Student Poster Awards Candidates

Day 1

(March 22, 13:00 - 14:00)

AC-1

Two calmodulin binding site model for the regulation of Cav1.2 channel

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Calmodulin (CaM) plays a critical role in the regulation of Cav1.2 Ca^{2+} channels. In general, CaM binds to the channel directly and changes channel activity in a Ca^{2+}-dependent manner (1-CaM model). We have reported that the channel activity increases and decreases in a CaM concentration dependent manner. Based on our experiments, we propose a model for the regulation of Cav1.2 channel by two CaM-binding sites, in which CaM binds to an activation site and then another CaM binds to an inactivation site. To test this hypothesis, we compared the activity of the wild-type channel (α1C) and mutant derivatives, C-terminal deleted (α1C δ) and α1C δ linked with CaM (α1C δ CaM) or Ca^{2+}-insensitive CaM mutants (CaM12, CaM34 and CaM1234), using the patch-clamp technique. In the whole-cell recording, Ca^{2+}-dependent channel inactivation (CDI) of these channels is larger in the order of α1C δ CaM1234 > α1C δ CaM34 > α1C=α1C δ =α1C δ CaM=α1C δ CaM12. However, the mean time constant (τ) for recovery showed similar values. In the inside-out recording, Ca^{2+}-dependent inactivation was observed in α1C with 1 μ M CaM, α1C δ with 1 μ M CaM and α1C δ CaM, but not in the channels liked with three types of Ca^{2+}-insensitive CaM mutants. The CaM concentration-dependent inactivation was observed in the all channels except for α1C δ CaM34 in 80 nM Ca2+-condition. These results support by 2-CaM binding site model, rather than 1-CaM model. (COI:No)

AC-2

Analyses of the voltage dependence and its structural determinant of the P2Y1 purinergic receptor

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It has been known that the signaling efficacy of some of the G-protein coupled receptors shows voltage dependence. We investigated the voltage dependence of Gq-coupled receptors, such as a muscarinic acetylcholine receptor M1R and a purinergic receptor P2Y1R, by analyzing the KCNK13 channel current co-expressed in human embryonic kidney 293T cells by whole cell patch clamp recording. An application of oxotremorine-M to M1R evoked an increase in the amplitude of the KCNK13 channel current in a concentration dependent manner. EC50 of oxotremorine-M for M1R at 0 mV was significantly lower than that at -80 mV, consistently with previous reports. As for P2Y1R, we newly observed that the dose-response curve of ADPβS was biphasic and the biphasic curve changed depending on the membrane potential. Efficacy of P2Y1R to increase the amplitude of the KCNK13 channel current was higher at 0 mV than at -80 mV. As a next step, we approached the structural background by mutagenesis. Mutants of residues at the putative ligand-binding site in the transmembrane (TM) regions, such as R128K and H132A in TM3 and H277A and K280R in TM6, did not change the voltage dependence of P2Y1R. Mutations of D97A in TM2 and D320A in TM7 abolished the voltage dependence. Taken together, the results suggested that P2Y1R shows the voltage dependence and both D97 and D320 are part of the structural determinants. (COI:No)

AC-3

Identification of a single amino acid residue involved in TRPA1 inhibition by HC-030031 utilizing species specific differences

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Pain is a harmful sensation that usually arises from noxious stimuli. Transient receptor potential ankyrin 1 (TRPA1), a member of TRP superfamily, is one of those targets for studying the pain mechanism. TRPA1 is known to be activated by various stimuli such as noxious cold (potentially in rodents), pungent natural products (like cinnamaldehyde; CA) and environmental irritants (like acrolein). Since TRPA1 is an attractive target for pain therapy, many TRPA1 antagonists have been developed and some of them function as analgesic agents. Responses of TRPA1 to these agonist and antagonists were known to vary among different species and species differences have been utilized to identify the structure basis for activation and inhibition mechanisms. Here, we show that HC-030031 (HC), one of the most potent mammalian TRPA1 antagonists, did not inhibit western clawed frog TRPA1 (fTRPA1). HC failed to inhibit fTRPA1 activation elicited by CA, but inhibited CA-evoked currents of human TRPA1 (hTRPA1) with a dose-dependent manner in a heterologous expression system with *Xenopus* oocytes. Chimeric studies between fTRPA1 and hTRPA1 as well as point mutant channel analyses revealed that one specific amino acid residue located within the transmembrane domain was partially involved in the inhibitory action of HC. These findings provide novel insights into the structural-function relationship of TRPA1. (COI:No)

AC-4

TRPC3 contributes to a slow force response to stretch on mice cardiomyocytes

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When cardiac muscle is stretched, its intracellular Ca^{2+} transient and twitch force slowly increase over several minutes. This response is called a slow force response to stretch (SFR). The stretch-induced release of angiotensin II (Ang II) has been implicated in the SFR, to raise intracellular Na^+, followed by an increase in intracellular Ca^{2+} via Na^+/Ca^{2+} exchanger. However, the cation influx pathway remains unclear. TRPC3 is known as receptor-operated cation channel. We focused on the functional relation between Ang II type 1 (AT1) receptor and TRPC3 via diacylglycerol (DAG) on SFR. Mice ventricular myocytes were enzymatically isolated. A pair of carbon fibers was attached to each cell end to apply stretch. The myocytes were electrically stimulated at 1 Hz. Ca^{2+} transient was measured with Fura-4F. The myocytes were stretched for 300 seconds. The stretch slowly increased the Ca^{2+} transient. AT1 receptor blocker (Olmesartan), DAG inhibitor (U-73122) and TRPC3 inhibitor (Pyrazole-3) significantly inhibited the SFR. The SFR was significantly suppressed on cardiomyocytes of TRPC3 knockout mice. Applying Ang II without stretch also slowly increased Ca^{2+} transient. This response was suppressed by U-73122 and Pyrazole-3. To investigate the potential location of TRPC channels, we used a mathematical cardiomyocyte model with cation channels on either sarcolemma or sarcoplasmic reticulum. The model with cation channels on sarcolemma successfully reproduced SFR, while the other did not. These results suggest that TRPC3, modulated by AT1 receptor on sarcolemma, contributes to SFR. (COI:No)

AC-5

The ionic mechanism of electrocardiogram examined using a one-dimensional array of human ventricular cell model

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The electrocardiogram is a useful tool of recording the cardiac electrical activity. To explain electrocardiographic waveform in terms of membrane ionic currents, we connected 100 endocardial and epicardial human ventricular cell models (HuVEC, Himeno. *et al.*, 2015) via a gap junction channels. To calculate the extracellular potential change, all cell models were grounded through a pair of parallel extracellular capacitance (C_o) and conductance (G_{sr}). The extracellular potentials of all cell models were summated after weighing them with a revers function of distance from a surface electrode. Two surface electrodes were positioned at different locations along the one dimensional array to record electrocardiographic waveform of bipolar lead.In general, the bipolar lead of the model array well corresponded with electrocardiographic waveform and we call characteristic waveforms qrs wave, j wave and t wave, respectively. We examined cause-result relationship between modification of ionic current and change of **surface potential**. In the control run, the basic interpretation of QRS, T and J waves could be applied to **surface potential**. The duration of qrs wave was modified when ionic current or gap junction conductance were changed in simulation. Activation of ATP-sensitive K^+ channel shifted the st segment upward. The j wave became more evident by increasing I_{Kto} amplitude or by enhancing I_{Na}. One can discriminate between these two cases of simulation result by thoroughly examining waveform changes in other components. (COI:No)

AC-6

Ionic mechanisms underlying ventricular fibrillation examined in a one-dimensional array of human ventricular myocyte model

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It is now largely accepted that the delayed inactivation component of the fast Na^+ current (I_{NaL}) is further delayed under various pathophysiological conductions. We recently demonstrated that the early afterdepolarization (EAD) was evoked by further retarding the inactivation of I_{NaL} in the human ventricular cell model (Asakura et al., 2014). We aim at clarifying whether this EAD is capable of evoking ventricular fibrillation in an array of the ventricular cell models. The array was composed by connecting the ventricular cell models through gap junctions; 1000 control models and the same number of EAD models in series. In the EAD models, the rate (k_{I12}) of I_{NaL} was scaled by 0.1 and amplitudes of I_{Kr} and I_{K1} were reduced to various levels. When the cell array was stimulated at its normal end, the propagated action potential into the EAD array was followed by several events of EAD. However, the EAD rhythm was regular and ceased after several events by a progress of I_{NaL} inactivation. Long lasting and stochastic EAD events were initiated when I_{K1} was scaled down by ~30% and I_{Kr} by ~65%. Under this condition, a small fraction of the EAD cell array intermittently showed full repolarization to ~ -90 mV, resulting in a full recovery of I_{NaL} from inactivation. Those cells released from inactivation served as a refreshed focus of EAD initiation in the cell array, when excited by neighboring EAD cells. The fibrillation ceased in a stochastic manner when EAD failed to excite the refreshed focus. (COI:No)

AC-7

Is there an effect of low birth weight on current low-grade inflammation, blood pressure, and autonomic function in healthy young Japanese adults?

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Numerous epidemiological studies have shown an association between low birth weight (LBW) and increased risk of adult hypertension. Low-grade inflammation and deterioration of autonomic function play important roles in hypertension; however, the underlying mechanisms of these associations remain poorly understood. The purpose of our study was to investigate possible risk factors for hypertension related to LBW in healthy young Japanese adults. We measured blood pressure and heart rate variability at rest and during postural change from a supine to a sitting position, in 33 Japanese healthy volunteers aged 18 to 23 years. We measured blood cell counts, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and high-sensitivity C-reactive protein levels. None of these measures significantly varied between the volunteers with LBW and normal birth weight (NBW). However, resting parasympathetic nerve activity was lower in the LBW group than in the NBW group($p<0.05$). Following postural change, systolic blood pressure significantly increased among volunteers in the NBW group ($p<0.001$), but not among those in the LBW group. We propose that among healthy young Japanese adults, those with LBW have higher risk for hypertension than do those with NBW. (COI:No)

AC-8

Measurement of cardiac action potential in anesthetized guinea pig for estimating drug action on ion channel conductance

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(Dept Bioinformatics, Grad Sch Life Science, Ritsumeikan Univ, Shiga, Japan)

Since the evaluation of side effects of drug discovery candidate compounds in physiological environment is important, we have been developing an in silico drug action estimation system which uses cardiac action potentials (APs) in guinea pig heart. The system uses APs both before and after drug application as input data. Changes of the ion channel conductance induced by the drug application can be estimated by finding similar APs from the AP database generated by varying the conductance of several ion channels of the simulation model. For the accurate estimation, it is important to use APs that are stable and less susceptible to changes in the location of the electrode or the elapsed time of the experiment. In previous reports, input data had been acquired from guinea pig isolated cardiomyocytes or myocardial strips with the microelectrode method or a guinea pig Langendorff heart with the suction electrode method. However, recorded APs with these methods had been generally unstable, which made it difficult to obtain accurate analysis results. Therefore, in the present study, APs were recorded by pressing a suction electrode on a guinea pig ventricular wall through ventilated thoracotomy. In this way, APs had relatively constant amplitudes of 50 - 60 mV, and were quite stable even when the locations of the recording electrode were changed on the ventricular wall. By comparing AP obtained under application of channel blockers, we are evaluating the suitability of the recording method developed in this study. (COI:No)

AC-9

Changes in peripheral hemodynamics with progression of diabetes: a diffuse correlation spectroscopy study

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Diffuse correlation spectroscopy (DCS) is an optical measurement of a velocity of the capillary blood flow in the living tissue. Using DCS, we measured longitudinal changes in the peripheral blood flow velocity in diabetic model rats. Streptozotocin (STZ, 50mg/kg) was administered intraperitoneally to 10 male SD rats (6-7 weeks of age) to produce hyperglycemia state (blood glucose level more than 250 ml/dl). Two of 10 rats failed to show the increase in the blood glucose level and were therefore treated as controls. We measured the blood flow reactivity using a reactive hyperemia (RH) test with two optical probes attached to the hind limb, which is a frequent site of diabetic capillary disorder, of the anesthetized rats (2% Isoflurane, 1.5 L/min) at 1 week before and once in every week after the STZ administration. The blood flow speed during the rest period of RH test was gradually decreased after administration of STZ in diabetic rats but not in control rats. The peak value of blood flow speed after the release from the ischemia also decreased over time. The time-to-peak duration was almost remained unchanged throughout the experimental period. The gradual reduction of the baseline and the maximum blood flow speed after RH test in diabetic rats suggests the progression of capillary disorder under the influence of high blood glucose stress. DCS is suitable to monitor changes in peripheral hemodynamics with progression of diabetes. (COI:No)

AC-10

The role of pacemaker channel HCN4 against bradycardic response

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The physiological role of HCN4, major subtype of hyperpolarization-activated cyclic nucleotide-gated channels in sino-atrial node (SAN), is not fully understood. To study this, we generated two lines of transgenic mice; $HCN4^{+/tetA\_TRE}$ overexpressing HCN4 at their physiological locus (tTA mouse) ; $HCN4^{Luc/tetA\_TRE}$, a double knock-in mouse that enables complete knockdown of HCN4 expression with doxycycline (TET-off mouse). We first recorded ECG in these mice under general anesthesia; TET-off showed significant bradycardia with intermittent sinus pause or irregular RR interval, whereas the heart rate (HR) of tTA was similar to that of wild type (WT). After intraperitoneal application of isoproterenol (ISO), HR was not significantly different between three groups. We next compared parasympathetic response by electrical stimulation of right cervical vagal nerve. During the stimulation, WT and tTA equally showed bradycardia. In contrast, complete sinus pause was induced in TET-off. To investigate this mechanism, we recorded action potential (AP) in SAN of right atrium tissue using microelectrode technique. TET-off exhibited unstable spontaneous firing. Cumulative application of acetylcholine gradually decreased the rate of spontaneous AP, and finally hyperpolarized the membrane potential at stable level. The stable level was more depolarized in tTA than those of WT and TET-off. These findings indicate that HCN4 may act as a limiter for parasympathetic hyperpolarization of SAN. (COI:No)

