNT ($P<0.001$), suggesting greater suppression of pro-inflammatory cytokines in HD. Thus, PEDPI enhanced the increases in thigh muscle strength and *NFκB2* methylation by IWT in middle-aged and older women.

(COI: No)

Meeting Symposium 3

Neuronal Specializations of Auditory Temporal Coding

(March 21, 14:00～15:30, Room G)

MS03-1

Synaptic plasticity interacts with postsynaptic membrane kinetics in the chick cochlear nucleus

Burger, Michael R.; Oline, Stefan (*Lehigh University, Bethlehem, PA, USA*)

Auditory stimuli are processed in parallel, frequency-tuned circuits. Auditory nerve fibers (nVIII) impart frequency tuning onto their postsynaptic targets in nucleus magnocellularis (NM). NM neurons express specializations that reflect the characteristic frequency (CF) of their nVIII inputs, and may confer computational specificity. Our previous work demonstrated a gradient of synaptic input properties according to CF where short term depression was strongest for neurons processing low frequencies. We are now evaluating how the postsynaptic neurons integrate inputs in depressed and rested conditions. An efficient way to evaluate postsynaptic properties is by examining responses to injected ramp or frequency modulated Zap-currents. Low CF neurons exhibited longer integration times in response to ramp currents, with spikes following less steep and more prolonged stimuli compared with high CF cells. Responses to ZAP currents were almost all low pass for low CF neurons, while nearly a third of high CF neurons were band-pass. These responses were strikingly adaptive to input conditions. Depolarization shifted frequency selectivity of high CF cells toward higher peak response frequency (f0), enhancing the band pass output of these neurons. Together, these data suggest that computational strategy for spike initiation in NM may depend on both input conditions and frequency selectivity. Interestingly, the ability for high CF neurons to alternate between low- and band-pass filtering may indicate a stimulus-dependent switch, between relay and integrate-and-fire strategies for neural computation.

(COI: No)

MS03-2

Roles of phasic inhibition in coincidence detector neurons of birds

Yamada, Rei (*Dept Cell Physiol, Grad Sch Med, Nagoya Univ, Nagoya, Japan*)

The interaural time difference (ITD) is crucial in localizing the sound source particularly for low-frequency sounds. In birds, neurons in nucleus laminaris (NL) detect the coincidence of bilateral excitatory inputs and change their firing rate as a function of the ITD. Inhibitory synapses are proposed to be significant for accurate ITD detection and tonic inhibition is known to control the gain of coincidence detection, which enhances the ITD sensitivity of NL neurons for strong-intensity sound. In this symposium, I will introduce a phasic inhibition that was found in chicken brain slices, which may further enhance ITD sensitivity for low-frequency sound. During whole-cell recording from low-frequency NL neurons, stimulation to the ipsilateral projection fibers generated a polysynaptic IPSC that follows EPSC with 1-2 ms latency. In addition, GABA-positive neurons are distributed near the NL and generated the IPSCs in NL neurons when photoactivated by a caged glutamate. These results suggest that these GABAergic neurons are interneurons that mediate phasic inhibition to the NL neurons. Model simulations demonstrated that these phasic IPSCs narrow the time window for coincidence detection and increase the contrast of ITD-tuning particularly when the excitatory inputs are weak. Furthermore, cooperation of the phasic and tonic inhibitions effectively increases the contrast of ITD-tuning over a wide range of excitatory input levels. We propose that the complementary interaction between phasic and tonic inhibitions is the neural mechanism that improves ITD sensitivity for low-frequency sound in the NL.

(COI: No)

MS03-3

Inhibition Tunes Coincidence Detection in the Auditory Brainstem

Myoga, Michael Hideki[1,2] ([1]*Max-Planck-Inst. für Neurobiologie, Germany;* [2]*Ludwig-Maximilians-University Munchen, Grosshaderner Str.2, Planegg-Martinsried, Germany*)

Neurons in the medial superior olive (MSO) detect microsecond differences in the arrival time of sounds between the ears (interaural time differences or ITDs), a crucial binaural cue for sound localization. Synaptic inhibition has been implicated in tuning ITD sensitivity, but the cellular mechanisms underlying its influence on coincidence detection are debated. Here we determine the impact of inhibition on coincidence detection in adult Mongolian gerbil MSO brain slices by testing precise temporal integration of measured synaptic responses using conductance-clamp. We find that inhibition dynamically shifts the peak timing of excitation, depending on its relative arrival time, which in turn modulates the timing of best coincidence detection. Inhibitory control of coincidence detection timing is consistent with the diversity of ITD functions observed in vivo and is robust under physiologically-relevant conditions. Our results provide strong evidence that temporal interactions between excitation and inhibition on microsecond timescales are critical for binaural processing.

(COI: No)

MS03-4

Interplay between inhibition and voltage-gated K channels during binaural computations

Golding, Nace[1,2] ([1]*Univ.Texas, Austin, Texas, USA;* [2]*University of Leuven, Leuven, Belgium*)

Neurons in the mammalian medial superior olive (MSO) detect interaural time differences (ITDs), cues that reflect the location of sounds along the horizontal plane. Feedforward glycinergic inhibition has been proposed to control ITD detection but its role and mechanism of action are controversial. To understand how inhibition shapes binaural processing we made intracellular whole-cell recordings from gerbils in vivo and recorded responses to binaural stimuli. Application of the glycine receptor blocker strychnine through a second pipette increased spike rates and usually widened ITD functions or enhanced side lobes, but did not alter the best ITD. Using dynamic clamp and dual somatic recordings from MSO neurons in brain slices, we found that the interplay between fast glycinergic IPSPs and low voltage-activated K channels reduces distortions in the shape and timing of EPSPs. During inhibition, the sharpening of EPSPs due to inhibitory shunting was offset by the deactivation of low voltage-activated K channels that are expressed at high density in MSO neurons. The primary effect of inhibition was to reduce spike probability and sharpen ITD curves through an iceberg-type mechanism, consistent with in vivo results. We conclude that the interplay between inhibition and K channels provides a mechanism that enables inhibition to modify the resolution but not location of spatial receptive fields

(COI: No)

Meeting Symposium 4

NO, the subsequent evolution

(March 21, 17:00～18:30, Room F)

MS04-1

NO, the subsequent evolution

Maeda, Masanobu (*Dept Physiol, Wakayama Med Univ Sch Med, Wakayama, Japan*)

In the 1990s, the number of the papers related with NO (nitric oxide) had become very huge. In 1993, NO became Moleculara of the year. In 1998, Dr. F. Murad, Dr. L. J. Ignarro and Dr. R. F. Furchgott got Nobel prizes. The NO research had reached peak in 1990s, and almost twenty years have passed. We would like to discuss NO research at the present time in this symposium.

(COI: No)

MS04-2

Expression of nitric oxide synthase (NOS) in bone

Ambe, Kimiharu; Watanabe, Hiroki (*Div. Oral Histol., Dept. Morphol. Biol., Ohu Univ. Sch. Dent., Koriyama, Japan*)

Nitric oxide (NO) is a free radical which is produced from a wide variety of cells. NO is involved in the regulation of many physiological processes, such as vascular relaxation, neurotransmission, and immune regulation. NO is generated by nitric oxide synthase (NOS), which has three identified isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Different isoforms are expressed depending on the organs, tissues, and cells, and investigation of the types and functions of enzymes expressed in various tissues is underway. We investigated the expression and cellular localization of NOS isoforms in mice calvaria and femur using immunohistochemistry and *in situ* hybridization to clarify their possible roles in bone. The immunoreactivity of eNOS was weakly positive in the osteoblasts of calvaria at 3 weeks of age, but immunoreactivity of nNOS or iNOS was not observed in the osteoblasts. In contrast, the immunoreactivity of nNOS, iNOS, and eNOS was positive at 3 weeks of age in femur in which bone matrix was formed. At 18 weeks of age, weakly positive reaction of nNOS and eNOS was also noted in osteoblasts, similarly to that at 3 weeks of age, but reaction of iNOS was negative. Expression of NOS mRNA for *in situ* hybridization showed results approximately similar to those of immunohistochemistry. On the other hand, in each NOS knockout mice, the expression levels of the remaining NOSs were increased. These results suggest that each isoform of NOS may be related to bone growth and remodeling in mice calvaria and femur.

(COI: No)

MS04-3

NO functions in plant defense responses against pathogen attack

Kawakita, Kazuhito (*Plant Pathol Lab, Grad Sch Bioagr, Nagoya Univ, Nagoya, Japan*)

In plants, nitric oxide (NO) and reactive oxygen species (ROS) play crucial roles in the regulation of various physiological processes. NO and ROS have been also shown to be an important messenger in plant defense signaling against microbial pathogens. They participate in the induction of resistance reactions such as the expression of defense-associated genes, accumulation of antimicrobial compounds (phytoalexins) and induction of hypersensitive cell death.

In most living organisms, NO is supposed to be mainly produced by nitric oxide synthase (NOS), which catalyzes NO and L-citrulline formation from O_2 and L-arginine. NOS-like activity in plants has been reported widely. However, it is speculated that there is no plant protein corresponding to mammalian NOS. Nitrate reductase, a key enzyme of nitrogen assimilation, is considered to be another enzyme that is capable of producing NO in plants.