AC-11

NA-induced Automaticity of the Rat Pulmonary Vein Cardiomyocyte demonstrated in a Mathematical Model

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In several types of atrial fibrillation (AF), β_1 and α_1 adrenoceptor stimulation by noradrenaline (NA) may be responsible for the ectopic activity in pulmonary veins (PVs). Repetitive spontaneous Ca^{2+} release has been suggested underlying the arrythmogenic membrane excitation in PV cardiac myocytes. To examine this hypothesis, we assembled key experimental findings reported on influences of β_1 and α_1 adrenoceptor stimulation on the L-type Ca^{2+} channel (LCC), the sarco-/endoplasmic reticulum Ca^{2+} pump (SERCA) and the inositol 1,4,5-trisphosphate receptor (IP_3R) into a mathematical model of PV myocytes. We found that the three influences of β_1 and α_1 stimulation evoked a repetitive spontaneous APs in our PV myocyte model. As a minimal requirement, the maximal conductances of LCC and SERCA were multiplied by two and three, respectively, and the $[IP_3]$ was increased above ~2 μM. Under this condition, the $[Ca^{2+}]$ in submembrane space near the Ca^{2+}-releasing site was gradually increased by SR Ca^{2+}-release to finally evoke a massive Ca^{2+}-induced Ca^{2+} release from SR. The resultant Ca^{2+} transients triggered AP through NCX-mediated inward current. Behind this sequence of events for spontaneous AP discharge, a certain range of Ca^{2+} overload of the model cell was always observed. The automaticity was hardly initiated with the β_1 adrenoceptor stimulation alone. (COI:No)

AC-12

The elevated orexin sensitivity via OX2R in RVLM involves with an increased arterial pressure in rats fed a high fat diet

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Rats fed a high fat diet have been used as an animal model of metabolic syndrome, i.e., increases in body mass, arterial pressure (AP), and leptin in plasma. We hypothesized that sympathoexcitation caused by the elevated orexin sensitivity in rostral ventrolateral medulla (RVLM) induces an increased AP in HFD. We employed Sprague Dawley rats fed a high fat diet (HFD) or a normal diet (ND) for 10 weeks from 3 weeks old. We measured orexin A in cerebrospinal fluid by ELISA and orexin receptors (OX1R, OX2R) mRNA expression in RVLM by RT-PCR. There was no significant difference in orexin A between HFD and ND (HFD: 62.5 ± 2.1 pg/mL, ND: 58.1 ± 3.0 pg/mL). On the other hand, OX2R mRNA in HFD was significantly higher than that in ND (HFD: 0.98 ± 0.21, ND: 0.50 ± 0.10). To test the hypothesis, we injected orexin B (175 pmol in 50 nL) to RVLM under urethane + α-chloralose anesthesia with measuring AP. Orexin B induced a larger AP response in HFD than ND (area under the curve in HFD: 1675 ± 318 mmHg×180 s, ND: 814 ± 255 mmHg×180 s). We also injected OX2R antagonist, TCS-OX2-29 (20 nmol in 2 μL) to the lateral ventricle. Although OX2R antagonist had no effect on AP in ND, it decreased AP in HFD (area under the curve in HFD: - 509 ± 113 mmHg×180 s, ND: 171 ± 50 mmHg×180 s). These results suggested that the elevated orexin sensitivity in RVLM induces a chronic increased AP in HFD. To further examine this, whether AP in HFD is decreased by chronic injection of OX2R antagonist has to be determined in a future study. (COI:No)

AC-13

Vitamin B1 pretreatment preserves cardiac function against ischemia-reperfusion injury

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Vitamin B1 (VitB1) deficiency was recognized as a cause of Beriberi (Kakke; a neurological disease and heart failure). We previously investigated that the pretreatment of thiamine pyrophosphate (TPP), an active form of VitB1, preserved cardiac contraction after ischemia/reperfusion (I/R). To investigate the mechanism of preserved cardiac function by TPP after I/R injury, we performed biochemical analysis in this study. Male Sprague-Dawley rats (around 10 weeks old) were used. After 5 min perfusion of Tyrode's solution with or without 300 μM TPP in the Langendorff system, the hearts were treated with 40 min global ischemia followed by 60 min reperfusion. In the biological experiments, the phosphorylation level of AMPK in TPP-treated heart was reduced in comparison with untreated heart (n=5 each, $p<0.05$). In metabolome analysis, levels of AMP and ADP in TPP-treated heart were decreased, however, ATP level was not different between TPP-treated heart and control. We also measured tension in the papillary muscle of the right ventricle. TPP (100 μM) did not increase muscle tension. However, in the presence of 100 μM TPP, lactic acid (10 mM) increased muscle tension (n=4, $p<0.05$). Without TPP, lactic acid did not increase muscle tension. Taken together, 1) inhibition of AMP production by pretreatment of VitB1 and 2) the positive inotropic effect by pretreatment of VitB1 in the lactic acid accumulation could explain the mechanisms in the cardioprotective effect of VitB1 pretreatment against I/R injury. (COI:No)

AC-14

Multispectral Fluorescence Imaging for Evaluation of Arteriosclerosis

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We aimed to diagnose arteriosclerosis by a multispectral imaging that can identify the fluorescence which originates from the substance in the arterial wall. The inner surface of the coronary arteries extracted from human cadavers was illuminated by an excitation light (405nm), and multispectral fluorescence (450-700nm) images were obtained. The fluorescence spectra in arteriosclerotic sites were shown to be different from those in normal sites. We then calculated an index of fluorescence intensity at a wavelength of two significant differences for each pixel, and reconstructed the index images. As a result, we succeeded in "disease mapping", by which arteriosclerotic sites can be discriminated from normal site. In addition, a ratio of the index of normal sites to arteriosclerotic sites was shown to correlate to a thickness of arterial wall. The differences in fluorescence spectra may be ascribed to the differences in fluorophores contained in the intima/media of artery. (COI:No)

AC-15

Development of a microcirculation model to calculate glucose supply to meet the cellular requirement

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Glucose transport through capillary membrane to tissue cells rely upon convection and diffusion. This study aims at clarifying relative contributions of these two mechanisms to the total supply of glucose by constructing a microcirculation model. The convection of glucose was determined by the trans-capillary fluid exchange according to the Starling principle and the reflection coefficient for glucose. Diffusion was calculated using the experimental permeation coefficient and the concentration gradient across the capillary membrane. It was confirmed that the glucose transport was mainly dependent on diffusion, which was largely determined by the rate of metabolic rate of the tissue cells, and less dependent on convection. When glucose catabolism was ceased, the glucose diffusion rapidly decreased within 21 min accompanying the decrease in the concentration gradient across the capillary membrane, and no diffusion was observed in steady state. Vice versa, the diffusion rate was accelerated when the cellular consumption was enhanced. We assumed that the tissue cells demanded glucose at 70 % of its own glucose consumption, and examined the glucose supply capacity at various levels of cellular consumption. In the standard condition, the capillary allowed the cellular consumption to increase only by 3.1-fold. Increasing the number of capillaries carrying blood flow by 4-fold allowed the consumption to increase by 13.4-fold, whereas increasing the blood flow scarcely increased the supply to meet the consumption. (COI:No)

AC-16

Changes in posterior cerebral artery blood flow velocity and vertebral artery blood flow during static exercise

Washio Takuro、Sasaki Hiroyuki、Ogoh Shigehiko
(Tokyo)

It has been established that vertebral artery (VA) blood flow increased during dynamic heavy exercise. More recently, in contrast, it was reported that blood flow velocity at posterior cerebral artery (PCA), which was bifurcated from VA, decreased during dynamic exhaustive exercise. Under these background, we hypothesized that PCA blood flow velocity (PCAv) could not be used as an index of blood flow at the artery of posterior circulation during exercise because of a change in PCA diameter. To test this hypothesis, we examined whether change in PCA velocity reflected that of VA blood flow during handgrip exercise (HGex). Nine healthy subjects performed HGex for 150 seconds at 30% maximum voluntary contraction. PCAv and VA blood flow were measured continuously throughout each exercise trial by transcranial Doppler ultrasonography (TCD) and Doppler ultrasound, respectively. In the present study, our comparison was based on the idea that change in PCAv was the similar with that of VA blood flow. HGex significantly increased both VA blood flow and PCAv from the resting baseline (34.9±17.3 %, $P<0.001$; 12.16±5.3 %, $P<0.001$). Interestingly, the PCA conductance index was significantly decreased from rest (-8.75±8.8%, $P=0.02$) despite no change in VA conductance (9.9±18.3 %, $P=0.14$). These findings clearly showed that the response of PCAv to HGex unlikely reflected change in blood flow at posterior circulation. Therefore, PCAv measured by TCD may not be useful as an index of PCA blood flow especially during resistance exercise. (COI:No)

AC-17

Acute effect of high-intensity short-term endurance training on arterial stiffness in elite endurance runners

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We hypothesized that an intense exercise-induced sympathoexcitation enhanced arterial stiffness. To test our hypothesis, we examined the relationship between responses of arterial stiffness and the balance of sympathetic and parasympathetic vascular tone after an intense short-term training camp. Thirty three male elite college endurance runners participated in the study. Each participant performed a short-term intense endurance training at the one-week endurance training-camp. All physiological parameters were measured at the same time of day in the morning on the days immediately before and after the training camp. As expected, the training-camp increased heart-to-ankle pulse wave velocity (haPWV) significantly ($P=0.049$), but the ratio of low and high frequency spectral power of R-R interval (LF/HF ratio), which is an index of the balance between sympathetic and parasympathetic tone, was unchanged ($P=0.930$). However, when we divided all participants into subgroups (increase or decrease group in the LF/HF ratio), the haPWV was increased significantly after training camp in the LF/HF increase group ($P=0.017$). In contrast, the haPWV was unchanged in the LF/HF decrease group ($P=0.459$). These findings suggest that the short-term intense endurance training induced-change in sympathetic vascular tone partly determines arterial stiffening after training camp. (COI:No)

AC-18

Effects of eicosapentaenoic acid supplementation on prognosis and clinical parameters in the elderly

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The purpose our study was to investigate the cause of eicosapentaenoic acid (EPA) deficiency in care house residents and the effect of EPA treatment on clinical parameters in total 130 elderly in their mid-eighties.We recruited the age- and sex- matched three groups: out-patients clinic attendees (OPC), group home residents (GH) and elderly in a geriatric welfare home for the elderly (GWHE).GH had higher mortality and lower EPA/AA values (0.20 vs 0.55 in median) than the OPC group. EPA supplementation (1800 mg daily) reduced relative mortality by 83% in the GH ($p<0.001$) in contrast with a reduction of 8% in the OPC patients ($p=0.759$).EPA/arachidonic acid (AA) ratio was lower in the GH and GWHE residents than in the OPC (OPC; 0.56±0.3, GH; 0.23±0.12, GWHE; 0.31±0.1). Fish consumption was lower in the two groups than in the OPC group. EPA treatment increased serum EPA levels (54.0±29.0 to 210.5±50.6 μg/ml, $p<0.001$), decreased AA, docosahexaenoic acid, triglyceride and LDL cholesterol levels at 4.5±3.4 months after administration and reduced the severity of supraventricular arrhythmias on ambulatory electrocardiogram at 12.5±4.5 months ($p<0.05$). EPA supplementation reduced mortality in the GH residents with EPA deficiency related to the nutritional characteristics of the elderly care facility. EPA treatment induced changes in various lipids and reduced the severity of supraventricular arrhythmias. (COI:No)

AC-19

ATP consumption by the cardiac muscle contraction implemented in a Huxley-based contraction model

Yuttamol Muangkram, Kosuke Taniguchi, Akinori Noma, Akira Amano
(Graduate School of Life Sciences, Ritsumeikan University, Shiga, Japan)

A variety of mathematical models have been developed to reconstruct the cardiac muscle contraction evoked by the membrane excitation. However, to our knowledge, no contraction model has been available which simultaneously calculates both the ATP consumption by the S1-segment of myosin and the force of contraction triggered by the intracellular Ca^{2+} transient. The aim of this study was to develop a myofilament model which reconstructs molecular sequences of ATP binding to S1-myosin and subsequent hydrolysis during the muscle contraction initiated by the Ca^{2+} binding to troponin C, based on the Huxley-type contraction model (NL model, Negroni & Lascano, 2008). Our seven-step model of cross-bridge cycle could well reproduce the developed tension evoked by Ca^{2+} binding to troponin as in the original NL model. The ATP hydrolysis was calculated by incorporating the principle three reaction cycle as a short loop to the NL model; (1) the binding of ATP to the catalytic site of S1-segment in place of ADP, (2) hydrolysis of ATP to ADP + Pi accompanied with temporal dissociation of cross-bridge, (3) release of Pi to induce the power stroke. Additional ATP consumption was also estimated according to the cross-bridge average work stroke per 1 ATP hydrolysis. The ratio of ATP usage by the muscle contraction to that of ionic pumps was in rough agreement with those reported estimation based on macroscopic energetic study, when the new contraction model was implemented in the whole cell model of ventricular myocytes. (COI:No)

AC-20

Optogenetic mapping of cerebro-cerebellar communication loop in mice

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Interplay between the cerebellum and the neocortex is crucial for the execution of skilled movement and motor learning. Although the structure and function of cerebro-cerebellar communication loop have been extensively studied, the details of functional connectivity between the two brain structures are still largely unknown.
To elucidate comprehensive functional connections between the neocortex and the cerebellar cortex, here we performed cell-attached recordings from single cerebellar Purkinje cells while layer 5 pyramidal neurons located at various areas of the cerebral cortex were optogenetically stimulated in Thy1-ChR2-EYFP mice. Photostimulation reliably evoked simple and complex spikes in Purkinje cells. Accordingly, we have identified spatial and temporal spike maps on the cerebral cortex including the sensory, motor and association areas.
The results indicate that specific areas of the cerebellum are closely associated with the motor, sensory and association areas. We assume that different aspects of information from these cerebral cortical areas are integrated in the specific cerebellar regions, which enables spatially and temporally accurate coordination of movement. (COI:No)

AC-21

Imaging of the excitatory transmission in a slice of the trigeminal subnucleus caudalis of mice, evoked by afferent pathway stimulation

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The spinal trigeminal caudalis (Vc) has layered structures similar to the spinal cord and plays an important role in the transmission of nociceptive stimulus. However, the pathway from the trigeminal nerve root to the Vc has a complex structure and to make preparations presents considerable difficulty. In this study, we applied calcium imaging techniques to observe excitatory signal propagation using slices of brainstem from mice. Brainstems were removed from 6-7 week-old male mice (C57/BL6J). They were sliced in thicknesses of 600μm in an oblique plane which contained the trigeminal nerve root, tract and the Vc. The slices were incubated with calcium imaging dye Rhod-2. The trigeminal nerve root was stimulated by an electrode which was inserted in the vicinity of the root (intensity: 200μA; duration: 200μsec). Successive fluorescence images of the slices were recorded every 1.2msec. The stimulation to the bundle of fibers around the trigeminal nerve root evoked an increase in intracellular calcium concentration ([Ca2+]in) in the Vc within several msec. The evoked elevation of [Ca2+]in was inhibited in the presence of CNQX and MK-801. This novel approach has potential for investigating trans-synaptic excitation within the Vc. (COI:No)

AC-22

Microglia modify spatial pattern of neuronal activity through the regulation of synaptic activity

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Microglia are the primary immune effector in the central nervous system. Recent studies showed that microglia are highly motile cell in the healthy brain, extending and retracting their processes. They make direct contacts with synapses to monitor synaptic activity in an activity dependent manner. Recent evidence using embryonic zebrafish optic tectum showed that microglia also have direct contact on neuronal cell body to modify neuronal activity. These results lead our hypothesis that microglia also could modify synaptic activity by their direct contacts. In this study we showed using in vivo two photon microscopy that local calcium responses in the postsynaptic spines increased significantly when microglial processes have contacts with spines. Activation of microglia by applying Lipopolysaccharide (LPS) inhibit spine activation with microglia contacts. Since microglia have several processes and could contact on several spines to modify their activity, they could regulate spatial firing pattern of the neuron. To elucidate this possibility, we compare the correlation co-efficiency of Ca2+ elevation of paired two neurons in wild-type and in LPS injected mice. The averaged correlation co-efficiency is higher in wild type compared with LPS injected mice. Those results suggest that microglia have crucial roles of regulating synaptic activity with direct contacts with synapses to regulate spatial neuronal activity. (COI:No)

AC-23

Imaging of signal transmission in mouse brain slices including cerebral cortex and hippocampal formation; Characteristics of signal transmission in the hippocampal formation from the entorhinal cortex and fimbria

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To spatio-temporally characterize the signal transmission to the hippocampal formation from the entorhinal cortex (EC) and fimbria, an imaging technique was applied to visualize the regional changes in membrane excitation using mouse brain slices. Brain slices of C57BL6J mice prepared by cutting along the plane of the front elevation angle of 30 degrees from the horizontal plane were dyed with a voltage sensitive dye (di-4-ANEPPS) and bathed in artificial CSF. Fluorescence changes of the slices evoked by an electrical stimulation with a microelectrode to the fimbria or EC were detected every 1.2 ms, and the areas revealing excitation were recorded using an image processor equipped with a high-speed camera. Single pulse stimulation to the fimbria evoked a transient excitatory response in the hippocampal CA2, CA1 and subiculum. Stimulation with repetitive inputs (20 Hz) elicited gradually increased excitatory responses. Single pulse stimulation to the EC also evoked a transient excitatory response in the DG. The responses in the DG evoked by the repetitive inputs from the EC diminished and became very weak at the sixth of the stimulus. The responses in the hippocampal formation to both single and repetitive stimulations almost disappeared in the presence of a glutamate receptor antagonist CNQX. The findings suggest that the repetitive inputs from the fimbria-fornix is amplified and those from the ipsilateral cortex were attenuated in the hippocampal formation. (COI:No)

AC-24

High fidelity spike propagation along single axon of hippocampal mossy fiber

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Axons carry output signal of neurons as the consequence of somato-dendritic integration of numerous synaptic inputs, and therefore could be the potential site for modulation of neuronal information dynamics. Direct recordings from single axons or the terminals are the promising approach to understanding the mechanism of fine-tuning of spike propagation along the axons. In this study, we adopted loose-patch clamp recordings from the visually identified single axon terminals of the mossy fibers in mouse hippocampal slices. Electrical stimulation to the granule cell layer of dentate gyrus elicited action potentials on the recorded terminals in all-or-none fashion. These responses are sodium spikes of the axon terminals, since they were abolished by focal application of 0.5μM tetrodotoxin to the recording site. Using supra-threshold stimulus intensity (approximately 50 % stronger than threshold stimulus intensity) at room temperature of around 25°C, these spikes rarely failed in response to low frequency stimulation at 0.1Hz. Even at the high frequency stimulation at 50 or 100 Hz, failures of axonal spike occur only occasionally (1 ± 1 % and 10 ± 5 %, respectively, mean ± SE, n=14). These results suggest that spike propagation along hippocampal mossy fibers are highly reliable process, in contrast to the prediction as expected from impedance mismatch of thin axon shafts and large axon terminals on en passant axons. High fidelity of spike propagation along the mossy fibers is possibly due to expression of high density of voltage-dependent sodium channels in the axon terminal membranes. (COI:No)

AC-25

Distributions of Doc2A and B in the central nervous tissue of rodents

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Doc2 is a soluble protein containing two C_2 domains. Its two isoforms (A and B) are expressed in the brain and have been thought to be involved in exocytosis of synaptic vesicles based on electrophysiological findings in Doc2-deficient neurons. Although it is reported that mRNAs coding Doc2A and B are expressed in neurons, it is still unclear whether the proteins are present in presynaptic terminals. In this study, we have generated isoform-specific antibodies against Doc2A and B and studied their distributions in rodent brains. Antibodies were raised by immunizing rabbits with a synthetic peptide corresponding to a carboxy-terminal sequence of either rat Doc2A or B. In immunoblotting experiments, the antibodies specifically reacted with each isoform in rat brain homogenate as well as recombinant proteins. Immunohistochemistry revealed that Doc2A and B were expressed in synaptic and non-synaptic regions in rat cerebellum. In primary cultures of mouse hippocampal cells, immunoreactivies against Doc2A and B were observed in glia as well as neurons. In neurons, Doc2A and B were found to be colocalized with a synaptic vesicle marker (synaptotagmin), but not a postsynaptic marker (PSD-95), supporting the idea where Doc2A and B play important roles in neurotransmitter release from presynaptic terminals. In addition, these results suggest that Doc2A and B are also involved in functions other than synaptic vesicle exocytosis in neurons and glia. (COI:No)

AC-26

Optogenetic activation of serotonergic terminals facilitates inhibitory input to orexin/hypocretin neurons

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Orexin/hypocretin neurons play a crucial role in the regulation of sleep/wakefulness, especially in the maintenance of wakefulness. These neurons innervate wide area in the brain and receive many inputs including serotonergic (5-HT) neurons in the raphe nuclei. Although, previous studies revealed that 5-HT inhibited orexin neurons, it is still not well understood so far that how 5-HTergic neurons regulate orexin neurons since 5-HTergic neurons contain not only 5-HT but also other neurotransmitters. To reveal this, we generated a new triple transgenic mice (orexin-EGFP;Tph2-tTA; TetO ChR2) in which orexin neurons express EGFP and 5-HTergic neurons express channelrhodopsin2 (ChR2). Immunohistochemical studies showed that 5-HTergic terminals in the lateral hypothalamic area expressed ChR2. We generated brain slice preparations, recorded from orexin neurons and stimulated 5-HTergic terminals by illuminating blue light. Optogenetical activation of 5-HTergic terminals induced outward current and increased IPSCs, that is increased GABAergic inhibitory inputs to orexin neurons. Accordingly, firing frequency of orexin neurons decreased upon activating 5-HTergic terminals. However, EPSCs were not affected by activating 5-HTergic nerve endings. These results suggest that orexin neurons are inhibited by 5-HTergic neurons by both direct and indirect manner. (COI:No)

AC-27

The endogenous opioid system mediates odorant-X induced analgesia in mice

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We previously reported that odor exposure to odorant-X, one of the terpenoids involved in plant extracts, induced significant analgesic effects in mice. The analgesic effects were not observed in anosmic model mice, indicating that olfactory input triggers the effects. Furthermore, analyses of mutant mice indicated that hypothalamic orexinergic neurons mediate the effects. However, most of the central neuronal mechanisms underlying the analgesia remain unrevealed. In this study, we examined the contribution of the endogenous opioid system to the odor-induced analgesia using the hot plate test. Mice were injected i.p. with eitheir an opioid receptor antagonist (3.0 mg/kg naloxone hydrochloride) or saline 10 min prior to a 5 min exposure to either odorant-X or odorless air. The latency to pain response in the hot plate test (54.5 °C) wa then measured in order to determine the pain threshold. The latency of odorant-X exposed group was 30.2% longer ($p < 0.05$, Mann-Whitney U test) than that of the odorless-air exposed group under vehicle injection. The latency was, however, not significantly different (-1.61%) between the two groups under naloxone injection. These results indicate that the endogenous opioid system mediates the odor-induced analgesic effects. (COI:No)

AC-28

Merkel cells transduce mechanical stimulation into intercellular transmitter release

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Merkel cells (MCs) have been proposed to form a part of the MC-neurite complex with sensory neurons. However, the mechanism underlying neurotransmission between MCs and nerve endings is not well understood. By using fura-2 fluorescence, we examined the increase in intercellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in response to membrane deformation, elicited by direct mechanical stimulation. MCs were acutely isolated from the buccal mucosa of 3-5 week-old golden hamsters after intraperitoneal injection of quinacrine (MC marker) 24 h prior to isolation. Application of direct mechanical stimuli by glass micropipette to MCs induced an increase in $[Ca^{2+}]_i$, which was dependent on the intensity of the mechanical stimuli. Direct mechanical stimulation induced $[Ca^{2+}]_i$ increase in not only the stimulated MC but also the adjacent MCs. The amplitude of $[Ca2+]_i$ increase was inversely proportional to the distance between mechanical stimulation and nearby MCs, suggesting that the effect was distance dependent. These results indicate that MCs respond to mechanical stimulation by releasing diffusible chemical signaling molecule(s) into the extracellular space. (COI:No)

AC-29

Serotonin improves visual detectability of freely moving rats

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Efferents from serotonergic neurons localized in the pons and upper brain stem reach the whole cerebral cortex, releasing serotonin (5-HT) in a behavioral context-dependent manner and modulating various brain functions to fit the underlying behavioral situation. In our previous study on the primary visual cortex (V1) of anesthetized monkeys, iontophoretically administered 5-HT1B and 2A receptor-selective agonists improved the signal-to-noise ratio and enhanced the response gain, respectively, for visual inputs in V1 neurons. It suggests that endogenously released serotonin optimizes the neuronal visual information processing and improves behavioral visual detectability, but it remains unknown. To examine this point, we tested the effect of a fluoxetine (FLX), a selective serotonin reuptake inhibitor, on the behavioral contrast sensitivity (CS) as visual detectability. We first trained Long Evans rats to perform a two-alternative forced-choice grating detection task (2AFC). And then, the CS was measured by the 2AFC combined with a staircase method where grating contrast is decreased or increased step-by-step according to animal's correct or miss response in the prior trial. We compared the CS obtained from control and intraperitoneally injected FLX groups, finding that the CS was significantly higher in FLX than in control condition. Thus, serotonin level in the brain involves visual detectability of freely moving rats. We are currently testing the effects of FLX on neuronal activity in rat V1. (COI:No)

AC-30

Hypothalamic MCH neurons negatively control spatial recognition memory in mice

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[Background] Melanin-concentrating hormone (MCH)-producing neurons project widely throughout the brain, including hippocampus, from lateral hypothalamus. We have demonstrated MCH neurons control NREM and REM sleep. As sleep is suggested to be linked to memory, possible involvements of MCH neurons in memory formation were examined.
[Methods] Ablation of MCH neurons were achieved with diphtheria toxin A in MCH-tTA; TetO DTA bigenic mice by removing doxycycline in chow. Activation of MCH neurons were induced by clozapine-N-oxide through MCH neurons-specific hM3Dq designer receptor exclusively activated by designer drug (DREADD), in MCH-Cre mice. The MCH neuron ablated or activated mice were subjected to the object recognition test and the contextual fear conditioning test as the tasks of memory.
[Results] MCH neuron ablated mice spent more time for exploration of a novel object compared to MCH neuron intact control mice. MCH neuron ablated mice given foot-shock-conditioned fear memory showed higher freezing rate than the control mice. In contrast, MCH neuron activated mice spent less time for a novel object and showed lower freezing rate than the control mice.
[Conclusion] The findings that the ablation and activation of MCH neurons improved and decreased memory formation respectively suggest that MCH neurons control memory negatively. (COI:No)

AC-31

Splicing regulation of cold-inducible RNA-binding protein (CIRP) mRNA in hibernating Syrian hamsters.