We searched for compounds that elicit NO production in plants and found *bis*-arylmethanone compound. The compound elicited hypersensitive cell death but no phytoalexin in potato. Resistance against *Phytophthora infestans*, the potato late blight pathogen increased in potato leaves treated with the compound. In *Nicotiana benthamiana*, a model tobacco plant treated with the compound, NO production was induced without the induction of ROS or hypersensitive cell death. The compound also induced resistance in *N. benthamiana* against *P. infestans*. These results suggested that both NO production and ROS production are required for the induction of hypersensitive cell death.

(COI: No)

MS04-4

Redox regulation of soluble guanylate cyclase -from the point of vascular function study-

Tawa, Masashi; Okamura, Tomio (*Dept. Pharmacol., Shiga Univ. Med. Sci., Otsu, Japan*)

Nitric oxide (NO) plays an essential role in regulating vascular tone. This molecule activates soluble guanylate cyclase (sGC) by binding to its reduced (Fe^{2+}) heme moiety, leading to elevation of intracellular cGMP levels, but once the prosthetic heme moiety is oxidized (Fe^{3+}) or lost, NO loses its ability to stimulate sGC. As valuable tools for elucidating the redox state of sGC, two different types of compounds that act directly on sGC (sGC stimulators and sGC activators) have been recently utilized. sGC stimulators can activate the reduced form of sGC in the absence of NO, whereas sGC activators preferentially and effectively stimulate this enzyme when it is in the NO-unresponsible, heme-oxidized or heme-free state [Follmann et al., doi: 10.1002/anie.201302588]. In organ chamber experiments, exposure to the intracellular superoxide generator menadione impaired the relaxant response of endothelium-denuded rat iliac arteries to the sGC stimulator BAY 41-2272, whereas it augmented that to the sGC activator BAY 60-2770. Similar results were obtained in the arteries exposed to peroxynitrite. These influences of intracellular superoxide, but not of peroxynitrite, on the BAY compound-induced vasorelaxations were eliminated in the presence of tempol. On the other hand, hydrogen peroxide exposure did not affect the relaxation induced by either BAY 41-2272 or BAY 60-2770. These findings suggest that sGC redox equilibrium is shifted towards the NO-insensitive oxidized/heme-free state in the diseased blood vessel associated with the increase in certain reactive oxygen and nitrogen species level.

(COI: No)

MS04-5

Crucial Role of Endogenous and Exogenous NO Production Systems in the Pathogenesis of Cardiovascular and Metabolic Diseases

Tsutsui, Masato (*Department of Pharmacology, Graduate School of Medicine, University of the Ryukyus*)

Nitric oxide (NO) is endogenously synthesized by three distinct NO synthases (NOSs), all of which are expressed in the human body The roles of NO derived from all NOSs have been examined in pharmacological studies with non-selective NOS inhibitors. However, due to non-specificity of the NOS inhibitors, the authentic roles of NOSs-derived NO are still poorly understood. To address this issue, we developed mice in which all three NOS genes are completely disrupted. The triple NOSs null mice were not embryo-lethal; however, their fertility and survival were markedly reduced as compared with wild-type mice. The triple NOSs null mice exhibited a variety of phenotypes, including acute myocardial infarction, diastolic heart failure, and metabolic syndrome. These results suggest that endogenous NOSs deficiency leads to cardiovascular and metabolic diseases in mice. On the other hand, it has recently been discovered that NO is produced from NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), the latter of which is rich in green leaf vegetables. Based on the background, we have recently elucidated that long-term exogenous NO_2^-/NO_3^- deficiency in a diet resulted in metabolic syndrome in mice, identifying the specific dietary ingredient that causes metabolic syndrome even in the absence of calorie excess. These findings provide the first evidence for the crucial role of endogenous and exogenous NO production systems in the pathogenesis of cardiovascular and metabolic disorders.

(COI: No)

Meeting Symposium 5

"La raison d'être" of the Associations, Councils, Committees and Unions of the Academic Societies

(March 22, 9:00〜10:30, Room A)

1. 日本学術会議
本間 さと　北海道大・医〈生理〉
岡部 繁男　東京大・医〈解剖〉

2. 日本医学会連合
加藤 総夫　慈恵医大〈生理〉
河田 光博　京都府医大〈解剖〉

3. 日本医学会連合用語管理委員会
松村 譲児　杏林大・医〈解剖〉
坂井 建雄　順天堂大・医〈解剖〉

4. 日本医学雑誌編集者会議
石川 義弘　横浜市大・医〈生理〉

5. 日本脳科学関連学会連合
伊佐 正　生理研〈生理〉
岡部 繁男　東京大・医〈解剖〉

6. 生物科学学会連合
仲嶋 一範　慶應・医〈解剖〉
小西 真人　東京医大〈生理〉

7. 総合討論

Meeting Symposium 6

Molecular mechanism and physiological function of cell polarity: through the function of transporters

(March 22, 17:30〜19:00, Room C)