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Hibernators can survive even under severe hypothermic conditions less than 10°C during hibernation. We hypothesized cold-inducible RNA-binding protein (CIRP), which is a family of cold shock proteins, might participate in the tolerance to hypothermia, since CIRP plays important roles in protection of various types of cells against harmful cold temperature. In the present study, we investigated expression patterns of CIRP mRNA in various organs in hibernating hamsters. Hamsters were kept in cold environment to induce hibernation. Then we analyzed the expressions of CIRP mRNA in the brain, kidney, lung and heart using RT-PCR. The RT-PCR analysis revealed that CIRP mRNA is constitutively expressed in all the organs of non-hibernating euthermic hamsters with three alternative splicing variants. The short form contained open reading frame for full-length CIRP. On the other hand, the long form was inserted sequences containing a stop codon, suggesting production of C-terminal deletion isoform of CIRP. In contrast to non-hibernating hamsters, only the short product was amplified in the hibernating animals. These results indicate that CIRP expression is regulated at the levels of alternative splicing in the hamster organs including the brain and heart, which would permit a rapid expression of functional CIRP during entering hibernation. Considering the expression pattern is commonly observed in most of the organs, the hibernation-specific splicing of CIRP gene could be important for tolerance to hypothermia. (COI:No)

AC-32

The timing-regulatory signaling via neural connections from the suprachiasmatic nucleus is necessary for estrous cyclicity

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The central circadian clock in the suprachiasmatic nucleus (SCN) generates daily oscillations responsible for organizing the timing of most physiological events in mammals, including the luteinizing hormone (LH) surge. In female rodents with normal estrous cycle, LH surge occurs on the day of proestrus in the late afternoon. Given the fact that SCN lesioning abolishes LH surge, timing-regulatory signals from the SCN are required for estrous cyclicity. It is known that these signals travel via neural connections and diffusible factors. However, which pathway is critical for the estrous cyclity remains unclear. In the present study, we employed a micro-knife to sever nerves originating from the SCN. To determine the effect of the knife-cut on estrous cyclicity, we recorded wheel-running activity and vaginal smears of female C57BL/6J mice (n = 17) showing normal 4 or 5 days-estrous cycle before and after the knife-cut. In 7 of 17 mice, their estrous cyclicity was disappeared and the SCN were completely isolated by the knife-cut. 4 of 17 mice with partially SCN isolated had a normal estrous cycle. Remaining 6 sham-operated mice showed normal estrous cycle. In addition, mice which had disappeared estrous cyclicity by the knife-cut showed light-entrained behavioral rhythms and circadian rhythms in constant darkness, indicating their SCN are intact. Our results strongly suggest that the timing-regulatory signaling via neural connections from the SCN is critical for estrous cyclicity. (COI:No)

AC-33

Caffeine improves the contrast sensitivity of freely moving rats

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Caffeine (1, 3, 7-trimethylxanthine) is a well-known CNS stimulant, affecting positively arousal, attention, and wakefulness by antagonism of adenosine receptors. Since adenosine exerts a tonic inhibitory influence in the CNS as an endogenous neuromodulator, caffeine's action is thought to be the disinhibition of CNS activity from the adenosinergic modulation. This suggests that caffeine modulates various CNS functions including visual function. However, it remains unclear whether and how caffeine modulates visual ability such as contrast sensitivity (CS) and CS-spatial frequency (SF) relationship. To investigate these points, we tested the effects of caffeine on the behavioral CS of freely moving rats. Long Evans rats were trained to perform a two-alternative forced-choice visual grating detection task (2AFC). After the animal's learning, the CS was measured with or without an intraperitoneal injection of caffeine using the 2AFC combined with a staircase method. The grating contrast was decreased or increased step-by-step according to animal's correct or miss response in the prior trial. Caffeine improved the CS at low SF (0.1 cycles/degree), suggesting that neuronal visual information processing is modulated by caffeine presumably via adenosine receptors. Therefore, we are currently examining whether and how adenosine receptors are involved in the improvement of the contrast detectability in the same behavioral experiments and how caffeine modulates neuronal activity in the primary visual cortex in in vivo electrophysiological experiments. (COI:No)

AC-34

Effects of proteins on entrainment of mouse peripheral clock

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Insulin secretion is known to play an important role in the entrainment of the peripheral clock via restricted feeding (Tahara et al., 2011; Sato et al., 2014). We also found that fast-digesting carbohydrates cause a large phase-shift in the peripheral clock (Itokawa et al., 2013). In addition, we found fish oil containing rich DHA/EPA could facilitate the RF-induced entrainment by the activation of insulin secretion through GPR120 located on the lower ileum and upper large intestine (Furutani and Ikeda et al., 2015). However, we have not yet established a clear relationship between the amounts of proteins in food and entrainment of the peripheral clock. In this study, we prepared 100% casein (100C), equal amounts of casein and β-cornstarch (βCS) (50C), or 100% βCS (0C). Mice were fed with each of these diets at daytime for two days. We measured the phase-advances in the liver, kidney, and, submandibular gland (Sub Gla) clocks using in vivo imaging system (IVIS). Unlike the liver and the kidneys, the phase-advance of the Sub Gla was positively dependent on the carbohydrate content of the food. These results suggest that protein-rich foods elicit signals that are independent of insulin secretion. And, 100% casein food increased significantly glucagon secretion. At present, we are verifying pCREB that downstream of glucagon. This work was partially supported by the Council for Science, Technology and Innovation, SIP. (COI:No)

AC-35

Characteristics of circadian behavioral rhythms in CBA mouse substrains

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Currently, six substrains of the CBA mouse inbred strain are commercially available. The differences in the circadian phenotype between the substrains have not been studied yet. In this study, we determined the characteristics of circadian behavioral rhythms in CBA/N and CBA/J substrains. 8-week-old male mice were housed in a cage with a wheel and maintained under a 12:12 h light-dark (LD) cycle. Their wheel running activities were recorded under LD and constant dark (DD) conditions. Then, the animals were re-entrained to the LD cycle and their activities were recorded under constant light (LL) conditions. The mice were also subjected to forced swimming test (FST) and tail suspension test (TST). Under DD conditions, CBA/N mice showed a significantly shorter free-run period than CBA/J mice (CBA/N: 22.59h CBA/J: 23.12h) and the total activity of CBA/N mice (53152 revolutions/cycle) was significantly higher than that of CBA/J mice (35932 revolutions/cycle). Under LL conditions, CBA/N mice showed a significantly longer free-run period than CBA/J mice (CBA/N: 25.6h CBA/J: 25.04h). Furthermore, CBA/N mice showed a statistically longer immobility time in the TST than CBA/J mice, indicating the differences in psychiatric state between two the substrains. We also observed circadian behavioral rhythms in CBA/Ca and CBA/CaJ mice, which showed CBA/N-type circadian behavioral phenotypes. Taken together, the data suggest the existence of an unknown mutation that affects circadian and psychiatric behaviors. (COI:No)

AC-36

The quality of elderly's sleep and the circadian rhythm of their autonomic nerve

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[Purpose]It is said that the quality of sleep deteriorates with aging. We studied elderly's autonomic-nerve functional circadian rhythm and their sleep.[Subjects and Method]Twenty two healthy elder males (77.0±3.1 years old). Controls are 12 healthy young males (23.6±3.9 years old).We calculated sleeping hours (SH), sleep latency (SL), frequency of halfway wakening episode (WEF), halfway wakening time (WET), and sleep efficiency by using the record of the Actigraphy. We evaluated parasympathetic function (HF) and sympathetic function (LF/HF) by using frequency analysis of the R-R interval of ECG. ECG was recorded by Holter recorder. [Result and Discussion]There was no significant difference in the WEF (elderly 11.0±7.0, youth 13±7.0, p=0.486) and SH (295.7±97.8, 325.9±97.1, p=0.424) between elder group and young group. WET (49.4±33.2, 12.5±6.9, p=0.0002) and SL (12.1±8.3, 4.4±4.1, p=0.01) of elderly were significantly longer than those of youth. The elderly might not go to sleep easily at hypnagogic or after halfway wakening. They were considered not to be satisfied. Parasympathetic function of youth had significant circadian rhythm (p=0.001, 2way-ANOVA). It rose during sleep time and it fell during awaking time. In elderly, this circadian rhythm was decreasing definitely. [Conclusion]In elderly, in order that the circadian rhythm of a parasympathetic-nerve function might decrease at night , it was suggested that neither the energy storage nor tissue recovery is performed to sufficiently. (COI:No)

AC-37

Cognitive function and nutriture of healthy elderly

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[Purpose]It is known that volume of dementia patients' prandials decreases and their nutriture gets worse. Not only in dementia patient also in healthy elderly in whom ADL is independent, nutriture may get worse. We examined the healthy elderly's cognitive function and nutriture. [Subjects and Method]Sixteen healthy elderly males (75±3.4 years old) and 2 healthy elderly females (79.5±3.5 years old), whose ADL are independent were recruited. Controls are 12 young healthy males (22.3±1.2 years old). We evaluated the cognitive function by using event-related-potential P300 latency. The event-related potential was recorded with the hearing odd-ball task. We evaluated the nutriture using BMI and serum nutrition index.[Result and a Discussion] The latency of elderly's P300 (Fz405.6±85.0,Cz403.0±87.6,Fz409.0±82.6,response time431.5±79.8) were significantly longer than those of healthy youth(345.3±24.9,341.1±21.4,340.8±19.0,336.8±60.5). Even if the cognitive function and ADL of elderly were with in normal limit, their P300 latency increased. We thought that the degeneration of medullary sheath because of aging made the latency prolonged. The significant negative correlation between Prealbumin (PAL) and age was observed (correlation coefficient 0.624, p= 0.006). The P300 latency tended to prolong in aging (0.450,p=0.06). It has been said that arteriosclerosis was the cause of medullary-sheath denaturation of elderly. A poor nutriture may be the cause of a medullary-sheath denaturation.[Conclusion]Nutriture and cognitive function may be related. (COI:No)

AC-38

Orexin regulates noradrenergic signaling in lateral amygdala to modulate fear-related behavior

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Orexins (OX) play a key role in maintaining wakefulness. Salient emotional signals are known to activate OX neurons and increase arousal, suggesting the involvement of OX in increase of vigilance levels. We found orexin-1 receptor (OX1R)-KO mice exhibited reduced freezing behavior against fear-conditioned cues and contexts. OX1R-mediated activation of noradrenergic neurons in the locus coeruleus (LC-NA neurons) was shown to be essential for cued fear memory retrieval and/or consolidation. Here, we tested roles of the OX-LC-LA circuit in the fear-related behavioral response. After cued fear conditioning in a context A, photostimulation of OX fibers in the LC or LC-NA fibers in the LA induced freezing behavior even in altered context B. These mice showed increased freezing time when exposed to the context B again. These data exhibit the OX-LC pathway modulates fear-related behavior and fear generalization often seen in PTSD. We also found that pharmacogenetic or optogenetic inhibition of LC-NA neurons reduced the cued fear memory retrieval. OX1R antagonist (SB-334867) administered just before test session also decreased cue-induced freezing behavior. Finally, selective deletion of OX1R in the LC-NA neurons showed decrease in the cue-induced freezing time. These results demonstrate that OX activates the LC-NA neurons to modulate LA function and this pathway can drive fear memory retrieval, indicating a possibility that OX1R antagonists are effective for treating psychiatric diseases with excessive fear responses, such as PTSD and phobia. (COI:No)

AC-39

Functional characterization of FTSJ1, a X-linked mental retardation-related gene

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Genetic mutations in X chromosome-linked genes have been associated with mental retardation (XLMR). Recently, linkage analyses performed in Belgian, Chinese and Japanese families have identified Ftsj1 gene as a novel candidate gene. Ftsj1 shares homology with a bacterial 23S rRNA methyltransferase FTSJ.However, the molecular function of Ftsj1 and its pathological relevance in mental retardation have remained unknown. Using Ftsj1 knockout mice, we demonstrate that Ftsj1 methylates cytosolic transfer RNAs (tRNAs) at position 32and 34. While the FTSJ1 KO mouse developed normally, we observed a decreased protein synthesis level in hippocampus of FTSJ1 KO mice using puromycin-mediated in vivo pulse-labeling technique. Especially, there was a marked decreased of synaptic proteins including glutamate receptors and signaling molecules. The decreased protein synthesis level resulted in the electrophysiological and morphological abnormalities in hippocampal neurons of FTSJ1 KO mice. There results suggest that the accumulation of hypomodified tRNAs disturbsneuronal protein synthesis, which ultimately contributes to the developmentof mental retardation in Ftsj1-deficient mice and human. (COI:No)

AC-40

Sympathectomy and sympathetic blockade reduce pain behavior via alpha-2 adrenoceptor of the dorsal root ganglion neurons in a lumbar radiculopathy model

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The abnormal sympathetic-somatosensory interaction may underlie some forms of neuropathic pain. We investigated whether sympathectomy and pharmacological sympathetic blockade reduced pain behavior and reversed adrenoceptor mRNA expression of the dorsal root ganglion (DRG) in a lumbar radiculopathy model. We used 91 male Sprague-Dawley rats. Just after root constriction (RC), the rats underwent sympathectomy or received three local injections of subtype-specific α-adrenergic receptor antagonists around the DRG. We evaluated the analgesic effects of sympathectomy and sympathetic blockade using behaviors indicative mechanical allodynia and thermal hyperalgesia. We estimated the mRNA expression levels of the DRG adrenoceptor subtypes using real time reverse transcription polymerase chain reaction (RT-PCR). Sympathectomy and α_2-antagonist significantly reduced the mechanical allodynia and thermal hyperalgesia following RC. Real time RT-PCR analysis indicated that sympathectomy possibly reversed α_{2A}- and α_{2B}-adrenoceptors mRNA overexpression in the DRG following RC. We considered that pain behavior of neuropathic pain are due, at least in part, to enhanced sympathetic noradrenergic transmission within the DRG. Suppression of sympathetic activity by reducing adrenergic release, α_2-adrenoceptor stimulation, and/or α_2-adrenoceptor upregulation in the DRG may relieve neuropathic pain. (COI:No)

AC-41

Mechanism of the colokinetic effect of spinal serotonin in rats

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We have previously demonstrated that serotonin enhances colorectal motility through activation of lumbosacral 5-HT2 and 5-HT3 receptors in rats. The aim of this study was to clarify neural pathway of the colokinetic effect. Rats were anesthetized with α-chloralose and ketamine, and colorectal intraluminal pressure and expelled liquid volume were recorded in vivo. Intrathecal administration of serotonin into the L6-S1 spinal cord elicited periodic increases in colorectal intraluminal pressure that were associated with increases in liquid output. This serotonergic colokinetic effect was unaffected even after disconnecting from supraspinal regions by severing the T10 spinal cord. On the other hands, transection of the pelvic nerves prevented the serotonin-induced enhancement of colorectal motility. Intravenous injection of hexamethonium, a ganglionic blocker, inhibited the action of serotonin on colorectal motility. In addition, after injection of atropine, a muscarinic acetylcholine receptor antagonist, the effect of serotonin was attenuated. These results suggest that lumbosacral serotonin acts on the pelvic efferents to enhance colorectal motility via the enteric neurons. (COI:No)

AC-42

Caudal lateral/ventrolateral periaqueductal gray projects to the rostral ventrolateral medulla in rats

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Periaqueductal gray matter (PAG) in the midbrain plays a role in sympathetic regulation in response to stress such as exercise. The rostral ventrolateral medulla (RVLM) contributes to determination of sympathetic nerve activity level. Although the PAG interplays with the RVLM to evoke stress-induced sympathoexcitation, little information is currently available as to neurocircuitry to link the PAG with the RVLM. Here, we examined detailed locations of cell bodies of PAG neurons projecting to the RVLM in rats. Cholera toxin subtype B (CTb), a retrograde tracer, was unilaterally microinjected into the RVLM (N=20). Eight-to-twelve days after the microinjection, coronal brain sections including the PAG at bregma -8.4 ~ -5.4 mm levels (every 0.6 mm) were processed for immunofluorescence staining to detect CTb-labeled cell bodies. CTb-labeled neurons were dominantly distributed in the PAG ipsilateral to the RVLM into which CTb had been microinjected as compared with those at the contralateral PAG at each bregma level ($P<0.05$). Moreover, in the ipsilateral PAG, the highest distribution of CTb-labeled neurons was observed at bregma -7.8 mm among all levels examined. Furthermore, in the ipsilateral PAG at bregma -7.8 mm, the distribution of CTb-labeled neurons in the lateral and ventrolateral subdivisions was more than threefold as compared with that in the dorsolateral subdivision. Taken together, we suggest that the caudal lateral/ventrolateral PAG subdivisions are a major area which sends efferent projections to the RVLM. (COI:No)

AC-43

Descending adrenergic pathways activate the spinal defecation center in rats.

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There are two defecation centers in the central nervous system, the lumbosacral spinal defecation center and a center in the brain stem. Although these two centers communicate with each other, underling mechanisms are not well understood. In the brain stem, there are abundant adrenergic neurons and some of them project to the lumbosacral spinal cord where the defecation center is located. In this study, we focused on noradrenaline (NA) and investigated whether intrathecal application of NA to the spinal defecation center affects colorectal motility in anaesthetized rats. Rats were anaesthetized with alpha-chloralose and ketamine, and both the distal colon and anus were cannulated to measure intra-colorectal pressure and expelled intraluminal liquid volume. Intrathecal application of NA at the level of the lumbosacral defecation center caused strong propulsive contractions of the colorectum. Pre-application of tetrodotoxin to the lumbosacral spinal cord blocked the effect of intrathecally applied NA. The alpha-1 adrenoceptor agonist phenylephrine applied intrathecally mimicked the effect of NA and the alpha-1 adrenoceptor antagonist prazosin applied intrathecally prior to NA blocked its effect. The effect of NA no longer occurred after bilateral severing of the pelvic nerves. Our results demonstrated that intrathecal NA activates the sacral parasympathetic preganglionic neurons through alpha-1 adrenoceptors. This indicates that NA could mediate signaling from the brain stem to the lumbosacral spinal defecation center. (COI:No)

AC-44

Mechanisms underlying impairment of sarcoplasmic reticulum Ca^{2+} release during recovery from prolonged low-frequency force depression

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Intense muscle contractions lead to a greater loss of force at low than high stimulation frequency, which is called prolonged low frequency force depression (PLFFD). Our previous study suggested that an impairment of Ca^{2+} release from sarcoplasmic reticulum (SR) causes PLFFD. The purpose of this study was to investigate the mechanism of impaired Ca^{2+} release during recovery of PLFFD. Intact rat gastrocnemius (GS) muscles were electrically stimulated via the sciatic nerve until force was reduced to ~50% of the initial. The superficial regions of GS muscles were excised 0 h, 0.5 h, 2 h, 6 h and 12 h after the end of fatiguing stimulation and used for skinned fiber analysis. PLFFD occurred at 0 h, 0.5 h, 2 h and 6 h of recovery in skinned fiber. To quantify the ability of the T-system, pairs of identical pulses were applied to the fiber with various time spacing. The T-system ability was decreased at 0.5 h of recovery, whereas it was increased at 6 h of recovery. A skinned fiber was depolarized by substituting Na^{+}-based solution. Depolarization-induced force was significantly reduced at 0 h, 0.5 h, 2 h and 6 h of recovery. The function of Ca^{2+} release channel (CRC), as evaluated by a caffeine-induced force response, was decreased at 0.5 h and 2 h of recovery. These results suggest that the impairments of T-system and CRC contribute to decreased SR Ca^{2+} release in the early stage of recovery, whereas decreased Ca^{2+} release is not ascribable to the failure of T-system and CRC function immediately after stimulation and in the late stage of recovery. (COI:No)

AC-45

Secretion of microRNAs during myogenic differentiation in C2C12 cells

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MicroRNAs (miRNAs) are small non-coding RNAs, which play important roles in the regulation of gene expression at the post-transcriptional level through mechanisms such as translational inhibition or mRNA degradation. There are many evidences regarding the roles and regulation of miRNAs in myogenic differentiating cells. Recently, skeletal muscle-specific miRNAs, which highly expressed in skeletal muscle cells, has been confirmed in the serum of patients with neuromuscular disorders. Therefore, circulating miRNA(s) may be a useful biomarker to evaluate the plasticity of skeletal muscle cells. However, it is still unclear whether skeletal muscle cells secrete miRNA(s) during differentiation. In the present study, we investigated the responses of skeletal muscle-specific miRNAs in the culture medium during the differentiation of culture muscle cells. C2C12 myoblasts were cultured in growth medium for 24 h, and then the culture medium was exchanged to differentiation medium. The medium was collected for every 48 h, and analyzed the level of miRNAs in the medium. Real-time reverse transcription-polymerase chain reaction (RT-PCR) analyses revealed that the level of miR-206 in the medium decreased in the early phase of differentiation, subsequently increased. Therefore, skeletal muscle cells may secrete miRNAs in response to the differentiation phase. Skeletal muscle-specific miRNAs in the circulation may be biomarkers for the skeletal muscle plasticity. (COI:No)

AC-46

The Discovery of Edible Plant Components Specifically Inhibiting Abnormal Vascular Contraction Leading to Vasospasm

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Vasospasm is a major cause of acute-onset sudden death induced by vascular diseases such as ischemic heart and brain disease in Japan, and is mediated by abnormal Ca^{2+}-independent vascular contraction, whereas Ca^{2+}-dependent contraction contributes to the maintenance of physiological blood pressure. As a causal factor of the abnormal contraction leading to vasospasm, we previously identified sphingosylphosphorylcholine, which induced abnormal Ca^{2+}-independent vascular contraction through the sequential activation of Fyn and Rho-kinase. Furthermore, we found that eicosapentaenoic acid (EPA) specifically inhibited such pathological pathway and thereby abnormal, but not normal, contractions, clinically preventing the cerebral vasospasm. However, lipophilicity of EPA precludes its intravenous injection for emergency. Therefore, in this study, we aimed to discover edible plant components inhibiting abnormal vascular contractions, which would enable us to develop not only preventive food for vasospasm but also an injection drug for emergency, because plants have many water-soluble components. We finally found that plant X extracts abolished abnormal contractions strongly and rapidly, while it has minimal inhibitory effect on normal contraction. Three known ingredients of Plant X were also examined as candidates. Among them, water-soluble component A inhibited abnormal contractions specifically, which shed light on both prevention and emergency treatment of vasospasm. (COI:No)

AC-47

A voltage-dependent K^{+} current in rat odontoblasts

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Odontoblasts play an important role in the sensory signal transduction sequence in generating dentinal sensitivity. However, the biophysical properties of voltage-dependent ionic currents in odontoblasts have remained to be clarified. We recorded plasma membrane voltage-dependent ionic currents in odontoblasts by whole-cell patch-clamp method in a voltage-clamp configuration. Voltage steps from -100 to +80 mV from a holding potential of -70 mV evoked outward currents. When we replaced equimolarly Cl^{-} to gluconate in both intracellular (150 mM) and extracellular solution (141 mM), the reversal potential (Erev) of the current showed a shift as expected for a K^{+} equilibrium potential (N = 22) during a series of changes in extracellular K^{+} concentration. The relatively slow activation kinetics exhibited dependence on the membrane potential. The steady-state inactivation was well described by a Boltzmann function with a half-maximal inactivation potential of -49 mV (N = 6), showing that K^{+} currents in odontoblasts exhibit voltage-dependency. Amplitudes of outward K^{+} currents were suppressed by extracellular tetraethylammonium. These results indicate that the odontoblasts express voltage-dependent K^{+} current showing slow activating/inactivating properties with residual Cl^{-} conductance. (COI:No)

AC-48

Occlusal discomfort-related brain activity in somatosensory and prefrontal areas

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We investigated somatosensory and prefrontal activities that respond to a simulated occlusal discomfort using Near-Infrared Spectroscopy (NIRS). Participants are 3 male young adults without any perceivable problem in dental occlusion. We used OMM-3000 (Shimadzu Corporation, 4x4 probe set, 48 channels) and Hb-13 (Astem Corporation, 4 probe set, 4 channels) to measure regional brain activity on bilateral somatosensory and prefrontal areas, respectively. Different intensities of occlusal discomfort were simulated by gently grinding dental aluminum foils that were stacked to 5 different thicknesses. Participants ground each thickness of foils 3 times using their first molar teeth on their habitual chewing side in a random order. We also recorded the positions of probes using 3D digitizer and identified the brain regions that responded to the change in perceived occlusal discomfort. Task-related hemodynamic responses were observed in the bilateral mouth area of the somatosensory-motor cortices and in the prefrontal cortices. Furthermore, the time-courses of these responses were gradually changed depending on the intensities of perceived discomfort in these two areas. These results suggest a possibility to develop a diagnostic system that can quantitatively evaluate the perceived occlusal discomfort of a dental patient from the cortical hemodynamic responses. (COI:No)

AC-49

The responses of D/L-Valine in taste sensory system in the isolated brainstem-spinal cord with tongue.