MS06-1

The role of polarized transport in epithelial cells

Yoshimura, Shinichiro; Nakajo, Atsuhiro; Iwano, Tomohiko; Kunii, Masataka; Harada, Akihiro (*Grad Sch Med Osaka Univ., Osaka, Japan*)

The transport pathway in epithelial polarized cells is directed to apical or basolateral plasma membrane, which are distinct in protein and lipid composition. Several findings suggest that newly synthesized protein exported from the trans-Golgi network (TGN) is delivered to the endocytic recycling compartment (ERC), which is also regarded as recycling endosome, and sorted to apical or basolateral plasma membrane. Rab8 is thought to be a key molecule to regulate apical transport from ERC. In Rab8-knockout small intestinal cells, apical cargo proteins are misorted to lysosome. Here we identify a novel Rab8 interacting protein complex, which provides a mechanical insight into Rab8-regulated apical transport.

(COI: No)

MS06-2

Tight Junction-based Building of apical microtubule network of epithelial cells

Yano, Tomoki[1]; Matsui, Takeshi[2]; Tamura, Atsushi[1]; Uji, Masami[1]; Kanoh, Hatsuho[1]; Tsukita, Sachiko[1] ([1]*Lab Biol Sci, Grad Sch Med, Osaka Univ, Osaka, Japan;* [2]*Lab. Skin Homeostasis, RCAI, RIKEN Center for Integrative Med. Sci. Yokohama, Japan*)

Our body is wrapped by epithelial cell sheets. The structure of epithelial cell sheets, in which cell-cell adhesion is highly organized, is critically dependent on the association of actin filaments with apical cell-cell adhering junctions. However, relatively little is known of the roles of microtubules (MTs) during epithelial morphogenesis and cell-cell contact. Here, we found novel non-centrosomal MT networks, in which the sides of the MTs bundles were associated with TJs. Then, we identified that cingulin is a MTs-binding protein which were integrated in TJ. And we found that head-domain of cingulin bind to α-tubulin. The binding of head domain of cingulin to MTs was depends on phosphorylation of serine residues by AMPK which is sensitive to metabolic homeostasis-relate kinase. The dephosphomimetic mutant of head domain of cingulin bind to C-terminus of cingulin. Using the low angle shadowing technique to visualize cingulin molecule structures, it was revealed that the molecules of phosphomimetic and dephosphomimetic mutants formed the thread form and pills form, respectively. These data showed that the conformational regulation was related to the phosphorylation of head domain of cingulin. In addition, although wild-type colonies formed spheres in 3-D culture, the cingulin knocked-down cells had anisotropic shapes. These findings collectively suggest that the cingulin-regulated MTs association has a specific role in TJ-related epithelial morphogenesis.

(COI: No)

MS06-3

Identification of a new heterodimeric amino acid transporter in the apical membrane of renal proximal tubule

Nagamori, Shushi; Kanai, Yoshikatsu (*Dept Pharmacol, Grad Sch Med, Osaka Univ, Osaka, Japan*)

The heterodimeric amino acids transporter (HAT) family, which consists of heavy chains (SLC3) and light chains (SLC7) linked with a disulfide bond, has important physiological roles in many types of organs. The light chains have 12 transmembrane domains and transport amino acids. The heavy chains, which have one transmembrane domain and a large extra-cellular domain, are considered as accessory chains. While the light chains are nine molecules, only two molecules are known as heavy chains and distinguished by the localization in polarized cells. rBAT (SLC3A1) localizes at the apical side and 4F2hc/CD98hc (SLC3A2) expresses at the basolateral side. The heavy chains define where the heterodimers should work in polarized cells.
One of heavy chains, rBAT has been known to form a heterodimeric complex only with $b^{0,+}$AT (SLC7A9). Mutations in the two genes cause cystinuria, an autosomal recessive disorder of renal reabsorption of cystine at the apical membranes of proximal tubules. However, the expression paradox of these proteins in the kidney has been remaining. While rBAT highly express in the S3 segment of the proximal tubules, the expression of $b^{0,+}$AT decrease from the S1 to the S3 segment. Thus, an unknown partner of rBAT in the S3 segment has been suggested. By proteomics combined with biochemical analysis, we have identified SLC7A13 as a new rBAT partner. Furthermore, our findings strongly indicate that SLC7A13 is the secondary cystine transporter at the apical membrane of the late proximal tubules in the kidney, which was postulated the presence in 1970's.

(COI: No)

MS06-4

The unidirectional K^+-transport across the epithelial tissue in the inner ear establishes the endocochlear potential essential for hearing

Hibino, Hiroshi[1]; Nin, Fumiaki[1]; Murakami, Shingo[2]; Kurachi, Yoshihisa[2] ([1]*Dept. Mol Physiol, Niigata Univ Sch Med, Niigata, Japan;* [2]*Div Mol Cel Pharm, Dept Pharm, Grad Sch Med, Osaka University*)

Cochlear endolymph, an extracellular fluid containing 150 mM K^+, exhibits a positive potential of +80 mV. This called endocochlear potential (EP), which is essential for hearing, has been thought to be achieved by unidirectional K^+-transport through the lateral cochlear wall. However, the mechanism has been uncertain. The lateral wall comprises inner epithelial layer whose apical surface faces the endolymph, and outer layer, of which basolateral membranes are exposed to an ordinary extracellular fluid, perilymph. Each layer expresses K^+ channels apically and K^+-uptake transporters basolaterally. Intrastrial space (IS), an extracellular compartment between the two layers, exhibits low K^+ and a potential similar to the EP. By using electrodes sensitive to potential and K^+, we found that the positive IS potential (ISP) dominates the EP and represents a K^+-diffusion potential elicited by a large K^+-gradient across the apical surface of the outer layer. Mathematical approaches revealed that the unidirectional K^+-transport underlies the K^+-gradient and depends on the function and polarized localization of the channels and transporters. Finally, ischemia and an ototoxic drug, which block the basolateral K^+-transporters in the inner layer, disrupt the unidirectional K^+-transport and diminish the K^+-gradient, leading to loss of the ISP and EP.

(COI: No)

Meeting Symposium 7

Neural development and neuropsychiatric disorder models

(March 23, 9:00～10:30, Room C)

MS07-1

A mouse model for 15q duplication towards understanding the pathophysiology of autism

Takumi, Toru (*RIKEN BSI, Wako, Japan*)

Autism is a complex psychiatric illness that has received considerable attention as a developmental brain disorder. Substantial evidence suggests that chromosomal abnormalities including copy number variations contribute to autism risk. The duplication of human chromosome 15q11-13 is known to be the most frequent cytogenetic abnormality in autism. We have modeled this genetic change in mice using chromosome engineering to generate a 6.3-Mb duplication of the conserved linkage group on mouse chromosome 7. Mice with a paternal duplication display autistic-like behavioral features such as poor social interaction and stereotypical behavior, and exhibit abnormal ultrasonic vocalizations. This chromosome-engineered mouse model for autism seems to replicate various aspects of human autistic phenotypes and validates the relevance of the human chromosome abnormality. This model is a founder mouse for forward genetics of a developmental brain disorder and an invaluable tool for its therapeutic development. I will present our analyses on these mice towards understanding the molecular pathophysiology of autism spectrum disorders.

(COI: No)

MS07-2

Dysregulation of RNA metabolism potentially causes neurodegenerative and developmental neuropsychiatric disorders

Takeuchi, Akihide[1]; Iida, Kei[1]; Ninomiya, Kensuke[1]; Tsubota, Tomoaki[1]; Denawa, Masatsugu[1]; Itou, Mikako[2]; Ohno, Kinji[2]; Hagiwara, Masatoshi[1] ([1]*Grad.Sch.Med.Kyoto Univ., Kyoto, Japan;* [2]*Grad.Sch.Med.Nagoya Univ., Nagoya, Japan*)

In mammals, several essential neuronal genes are expressed from extra-long pre-mRNAs, which are vulnerable to dysregulation during transcriptional elongation or processing. Several neurodegenerative and neuropsychiatric disorders are thought to be caused by the impairment of extra-long pre-mRNA expression. However, the identities and functions of essential molecules facilitating long neuronal gene expression are still largely unknown. Here, we discovered a novel function of the RNA-binding protein Sfpq in extra-long neuronal gene expression. Genome-wide Sfpq-binding mapping and transcriptome analyses showed that Sfpq binds co-transcriptionally across the entire span of pre-mRNAs of long genes and is required for the expression of pre-mRNAs > 100 Kbp. RNA polymerase II density analysis revealed that Sfpq is necessary for transcriptional elongation beyond 100 Kbp. Our findings using Sfpq-mutant mice indicated that Sfpq-dependent transcription of long genes is essential for neuronal development and that dysregulation of long genes potentially causes neurodegenerative and developmental neuropsychiatric disorders.

(COI: No)

MS07-3

The pathomechanism of Huntington disease: factors related to its pathological cascades

Nukina, Nobuyuki (*Juntendo University Graduate School of Medicine*)

Huntington Disease (HD) is a dominantly inherited neurodegenerative disorder caused by the accumulation of mutant huntingtin protein(HTT) containing an expanded polyglutamine (polyQ) tract. Most well established model mouse for HD is R6/2, transgenic mouse of HTT exon1 and we also established transgenic mouse of HTT exon1 with EGFP. Using those mice, we have been analyzing the pathomechanism of polyglutamine disease and searching the therapy for this disorder. We focused on nuclear inclusions, the main pathology of polyglutamine disease and polyglutamine aggregate interacting proteins(AIPs). AIPs include proteasome subunit, molecular chaperons, autophagy related molecules, RNA binding proteins and transcriptional factor. In this talk, I will introduce the pathological significance of those AIPs and recent discovery of new anatomy of basal ganglia based on the distribution of Scn 4b, which expresses in the striatum and decreased in HD.