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The tongue is composed of sensory system as a taste, and motor system such as mastication, swallowing and vocalization. Sense of taste on the surface of the tongue and sent to the brain can feel only sweetness, sourness, saltiness, bitterness and umami. In the rat, at first the gustatory nerve connects to the solitary tract nucleus, and toward reticular formation; secondly, connects to parabrachial nucleus and reaches taste area in cortex. We designed the preparation remained taste circuit keeping sensory-motor connection, so we produced isolated brain stem-spinal cord intact tongue preparation including solitary tract nucleus, parabrachial nucleus, facial nucleus. In this study, we examined the effects of sweet amino acid D-valine and bitter amino acid L-valine on tongue muscle activity as a tongue movement, comparing to the effect of sucrose as a sweetness. The tongue movement was recorded by bipolar-tungsten electrode inserted to tongue muscle. When sucrose was applied into the preparation, tongue movement was increased after 5 minutes. Application of D-valine to tongue increased tongue movement after 5-10minutes from application, but L-valine inhibited tongue movement or showed long delayed effect in postnatal 0-2-day-old rat. D-Valine and L-Valine distinguished each reception. In the circuit, the pons was required to the effect of Valine recognition because without pons has no effect of tongue movement with Valine application. These results suggested that D/L-Valine were detected with the sense of taste as different taste reception using pontine circuit. (COI:No)

AC-50

Augmentation of thyroid hormone receptor-mediated transcription and thyroid hormone-induced cerebellar morphogenesis by genistein and daidzein

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It has been believed that soybean contain various nutrients that regulate human health. Among such nutrients, soybean isoflavones may have bi-directional effect, either disrupt or promote human health. Soybean isoflavones especially genistein and daidzein may have various effects at the molecular, cellular and organ levels. Genistein and daidzein have been shown to modulate the thyroid hormone (TH) receptor (TR)- and various nuclear receptor-mediated pathways. Since THs play important roles in brain development, we examined the effects of genistein and daidzein on the TR-mediated transcription and TH-induced Purkinje cell dendrite arborization. We found that genistein or daidzein at doses 10-7 M to 10-5 M augmented TR-mediated transcription in a dose-dependent manner in CV-1 cells. Mammalian two-hybrid studies showed that genistein and daidzein augmented the interactions of coactivator and corepressor with TR. In primary rat cerebellar culture, low dose (10-8 M) of genistein and daidzein significantly augmented TH-induced dendrite arborization of Purkinje cells. These results suggest that genistein and daidzein augment TH action in brain. Unlike other endocrine-disrupting chemicals, these chemicals are readily catalyzed in body. Thus, these chemicals may possess health-promoting effect in body by augmenting TH action, rather than adverse effect. (COI:No)

AC-51

Aberrant Brain Function in Mild Perinatal Hypothyroidism Mice

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Thyroid hormones (THs) play crucial roles in the general growth and development of many organs. Decrease of TH secretion during perinatal period results in stunted growth, impaired brain development, and mental retardation in children (Koibuchi and Chin, 2000). Even if hypothyroidism is mild and does not show significant change in maternal TH levels, it may also induce the decrease of body weight, brain size, and deficiency of sensory/cognitive function in animal models (Sui and Gilbert, 2003). TH deficiency results in the change in neuronal excitability and aberrant neurotransmitter release. However, mechanisms underlying such changes are not fully understood. The aim of this study is to determine the effect of mild and moderate maternal hypothyroidism on development of brain function in female mice offspring. We focused on the behavior of female mice offspring including maternal behavior. We used C57BL/6j mice and divided them into three groups based on the dose of PTU that was given as a drinking water during perinatal period (from E14 to P21); control, 5ppm, and 50ppm groups. These concentrations induced the mild or moderate hypothyroidism (Amano et al., proceedings). We examined cognitive function, memory, motor coordination, and maternal behavior on female mice during 10-15 weeks old. We found that the motor coordination and cognitive function was affected in 5 and 50ppm groups.We conclude that even if perinatal hypothyroidism is mild, it induces aberrant brain function of adult female mice. (COI:No)

AC-52

The effect of mild perinatal hypothyroidism on development of postural reflex

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Thyroid hormone (TH) is necessary to control the basal metabolic rate of various organs. It also plays a critical role on brain development during perinatal period. Perinatal hypothyroidism induces retarded migration of granule cells from the external granule cell layer (EGL) of the cerebellum and morphological change of Purkinje cells (Koibuchi et al., 2001). Some effects of TH have critical period during development. Thus, adverse effect induced by the perinatal short-term hypothyroidism become irreversible. Particularly, even if hypothyroidism is mild, the defect may be observed. However, it is still under controversy whether mild perinatal hypothyroidism induces adverse effect in brain development. In this study, we generated a mild perinatal hypothyroid mice model by administering low dose (5 or 50 ppm) of 6-propyl-2-thiouracil from gestational day 14 to postnatal day 21 (P21) to C57BL/6 mice dams. We then measured righting reflex of offspring from P3 to P12. We also examined the mortor coordination at P21 by rotarod test. In addition, we measured several mRNA levels at P7 and P21 in the cerebellum by quantitative Real-time PCR. We found that retardation of reflex acquirement and disorder of motor coordination in mild perinatal hypothyroid mice. We also found the correlation between righting reflex at P7 and rotarod test at P21. These results indicate that even if perinatal hypothyroidism is mild, it may induce adverse effect in brain. (COI:No)

AC-53

Fetal environment failure causes maternal neglect in her adulthood

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Regarding biological mechanisms underlying the onset of maternal neglect, there have been considerable researches on the mother's childhood experience of maltreatment and findings on perinatal stress. Here we show that maternal neglect is already decided by the neuroendocrine environment during her fetal period. This was studied by using CIN85 knockout ($CIN85^{-ex2}$) mice which display a defect in maternal behavior towards her newborn mice. Despite that the mammary glands of $CIN85^{-ex2}$ mothers were completely normal, newborn mice were scattered within the bedding without receiving appropriate childcare and milk. As the result, almost all newborn mice born to $CIN85^{-ex2}$ mothers died in three days of birth. Importantly, the plasma level of prolactin immediately after delivery in $CIN85^{-ex2}$ was significantly lower than those in heterozygote and wild-type mice. By administrating prolactin to $CIN85^{-ex2}$ during pregnancy, $CIN85^{-ex2}$ born from the injected mice were rescued with smaller defect in maternal behavior. Interestingly, wild-type fertilized eggs transplanted and grown in the uterus of $CIN85^{-ex2}$ became neglecting mothers. Moreover, neglecting mothers showed decreased activity in brain areas associated with parental care. Taken together, our findings suggest that whether a mother mouse would expresses healthy maternal behavior or not in her adulthood, is already determined in her fetal period. (COI:No)

AC-54

Critical factors of fast consecutive visuomotor transformation task

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In a ping-pong game, players strike a ball together by maneuvering their rackets in a continuous way, in which the movements of the rackets are quickly and accurately controlled to hit the ball at the player's desired time and space. To know how the players make the sophisticated body movement and what accounts for their misplay, we established a psychophysical experiment model which mimics the ping-pong game. The subjects sit in front of a 27 inch LC display, grasping a force-sensor with thumb and index finger. In the display, a small Gabor patch (target) moved horizontally from the right lateral to the left one in a linear uniform motion one after another at various y-axis positions. The subjects were required to catch the target with a catcher which moves responding to the grasping force in a vertical axis along the left side of the display. The subject's basic catcher manipulation was composed of a rough approaching toward the target and the subsequent fine one (coarse-to-fine control). As the target speed was elevated higher than 125 cm/sec, the occurrence rate of a miss increased due to insufficient time period for fine control. The comparison between hit and miss trials at each target speed clearly demonstrated that the onset latency of rough approaching movement toward target is shorter at hit than miss trials. Thus, the time and accuracy for visual judgment of target position is critical to determine the performance of consecutive movements requiring a fast visuomotor transformation. (COI:No)

AC-55

Role of KCC2 downregulation for recovery after sciatic nerve injury

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Neuronal K^+-Cl^- co-transporter (KCC2) is the responsible for driving GABA/glycine mediated inhibitory responses. Increased excitability of neurons by KCC2 downregulation in several pathological conditions affects circuit remodeling and ultimately on functional behavior in the recovery phase after injury. However, the precise mechanism of those functional recovery promoted by KCC2 downregulation was still unknown. To elucidate the relation between KCC2 downregulation and recovery, we first check the time course of KCC2 expression after sciatic nerve injury. KCC2 at the injured side of the ventral horn of the spinal cord in wild type (WT) significantly decreased at 3 days after injury. We used KCC2 overexpression mouse regulated by tetracycline inducible system to rescue KCC2 downregulation after injury. Rescuing KCC2 impaired behavior phenotype measured by rotarod test. To test whether depolarizing effect of GABA by KCC2 downregulation could effect on the behavior recovery, we injected Bicuculline into the ventral horn at 3, 5 days after injury. Behavior recovery of mice injected with Bicuculline was reduced compared with ACSF injected mice. Finally, we focused on GAD and found WT significantly reduced GAD65, 67 expressions on 42 days after injury but not in KCC2 overexpression mouse. We then focus on excitability of motoneuron with electrophysiological approach. Those results suggest excitatory action by KCC2 downregulation is essential for acute recovery phase and those excitatory action resulted in reduced GAD expression in chronic phase promoting motor function. (COI:No)

AC-56

Erythropoietin facilitates endodermal differentiation of mouse embryonic stem cell via activation of MAPK pathway

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As a new therapy for diabetes due to lack of the absolute amount of insulin, it has been expected that the use of pancreatic β-cells derived from embryonic stem (ES) cells or induced pluripotent stem (iPS) cells. Therefore, it is important to develop more effective differentiation protocol for the differentiation into pancreatic β-cells. We previously reported that erythropoietin (EPO) increased the expression of *Sox17* gene, a marker for definitive endoderm. This effect was observed in cells directing differentiation, but not in undifferentiated cells. In this study, we investigated the mechanism of the action promoting the endodermal differentiation of ES cells by EPO treatment. Contrary to canonical function of EPO, EPO did not affect on the levels of phosphorylated JAK2, STAT3 and STAT5, but increased the levels of phosphorylated ERK1/2. Furthermore, we found that JAK inhibitor AG490 and STAT5 inhibitor pimozide did not repress the effect of EPO. On the contrary, AG490 treatment robustly increased *Sox17* expression independently of EPO. We suppose that AG490 could inhibit leukemia inhibitory factor (LIF)-JAK-STAT3 pathway, a main signal for pluripotency, down-regulate pluripotency genes and promote endodermal differentiation in the early stage of differentiation, continuously, EPO activate MAPK pathway, a key signal for endodermal differentiation. This study provides a new insight for the method to promote endodermal lineages from pluripotent stem cells. (COI:No)

AC-57

Analysis of inhibitory effects of menthol derivatives on contraction of isolated mouse intestine by using longitudinal muscle specimen

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We have reported inhibitory effects of menthol (M) and its derivatives (MD) on acetylcholine (ACh)-induced contraction of isolated mouse intestine and that the magnitude of their responses was different depending on intestinal positions. These inhibition by M/MD could be still obtained in the presence of ruthenium red (a non-specific TRP channel inhibitor) or capsazepine (a non-specific TRP channel antagonist), which suggested direct reaction of M/MD against intestines besides TRPM8. The present study used longitudinal muscle specimen prepared by peeling off in isolated intestines at 7 different positions; upper and lower parts of jejunum/ileum/colon, and rectum. Both M (100 μM) and MD (50 μM) inhibited ACh-induced contraction in longitudinal muscle specimen. M inhibited contraction about 20% on each position, but different inhibitory effects depending on intestinal positions were still observed by application of MD. At upper part of jejunum, about 40% inhibition occurred, but 20% inhibition at other positions. TRPM8 agonist icilin (50 μM) inhibited contraction about 60%-70%. As M/MD/icilin are lipophilic, they may adsorb on any parts of muscle layers and affect muscle contraction. But, by position-dependent inhibition of contraction by MD, a possibility of TRPM8 involvement is still remained. With immunohistochemical staining of TRPM8 distribution on gut, further studies are continued. (COI:No)

AC-58

Lysophosphatidylinositol triggers intracellular Ca^{2+} mobilization through GPR55 and TRPV2 in GLUTag cells

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Glucagon-like peptide-1 (GLP-1), a peptide hormone released from enteroendocrine L cells, stimulates insulin secretion from pancreatic β cells. Secretion of GLP-1 from L cells is regulated by not only luminal nutrients but also circulating hormones, cytokines or neurotransmitters. Although circulating lysophosphatidylinositol (LPI), one of lysophospholipids, and its specific receptor, GPR55, are increased in obese and diabetic patients, the relationship between LPI and GLP-1 secretion remains unclear. Here, we show by live cell imaging that LPI is involved in intracellular Ca^{2+} mobilization in murine enteroendocrine L cell line GLUTag cells. Application of LPI induced a long-lasting oscillatory intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase. Inhibition of GPR55, inositol triphosphate receptor, or Rho-associated kinase suppressed the LPI-induced $[Ca^{2+}]_i$ increase. Furthermore, inhibition and knockdown of transient receptor potential vanilloid 2 (TRPV2) channel, which was considered to be activated by LPI, also suppressed the LPI-induced $[Ca^{2+}]_i$ increase. These results suggest a functional link between GPR55 and TRPV2 underlying the $[Ca^{2+}]_i$ increase evoked by LPI. (COI:No)

AC-59

Molecular mechanism of GLP-1 secretion induced by L-glutamine from enteroendocrine GLUTag L cells.