(COI: No)

MS07-4

Synaptic defect in non syndromic autism: from the study in Neuroligin-3 mutant mice

Tabuchi, Katsuhiko[1,2] ([1]*Dept Mol Cell Physiol, Shinshu Univ Sch Med, Matsumoto, Japan;* [2]*PRESTO JST*)

Neuroligins and Neurexins are distinct families of cell adhesion molecules localized at post- and pre-synaptic terminals, respectively. They bind each other at synaptic cleft via their extra cellular domains and induce synapse maturation. R451C mutation in neuroligin-3 is the first identified neuroligin mutation that had been shown to affect the surface localization of Neurolign-3 protein by in vitro studies. We generated knock-in mice that recapitulate this mutation to examine its relevance to autism. These mice grew normally without exhibiting obvious physical phenotypes but showed behavioral abnormalities relevant to autism including impaired social interaction and enhancement of spatial learning and memory. We studied synaptic function of these mutant mice and found inhibitory synaptic transmission was selectively enhanced in the cerebral cortex. Administration of GABA receptor blocker ameliorated the impaired social interaction suggesting this mutation could be the cause of autistic behavior in these mice. We further found ratios of NMDA/AMPA and NR2B/NR2A, and synaptic plasticity were increased in hippocampus indicating synaptic maturation was impaired in these mice. We hypothesized that disturbance of synaptic maturation causes impairment in social behavior and extraordinary memory ability in certain type of autism patients.

(COI: No)

Meeting Symposium 8

Frontiers in biological application of microscopic measurements

(March 23, 9:00～10:30, Room G)

MS08-1

Dynamic property of transcription factors analyzed by fluorescence cross-correlation spectroscopy in living cells

Kinjo, Masataka (*Grad.Sch.Adv. Life Sci. Hokkaido Univ. Sapporo, Japan*)

Two-laser-beam fluorescence cross-correlation spectroscopy (FCCS) is promising technique that provides dynamic and quantitative information about the interactions of biomolecules. By using FCCS, we determined the dissociation constant (Kd) of the p50/p65 heterodimer, homodimer of that, and also homodimer of glucocorticoid receptor (GR) in living cells. GR is a well-known steroid-dependent nuclear receptor protein. The classical view is as follows; unliganding GR resides in the cytoplasm, translocates to the nucleus upon ligand binding, and then associates with a specific position, namely glucocorticoid response elements (GRE), where GR act as a transcription factor. On the other hand, it is still a puzzle whether GR forms a dimer in the cytoplasm or in the nucleus before DNA binding or after that. Our result using FCCS suggested that high values of Kd in nucleus and so the dimer-monomer equivalent state in the nucleus and also cytoplasm. These findings support the existence of a "dynamic monomer pathway"and regulation of GR function can be controlled by concentration and balance of monomer and dimer ratio.

(COI: No)

MS08-2

Measuring the distribution of small molecule compounds inside biological tissue via intrinsic molecular vibrations using nonlinear Raman spectroscopy

Kawagishi, Masahiko[1]; Obara, Yuki[2]; Suzuki, Takayuki[2]; Hayashi, Masumi[3]; Misawa, Kazuhiko[2]; Terada, Sumio[1] ([1]*Dept.Neuroanat.Cell.Neurobiol., Grad.Sch. Med.Dent.Sci., Tokyo Med.Dent.Univ., Tokyo, Japan;* [2]*Dept.Appl.Phys., Tokyo Univ. Agricul.Technol., Koganei, Japan;* [3]*Wired Co., Ltd., Komae, Japan*)

Distributions of small molecular weight (less than 300 Da) organic compounds inside biological tissue have been obscure because of the lack of appropriate methods to measure them. Raman spectroscopy is a technique to study molecular vibrational signature, specific to the chemical bonds and symmetry of the molecule. It can acquire information about chemical compounds without any labeling. Coherent anti-Stokes Raman scattering (CARS) is a third-order nonlinear optical process to produce a coherent and stronger Raman signal. We have been using CARS spectroscopy to detect and identify small molecule compounds. We have developed a time-resolved and phase-sensitive technique to remove non-resonant noise signals from water and to acquire resonant vibrational CARS signals from a target compound in aqueous environment. We applied this technique to detect small molecular weight drugs inside biological tissue. As an initial model experiment, we measured sevoflurane in squid giant axon. We also measured taurine inside mouse cornea and successfully characterized its depth profile. Our CARS spectra measurement can be a promising method to measure and visualize the distribution of small bio-related compounds in biological background without using any labeling, paving the way for new cell biological analysis in various disciplines.