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Glucagon-like peptide-1 (GLP-1), which is secreted from enteroendocrine L cells, promotes insulin secretion from pancreatic β cells. Given its important role for blood glucose homeostasis, and its potential to be a treatment target against type 2 diabetes, many studies have attempted to clarify the mechanism of GLP-1 secretion. Although recent studies revealed that amino acids, especially L-glutamine, increase GLP-1 secretion from enteroendocrine L cells, the mechanism of L-glutamine-induced GLP-1 secretion has yet to be elucidated. To address this question, we monitored intracellular Ca^{2+} and cAMP dynamics after the application of L-glutamine on enteroendocrine L cell line GLUTag cells. Application of L-glutamine increased intracellular Ca^{2+} and cAMP concentrations ($[Ca^{2+}]_i$ and $[cAMP]_i$, respectively). Depletion of extracellular Na^+ abolished the increase of $[Ca^{2+}]_i$, but not that of $[cAMP]_i$, suggesting that L-glutamine triggers membrane depolarization via Na^+ influx through sodium-coupled amino acid transporters and Ca^{2+} influx though voltage-dependent calcium channels. On the other hand, pan-G protein inhibiter BIM46187 suppressed both of $[Ca^{2+}]_i$ and $[cAMP]_i$ elevations. These results suggest that G_s coupled amino acid receptor with preference for L-glutamine is involved in GLP-1 secretion. (COI:No)

AC-60

D-Allose Inhibits GLUT1 Transcription by Down-regulating HRE-driven Promoter Activity and Suppresses Cancer Cell Growth

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Cancer cells require massive amounts of glucose as an energy source, and over-expressed glucose transporters (GLUTs) play a pivotal role in the dysregulation of cancer growth. Expression of GLUT1 and TXNIP (Thioredoxin Interacting Protein) is inversely correlated and a rare sugar, D-allose suppresses the growth of cancer cells through the up-regulation of TXNIP expression, thus our present study focuses on whether D-allose down-regulates GLUT1 expression and thus suppresses cancer cell growth. Western blot and real-time PCR analyses revealed that D-allose significantly induced TXNIP expression and inhibited GLUT1 expression in a dose-dependent manner in HuH-7 and other cancer cells. In these cells, D-allose inhibited the cell growth. The 2-deoxy D-glucose uptake assay revealed that either D-allose treatment or TXNIP over-expression decreased glucose uptake. Hypoxia response element (HRE) gene reporter activity was down-regulated by D-allose and TXNIP over-expression. Furthermore, a gene reporter assay using the GLUT1 promoter region containing the HRE consensus site showed that D-allose and the over-expression of TXNIP inhibited the promoter activity. This inhibition was reversed by mutating the HRE consensus sequence. Collectively, our results revealed a novel inhibitory mechanism of cancer cell growth by D-allose and the transcriptional regulation of GLUT1 expression by TXNIP via HRE-driven promotor activity. (COI:No)

AC-61

HDAC9 regulates alternative lengthening of telomere pathway

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Maintenance of telomeres is pivotal for the proper functioning of the cell. Extreme shortening of telomeres leads to cellular senescence. In contrast, cancer cells overcome this by the activating telomere maintenance mechanism, which can be either through telomerase or alternative lengthening of telomeres (ALT). The latter is mostly prevalent in subtypes of sarcomas and astrocytomas. Across all cancer subtypes, the prevalence of the ALT is approximately 4%. Being exclusive to cancer cells, targeting ALT provides a better avenue to the development of drugs against cancer. Histone deacetylase (HDAC) family plays significant roles in various cellular processes. In addition to the maintenance and regulation of the gene expression via deacetylation of acetylated histones, HDACs are also known to interact directly with many proteins. We focused on this family and compared the expression levels between ALT-positive and ALT-negative cells. We found that among the 11 HDACs present in a cell, HDAC9 were up-regulated in ALT-positive cells. Therefore we focused on the regulatory mechanism of ALT via HDAC9. On ALT-positive cells treated with HDAC9 siRNA, we found that there was a decrease in the telomere replicative capacity, which was evident from the C-circles assay. Furthermore, the formation of ALT-associated promyelocytic leukemia (PML) protein nuclear bodies (APBs), the hallmark of ALT, was inhibited by HDAC9 knockdown. In this study, it is suggested that HDAC9 regulates the formation of APBs and could be a candidate for the target of ALT-cancer therapy. (COI:No)

AC-62

Involvement of mitochondrial outer membrane protein in Ras-PI3K signaling-mediated endocytosis

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Ras, a member of small GTPases, regulates various physiological functions through spatiotemporal control of effector recruitments. We have previously reported that the Ras-phosphoinositide 3-kinase (PI3K) complex is preferentially translocated from the plasma membrane to the endosome upon epidermal growth factor stimulation, and Ras-PI3K signaling thereby promotes endocytosis. To reveal the mechanism underlying these phenomena, we compared the amino acid sequence of Ras-binding domain (RBD) of the effectors. A specific sequence was identified in PI3K and deletion of the sequence suppressed endosomal translocation of the Ras-PI3K complex. Next, we explored binding proteins of PI3K-RBD and identified 6 candidates. A mitochondrial outer membrane protein was subjected to further analysis after confirming the interaction with the above sequence. To examine whether the mitochondrial outer membrane protein is involved in endosomal translocation of the Ras-PI3K complex, cells in which the mitochondrial outer membrane protein was knocked down were subjected to time-lapse microscopy. Endosomal translocation was suppressed in the knockdown cells. In addition, endocytosis was downregulated and upregulated by knockdown and overexpression of the mitochondrial outer membrane protein, respectively. From the above, the mitochondrial outer membrane protein, which interacts with the specific sequence in PI3K-RBD, might be involved in endosomal translocation of the Ras-PI3K complex and subsequent regulation of endocytosis. (COI:No)

AC-63

Investigation of a molecule associated with the regulation of endocytosis through Ras-PI3K signal

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Although the small GTPase Ras has no enzymatic activity other than the GTP hydrolysis, it plays an important role in signal transduction involved in a range of physiological phenomena. Such multifunctionality would be accomplished by spatiotemporally specific recruitment of diverse effectors. Upon epidermal growth factor (EGF) stimulation, Ras and phosphoinositide 3-kinase (PI3K, one of the effector molecules) complex specifically translocates to the endosomes and promotes endocytosis. When we compared the Ras binding domain of the effector proteins, a specific sequence of 28-amino-acid-length was found in the RBD of PI3K. Deletion of the specific sequence suppressed the translocation of the Ras-PI3K complex, suggesting the presence of a protein that regulates Ras-PI3K signaling. Thus, we screened for interacting proteins by a mass spectrometry method, and focused on a cytosolic protein among identified candidates, which interacted the with the region in vitro. Upon EGF stimulation, localization of the protein changed from a diffuse pattern to granular structures, in which the Ras-PI3K complex was colocalized. Knockdown of the cytosolic protein suppressed translocation of the Ras-PI3K complex to the endosomes and subsequent endocytosis. These results suggest that the cytosolic protein interacts with PI3K through the newly identified domain, and the interaction is required for the spatiotemporal regulation of Ras-PI3K and subsequent endocytosis. (COI:No)

AC-64

Activation of ciliary beating by carbocistein via modulation of $[Cl^-]_i$ and pH_i in bronchiolar ciliary cells in mice

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The ciliary transport is controlled by two parameters, ciliary beat angle (CBA) and ciliary beat frequency (CBF). In this study, we examined the effect of carbocistein (CCis, a mucolytic) on CBA and CBF in mice bronchiolar ciliary cells. CCis respectively increased CBA and CBF by 30 % and 5% within 15 min associated with a decrease in $[Cl^-]_i$ and elevation of pH_i in bronchiolar ciliary cells. The CCis-induced CBA increase was inhibited by a CFTR blocker (NPPB or CFTR (inh)-172), which would elevate $[Cl^-]_i$. Removal of Cl^- in an extracellular solution, which would decrease $[Cl^-]_i$, mimicked the CCis action. These observations suggest that CCis increases CBA by decreasing $[Cl^-]_i$ via activation of Cl^- channels (CFTR). On the other hand, the CCis-induced increase in CBF or elevation of pH_i was not observed by removal of CO_2/HCO_3^- from an extracellular solution or addition of DIDS, which abolished NBC activity. This suggests that CCis increases CBF by elevating pH_i caused via activation of NBC. In conclusion, CCis increases CBA via a decrease in $[Cl^-]_i$ by activating CFTR, and increases CBF via pH_i elevation by activating NBC. (COI:No)

AC-65

Ciliary beat frequency modulated by PDE1A activity in procaterol stimulated mouse bronchiole

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Procaterol (an β_2-agonist, Proc: 1 nM), which stimulates cAMP accumulation, induced an immediate increase in the ciliary bend angle (CBA) associated with a delayed increase in the ciliary beat frequency (CBF). Treatment with 8MmIBMX (8-methoxymethyl-IBMX, an inhibitor of phosphodiesterase 1 (PDE1), a Ca^{2+}-requiring enzyme) hastened the time course of Proc-induced stimulation of CBF to a level identical to that of CBA. EGTA-induced Ca^{2+}-chelation in the extracellular solution mimicked the action of 8MmIBMX treatment on the Proc-induced time course of CBF, indicating that the Ca^{2+}-chelation enhances cAMP accumulation by inhibiting activity of PDE1. These observations suggest that the relatively slower time course of Proc-induced stimulation of CBF compared with CBA would be caused by a lower level of Proc-induced cAMP accumulation due to PDE1 activity in the CBF-regulated area in bronchial cell than that in CBA-regulated area of the cell. The immunohistochemical examination has demonstrated that PDE1A exists in the microdomain between the nine doublet tubules and the cell membrane, where the outer dynein arms (ODAs, molecular motors of CBF regulator) function. Thus, PDE1 regulates CBF by modulating cAMP accumulation in a spatially limited intracellular area. (COI:No)

AC-66

Effects of IL-13 on the ciliary beating of nasal epithelium of mice.

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Ciliary beating of nasal epithelium plays an important role in removing foreign particles. We examined the effects of IL-13 on the ciliary beat frequency (CBF) of nasal ciliary cells.
[Methods]Nasal mucosa was removed from mice sacrificed by pentobarbital Na (80 mg/kg ip). Nasal ciliary cells were isolated by an elastase treatment. We observed the beating cilia using a videomicroscope equipped with a high-speed camera (500fps). Experiments ware performed in accordance with the Guidelines of the Animal Research Committee of Kyoto Prefectural University of Medicine.
[Results]In unstimulated nasal ciliary cells, CBF fluctuated from 6 Hz to 20 Hz. The CBF fluctuation was not observed in a Ca^{2+}-free solution, suggesting that $[Ca^{2+}]_i$ fluctuates causing the CBF fluctuation. Application of IL-13 (10 ng/mL) abolished the CBF fluctuation observed under the basal condition by keeping CBF to 15-18 Hz, which was the highest level of CBF fluctuation observed under the basal condition.
[Conclusion]In unstimulated nasal ciliary cells of mice, CBF fluctuated, and stimulation with IL-13 increased CBF and maintained CBF at the highest level of the CBF fluctuation. (COI:No)

AC-67

Valosin-Containing Protein is a novel binding protein of eEF1BδL

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Heat Shock Proteins (HSPs) are essential to prevent protein aggregation caused by heat stress. Specific transcription factor, Heat Shock Factor 1 (HSF1) regulates expression of HSPs. Previously, We have identified a new transcription factor eEF1BδL, an alternative splicing variant of EEF1D. eEF1BδL binds to HSF1 and they corporate to control Heat Shock Element (HSE)-containing gene transcription. Without stress, inactive HSF1 forms a hetero-oligomer involving chaperones and other partners. Thermal stimulation causes HSF1 dissociation from its inhibitory proteins, and this activates transcriptional activity for protecting cellar environment. But, the detail of the HSF1-eEF1BδL complex remains to be elucidated.

This study is aimed to search novel elements in the complex by using proteome analysis. GFP-eEF1BδL and HA-HSF1 were co-expressed in HeLa cells. After heat shock, cell lysates were immunoprecipitated with anti-GFP antibody, and applied into two-dimensional-gel electrophoresis. Spots appeared in GFP-eEF1BδL overexpressed sample were proceeded to mass spectrometry analysis. As a result, Valosin-Containing Protein (VCP/p97) was identified as a novel binding protein of eEF1BδL. It is reported that VCP dissociates HSF1 from the hetero-oligomer. In conclusion, present data suggests that VCP has some contribution to heat shock response in corporation with HSF1 and eEF1BδL. (COI:No)

AC-68

Activation of ciliary beating by Seihai-to in distal airway epithelial cells of mice.

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Ciliary transport in airways is evaluated by two parameters; ciliary beating, ciliary beat angle (CBA) and ciliary beat frequency (CBF). We examined the effects of Seihai-to (an herbal medicine, TJ-90) on CBA and CBF in mice ciliary cells isolated from lungs of mice. Seihai-to increased both CBA and CBF in dose dependent manners. Seihai-to of extremely high concentration (400 μg/mL) immediately increased CBF to 160% of the basal level within 3 min after its application followed by a gradual decrease to a lower level (40%) than the basal one, while it immediately increased CBA to 150% keeping its increased level without any decrease. After cessation of Seihai-to application, both CBA and CBF gradually recovered to the pre-stimulation level. However, Seihai-to of lower concentrations (0.4-40 μg/mL) increased CBA and CBF keeping increased levels without any decrease, and Seihai-to of much lower concentrations (0.4-40 ng/mL) increased only CBA but not CBF. The dose response study of Seihai-to indicates that CBA has a higher sensitivity to Seihai-to than CBF. The results suggest that Seihai-to activates CBA and CBF mediated via different signal pathways in the distal airway ciliary cells of mice, and that Seihai-to of even very low concentrations has potential promoting expectoration by activating CBA, which essentially plays an important role in promoting expectoration. (COI:No)

AC-69

Modification of cellular proliferation by δ- aminolevulinic depends on heme oxygenase-1 expression

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δ-aminolevulinic acid (ALA) is a precursor for the synthesis of heme. Protoporphyrin IX (PPIX) are generated as its intermediates. In malignant cells, PPIX is excessively accumulated inside the cells. However,whether ALA application affects cellular kinetics is not clear. Excessively synthesized heme is degraded by HO-1 which is also known to affect cellular proliferation. Therefore we examined the relationship between HO-1 expression and cellular proliferation after ALA administration.Ten types of human cancer cells and two types of normal cells were incubated with ALA. A fluorometric measurement for PPIX and HO-1 expression by the western blot were carried out. Moreover, to examine the alteration in mitochondrial activity by ALA incubation, cells were incubated with TMRM and the fluorescence intensity TMRM using a flow cytometer.After ALA was applied, most of the cells decreased. However, A549 and AsPC-1 showed evident cellular proliferation.As a result, ALA induced cell death showed a negative correlation to the HO-1 expression in the pre-ALA administration phase. Flow cytometry revealed the positive correlation between mitochondrial inactivity and the cell decrease after ALA incubation. These results suggest cellular proliferation /cell death induced by ALA administration depends on HO-1 expression and mitochondrial activity. (COI:No)

AC-70

OCRL inhibitor suppresses dephosphorylation of Anillin in cell cycle

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Anillin, a scaffold protein linking actin and myosin, controls the contractile ring spatially and is required for cytokinesis. When the cell membrane ingresses, anillin binds to $PI(4,5)P_2$ located at the cleavage furrow. Such localization may regulate the contractile ring close to the cleavage furrow.