(COI: No)

MS08-3

Dynamics of protein assemblies in live cells revealed by monitoring orientation of individual molecules

Tani, Tomomi[1,2] ([1]*Marine Biological Laboratory, Woods Hole, Massachusetts, USA;* [2]*Dartmouth College*)

Monitoring orientation of individual molecules has yielded new insights in the structure and function of proteins and the assemblies. Current fluorescent single molecule methods are limited to measurement of orientation of single fluorophores and little has permitted robust imaging of orientation of multiple molecules simultaneously in living cells. We have developed a new fluorescence polarization microscope that instantaneously and efficiently sorts the emitted fluorescence along four polarization orientations to provide instantaneous imaging of position and orientation of single fluorescent molecules and their assemblies. The instantaneous imaging of fluorescence polarization has enabled analysis of molecular position and orientation in vitro and in living cells. Taking advantage of Alexa Fluor 488 phalloidin that reports local orientation of actin filaments, we have measured changes in the orientation of local actin filaments as they undergo retrograde flow at the leading edge of migrating human keratinocytes. We also used our system to study organization of septins, a highly conserved cytoskeleton critical for cytokinesis and intracellular compartmentalization. We found that individual septin molecules with constrained GFP in filamentous fungus bind to the cell cortex with consistent orientation with respect to the cell axis. Our single molecule fluorescence orientation imaging technique in living cells is also promising to explore conformational changes in single molecules or mechanisms of protein assembly.

(COI: No)

MS08-4

Subnanometer-scale measurements at solid/liquid interfaces by frequency modulation atomic force microscopy

Fukuma, Takeshi[1,2]; Asakawa, Hitoshi[2]; Inada, Natsumi[1]; Takao, Kazufumi[1]; Miyata, Kazuki[1]; Miyazawa, Keisuke[1] ([1]*Kanazawa Univ., Kanazawa, Japan;* [2]*Bio-AFMCenter, Kanazawa, Japan*)

Frequency modulation atomic force microscopy (FM-AFM) has been traditionally used for atomic-scale imaging of various materials in vacuum. Recently, we have developed liquid-environment FM-AFM that is capable of imaging atomic-scale surface structures at solid-liquid interfaces. With the developed technique, we have demonstrated subnanometer-scale imaging of biological systems in physiologically relevant solution. Furthermore, we recently developed a three-dimensional (3D) force measurement technique referred to as 3D scanning force microscopy (3D-SFM). We applied this technique to visualize 3D force distribution at a mica-water and lipid-water interface. The obtained force image shows atomic-scale contrasts that represent a 3D water density distribution (i.e. hydration structure) as well as spatial distribution of flexible surface structures such as lipid headgroups. The results demonstrate the possibility of 3D imaging of 3D hydration and flexible surface structures at a solid-liquid interface.In this talk, I will present a short summary of liquid-environment FM-AFM instrumentation and its applications to the investigations on soft interfacial structures.

(COI: No)

Meeting Symposium 9

Leading-edge of science advanced by new electron microscopic technology for 3D reconstruction

(March 23, 15:00〜16:30, Room G)

MS09-1

The principle and applications of 3-D subcellular morphological analyses by Serial block-face scanning electron microscopy (SBF-SEM)

Miyazaki, Naoyuki; Murata, Kazuyoshi (*NIPS, Japan*)

Elucidating the spatial distribution of organelles and molecules within a whole cell is critical for understanding the nature of cellular processes, and whole-cell structural analysis at a high resolution is necessary for precise structural interpretation. SBF-SEM is an advanced 3-D electron microscopy technique for investigating such large volumes at a resolution of a few tens of nanometeres. In this method, thin surface of a resin embedded specimen is cut off by a diamond knife attached to an in-chamber ultramicrotome, and then the newly exposed surface is imaged by SEM. The sectioning and imageing are automatically repeated to get a serial block-face images of the specimen. 3-D structure is reconstructed from the serial block-face images after image alignment. In this presentation, we will show the principle and the performance of SBF-SEM, and introduce some recent applications. For example, morphological changes in mitochondria regulated by Cdc48p/p97 ATPase was examined by SBF-SEM (Miyazaki et al., 2014). Cdc48p is a highly conserved cytosolic AAA chaperone that is involved in a wide range of cellular processes and regulates mitochndrial morphology. The 3-D morphological analyses of SBF-SEM showed that loss of the ATPase activity of Cdc48p leads mitochondrial fragmentations and aggregations without fusion of the mitochondrial outer membrane, which suggested that Cdc48p is involved in the mitochondrial fusion process. Our results demonstrate that SBF-SEM has considerable advantages in morphological and quantitative studies on organelles and intracellular structues in entire cells.

(COI: No)

MS09-2

Serial section scanning electron microscopy and its application for the morphological analysis of the Golgi apparatus

Koga, Daisuke; Kusumi, Satoshi; Ushiki, Tatsuo (*Niigata Uni. Grad. Sch. Med. Dent. Sci., Niigata, Japan*)

Serial section scanning electron microscopy (SEM) is based on the collection of backscattered electron images of serial ultrathin sections on solid substrates such as glass slides. This method is simpler than serial section transmission electron microscopy, and is useful for 3D reconstruction of cellular structures without using special instruments such as focused ion beam/SEM (FIB/SEM) or serial block face/SEM (SBF/SEM). Serial section SEM has a number of advantages in the following points. (1) it is free from handling of the grid. (2) Sections can be imaged repeatedly. (3) An extensive area of a specimen can be observed. Here, we will explain details of serial section SEM, and show its application for the 3D morphological analysis of the Golgi apparatus. This technique is suitable for 3D reconstruction of the Golgi apparatus which is known as a cell organelle with a complicated structure. In this study, we will introduce the entire shape of the Golgi apparatus in different cell types. The shape of the Golgi apparatus was various depending on cell types, and appeared as a single mass of Golgi cistern networks. The combination of serial section SEM and immunohistochemistry is expected to be used for clarifying the 3D structure of the Golgi apparatus in relation to its function.

(COI: No)

MS09-3

Analysis of synaptic connectivity with FIB/SEM: antidepressant-induced morphological changes in perforant path synapse in the dentate gyrus

Kitahara, Yosuke[1]; Ohta, Keisuke[2]; Sotogaku, Naoki[1]; Nakamura, Keiichiro[2]; Nishi, Akinori[1] ([1]*Dept of Pharmacol, Kurume Univ Sch of Med, Kurume, Japan;* [2]*Dept of Anatomy, Kurume Univ Sch of Med, Kurume, Japan*)

Neural circuit is composed of many neurons, which communicate each other via synaptic connections. When profiling neural circuits, it is important to visualize the details of synaptic connection (e.g., dendritic spine and presynaptic bouton), which are related to neural activity. For analysis of synaptic connection, high resolution imaging at the electron microscopy (EM) level is required. Focused ion beam (FIB)/scanning electron microscopy (SEM) can automatically obtain serial EM images, and 3D reconstruction of synaptic morphology in multiple samples becomes possible. By utilizing advanced technology of FIB/SEM, we investigated the fluoxetine-induced morphological changes in perforant path synapse in the middle molecular layer of DG, in which synaptic transmission is enhanced after chronic fluoxetine treatment. 3D analyses of dendritic spines revealed the appearance of the extremely large-sized spine after chronic fluoxetine treatment. The presynaptic bouton connected to the large-sized spine was large in volume, and the volumes of mitochondria and synaptic vesicle in the bouton were correlated with sizes of bouton. The fluoxetine-induced increases in sizes of pre- and post-synaptic structures of perforant path synapse may be involved in the enhanced glutamatergic neurotransmission. Thus, 3D image analysis with FIB/SEM is useful to visualize morphological features of synaptic connection under pathophysiological conditions.

(COI: No)

MS09-4

Space–time wiring specificity supports direction selectivity in the retina

Kim, Jinseop S.[1†]; Greene, Matthew J.[1]; Zlateski, Aleksandar[2]; Lee, Kisuk[1]; Richardson, Mark[1]; Turaga, Srinivas C.[1]; Purcaro, Michael[1]; Balkam, Matthew[1]; Robinson, Amy[1]; Behabadi, Bardia F.[3]; Campos, Michael[3]; Denk, Winfried[4]; Sebastian, Seung H.[1†]; the EyeWirers[5] ([1]*Departments of Brain and Cognitive Sciences;* [2]*Electrical Engineering and Computer Science, MIT, USA.;* [3]*Qualcomm Research, USA.;* [4]*MPI at Heidelberg, Germany.;* [5]*http://eyewire.org.* [†]*Present address: Princeton Neuroscience Institute, Princeton University, USA*)

What is the origin of neural computation? There are two possible theories. In biophysics, a neuron is considered to have internal mechanism that makes itself an already capable computer. The connectionism, on the other hand, claims that it is rooted in the connections between neurons. I will present a recent finding that supports the connectionism from a study about motion detection in the mammalian retina. The starburst amacrine cells (SACs) are known to exhibit direction selectivity (DS) and play crucial roles for DS of other neurons as well, yet the origin of their DS remains elusive. In search of clues, we reconstructed Off-type SACs and bipolar cells (BCs) in serial electron microscopic images with help from EyeWire, an online community of 'citizen neuroscientists'. We found quantitative evidence that one BC type prefers to wire with a SAC dendrite near the SAC soma, whereas another BC type prefers to wire far from the soma. The near type is known to lag the far type in time of visual response. A mathematical model shows how such 'space–time wiring specificity' could make SAC dendrites respond selectively to stimuli that move in the outward direction from the soma.

(COI: No)