On the other hand, Inositol 5-phosphatase Oculo Cerebro Renal syndrome of Lowe (OCRL) regulates $PI(4,5)P_2$ levels, and is involved in cytokinesis. Furthermore, OCRL is involved in remodel of F-actin accumulated in the intercellular bridge.

However, it is not known whether OCRL regulates cell cycle-dependent changes in anillin expression levels and phosphorylation levels.

To investigate such mechanism, HeLa S3 cells were synchronized by double thymidine block or thymidine-nocodazole block, and treated with OCRL inhibitor.

In the beginning of G1/S, expression and phosphorylation levels of anillin increased. On the other hand, in the beginning of G2/M, expression and phosphorylation level of anillin decreased. Addition OCRL inhibitor to cells in the beginning of G2/M had no effect on degradation of anillin, but suppressd dephosphorylation of anillin. These findings indicate the possible involvement of OCRL in dephosphorylation of anillin in cell cycle. (COI:No)

AC-71

N-terminal tyrosine phosphorylation-dependent intracellular localization of paxillin regulates stress fiber formation and the migration and invasion of breast cancer cells.

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The Rho-kinase-mediated stress fiber (SF) formation regulates cancer cell migration. Previously we found Fyn tyrosine kinase as an upstream molecule of Rho-kinase. Using functional proteomics for the molecule mediating between Fyn and Rho-kinase, we identified paxillin as the novel molecule which binds activated, but not inactive, Fyn. Surface plasmon resonance analysis of the recombinant proteins determined the binding site of paxillin for Fyn at its N-terminus.

In this study, we examined functional roles of paxillin and its N-terminal tyrosine phosphorylation by Fyn in the breast cancer cells. Chemotactic factors (EGF and TGF- β1) induced SF formation and the cell migration and invasion, and the activated Fyn colocalized with paxillin at the both edge of SF. All of these responses induced by chemotactic factors were abolished by the knockdown of paxillin by lentiviral shRNA. In addition, TGF- β1 markedly up-regulated paxillin expression and induced time-dependent (~ 60 min) phosphorylation at Y88 of its N-terminus. Among the N-terminal tyrosine of paxillin, Y88-phosphorylated paxillin localized to the both edge of SF upon the TGF- β1 stimulation, while Y118-phosphorylated one distributed in the cytosol. These results suggest that paxillin regulates breast cancer cell migration and invasion, presumably through the phosphorylation of Y88 by Fyn. (COI:No)

AC-72

Chloride dependency of cold stress induced cell damage in HeLa cell

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We already reported that Hela cells got severe damage under 4°C cold incubation in the 91th physiological society meeting. Our previous data showed that the intracellular small vesicles had been almost disappeared by over night incubation at 4°C condition, however the plasma membrane was still intact by the same condition. It is quite possible that the increase of the intracellular chloride concentration facilitate the diffusion of ion and water into the intracellular vesicles, followed by the rupture of those structures. To examine this hypothesis, we applied 4°C cold stress to HeLa cells under different chloride concentrations and measured the viability by MTT assay and trypan blue staining. The chloride replacement by gluconate improved the viability of cells after 4°C cold incubation for 24 hours in a chloride concentration dependent manner measured by both methods. The bright microscopic image showed the cellular swelling by cold stress and such swelling was reduced by chloride replacement. The structure of mitochondria was observed in the cells after 4°C cold incubation for 24 hours in chloride free condition by JC-1 staining, however, the mitochondria disappeared in normal chloride condition. These data support the hypothesis that the influx of chloride ion disrupts intracellular vesicular structures. To prove our hypothesis, further studies should be necessary. (COI:No)

AC-73

The effect of hypergravity on the antigen-specific antibody production

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It has been already established that the changes in gravitational environment cause various physiological systems including immune system in a living body. However it is still unknown whether there is a different effect of hypergravity on each immune response. Here we investigated the antibodies response to the immunized antigen under hypergravity environment. Antibodies (Ab) have several different classes and subclasses of immunoglobulin, such as IgG1, IgG2b and IgE. Each isotype of immunoglobulin is produced by class-switch recombination of Fc portion of immunoglobulin. The class-switch recombination is governed by different cytokines produced from different helper T cells. For instance, Switch to IgG1 is mainly controlled by Th2 cytokines, while IgG2b is by Th1. Ovalbumin (OVA)-immunized mice were divided into two groups, 1 *g* control group as Group1 and 2 *g* as Group2. We collected sera every week and examined the Ab production against OVA by ELISA. When we compared the amount of each IgG, IgG1 and IgG2b, we could not detect any difference between the two groups. However, the ratio of IgG1 to IgG2b in each group showed a significant difference. These data indicate that the change of gravitational environment might cause the quality but not the quantity of the immune responses. We will investigate further the T cell responses including cytokine profiles related to the Ab production. (COI:No)

AC-74

Variations in oral sensitivity to fatty acid and fat preference during the menstrual cycle in young women

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Energy intake varies across the menstrual cycle. Ovarian steroid hormones have effects on energy intake, although detail mechanisms are not well defined in humans. We examined whether alterations in fat taste perception and preference occur in response to ovarian hormone changes and affect fat intake in young healthy women. They were studied at three points during the menstrual cycle, corresponding to menstrual, periovulatory, and mid-luteal phases. The fatty acid detection threshold was examined using a three-alternative, forced-choice methodology (3-AFC) for oleic acid. The fat taste preference test was performed selecting favorite one among soups that contain dietary lipids of four different concentrations. Then subjects underwent the voluntary eating test in which they ate bread with butter and soup to satiety. In addition, they also self-reported their daily diets during 3 days near the experimental day. The results from 3-AFC revealed the fatty acid detection thresholds varied cyclically, with significant increase from the periovulatory phase to the mid-luteal phase. Furthermore, the subjects preferred dietary fat in the menstrual and the luteal phase more than the periovulatory phase. In addition, dietary survey showed that daily fat intake, especially saturated fatty acid, was decreased in the periovulatory phase compared with that in the mid-luteal phase. The present study suggests that oral fatty acid sensitivity, fat preference and intake vary during the menstrual cycle in young women. (COI:No)

AC-75

Estradiol replacement attenuates psychological stress-induced pressor response by inhibiting renal sympathetic nerve and renin-angiotensin system in ovariectomized rats

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The activations of renal sympathetic nerve and renin-angiotensin system (RAS) are involved in the development of hypertension. We examined whether chronic estradiol replacement has suppressive effects on psychological stress-induced pressor responses by attenuating renal sympathetic nerve and RAS activations in ovariectomized rats. Female Wistar rats aged 9 wk were ovariectomized. After 4 wk, the rats were assigned either to a placebo-treated (Pla) group or a group treated with 17β-estradiol (E2) subcutaneously implanted with either pellet, and implanted with radiotelemetry devices for blood pressure (BP) and heart rate (HR) measurements. Two wk later, the rats were denervated renally. These rats aged 17 wk underwent cage-switch stress. The stress-induced pressor responses were suppressed significantly in the E2 group compared with the Pla group. Moreover, the stress induced elevations of plasma renin activity, angiotensin II and aldosterone concentrations in Pla group, but not in E2 group. However, the renal sympathetic denervation abolished the differences in the pressor responses and elevations of plasma RAS components between the two groups. These results suggest that estradiol replacement may attenuate psychological stress-induced pressor response by inhibiting renal sympathetic nerve-RAS activations in ovariectomized rats. (COI:No)

AC-76

Changes in sympathetic nerve activity and heart rate in the development of DOCA-salt hypertension in rats

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Excess activation of sympathetic nerve activity has been implicated in pathogenesis of hypertension. We tested in the present study whether sympathetic activation is associated with the development of hypertension induced by deoxycorticosterone acetate (DOCA)-salt loading in rats. Wistar male rats were chronically instrumented with bipolar electrodes for measurements of renal and lumbar sympathetic nerve activity, and a telemeter was used for measurement of arterial pressure (AP). The time course of changes in AP, heart rate (HR), renal and lumbar sympathetic nerve activity were measured continuously and simultaneously before 3 days and 25 days after DOCA (100mg/rat) implantation with high sodium diet (4% NaCl). Systemic arterial pressure increased progressively after the onset of DOCA-salt loading over 25 days. HR gradually decreased after the onset of DOCA-salt loading. Renal and lumbar sympathetic nerve activity did not appear to increase in association the DOCA-salt loading. These results suggest renal and lumbar sympathetic nerves may not play a critical role in developing hypertension in this model. (COI:No)

AC-77

Effect of Rosmarinic Acid on Allergic Rhinitis in Rats

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Introduction: The symptoms of allergic rhinitis (AR) can be divided into two phases: namely, the early-phase response and the late-phase response. In the early-phase, IL-33 released from the nasal epithelium after allergen exposure is involved in the expression of rhinitis symptoms. In the late-phase, substance P (SP), calcitonin gene-related peptide (CGRP) and nerve growth factor (NGF) are associated with the exacerbation of symptoms. This study was performed to evaluate the antiallergic effect and the mechanism of rosmarinic acid, which is a type of polyphenol.Methods: Rats were sensitized with toluene 2,4-diisocyanate (TDI) to induce AR. Rosmarinic acid (3mg/kg i.p) was administered for 21 days. On day 22, the symptom of nasal allergy was evaluated by determining the frequency of sneezing and nasal rubs within 10 minutes. The level of IL-33 in the nasal lavage fluids and the blood plasma 1 hour after TDI administration, and the levels of SP, CGRP and NGF in the nasal lavage fluids 6 hours after TDI administration were measured.Results: AR model rats showed significant increases in the frequencies of sneezing and nasal rubs and the levels of IL-33, SP, CGRP and NGF. The administration of rosmarinic acid had no effect on NGF, but significantly suppressed the frequencies of sneezing and nasal rubs and the levels of SP and CGRP.Conclusion: These results suggest that rosmarinic acid can control the symptoms of nasal allergy. One potential mechanism by which this occurs is through suppression of the secretion of IL-33 in the early-phase and SP and CGRP in the late-phase. (COI:No)

AC-78

Suppressive effect of Hochuekkito on lung metastasis of B16 melanoma cells in mice

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Hochuekkito (HET) is well known to be one of Kampo (Japanese herbal) medicine consisted of ten component herbs, and used for the supplemental therapy of cancer patients with remarkable success. However, the precise mechanisms by which HET could favorably modify the clinical conditions of cancer patients are not well defined. The present study, therefore, was undertaken to examine the possible therapeutic mechanisms of HET on cancer using experimental mouse model.In the first part of the experiment, HET was thoroughly mixed with rodent's food with concentrations of either 0.1%, 1.0% or 3.0%, and administered orally ad libitum, which was started one week before tumor cell injection and continued throughout the experiment. Oral administration of HET at concentration 1.0% and 3.0% into C57BL/6 male mice significantly inhibited tumor metastasis in lungs, which was induced by the intravenous injection of 1×10^5 B16 melanoma cell. The second experiment was to measure the cytokine production ability of lienal lymphocytes from the mice. The third experiment examined the influence of HET for NK cell activity using asialo GM1 antibody.The metastasis of B16 melanoma cell was inhibited in HET concentration dependence, and increased by asialo GM1 antibody. HET caused increase of IFN-γ and IL12 production. These results suggest that oral administration of HET increases the production of IFN-γ and IL-12. These cytokines are responsible for the activation of NK cell, and therefore, inhibition of B16 melanoma cell metastasis. (COI:No)

AC-79

Simulation of Cl^- secretion in epithelial tissues: New methodology estimating activity of electro-neutral Cl^- transporter

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Epithelial cells play key roles in prevention of our body from environmental changes by regulating transepithelial transport of ions and water; e.g., the liquid covering epithelial apical membrane surface produced by Cl^--secretion-driven water movement plays an essential role in protection of our body from bacterial and viral infection. Transepithelial (transcellular) Cl^- secretion is, in general, mediated by two steps; 1) the entry step of Cl^- into the cytosolic space from the basolateral space across the basolateral membrane by Cl^- transporters, such as Na^+-K^+-$2Cl^-$ cotransporter, and 2) the releasing step of Cl^- from the cytosolic space into the luminal (air) space across the apical membrane via Cl^- channels, such as cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel.

In the present study, we established mathematical models describing transcellular Cl^- secretion based on three pathways and simulate transcellular Cl^- secretion using mathematical models combined with electrophysiological measurements, providing information on contribution of Cl^- channels/transporters to transcellular Cl^- secretion, activity of electro-neutral ion transporters and how Cl^- channels/transporters are regulated.

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AC-80

Oral osmotic laxative improved survival of cystic fibrosis mice

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Cystic fibrosis is an autosomal recessive disorder caused by mutations in the *CFTR* gene. CFTR functions as a cAMP-activated anion channel in epithelia cells. In CF, loss of CFTR function causes viscous and sticky luminal fluid and mucus in airway, intestine, pancreatic duct, *etc*. deltaF mouse is a cystic fibrosis mouse model in which F508del mutation was introduced. DF/DF mouse usually dies within a month due to ileus. In the present study, we examined the therapeutic effects of oral osmotic laxative on survival and weight gain in deltaF mouse.

Mice of both sexes were weaned at 21 days after birth and randomly assigned to treatment group or non-treated control group. Both groups were fed solid chow. In the treatment group, oral laxative solution was given instead of drinking water. The composition of the laxative solution was 60 PEG (polyethylene glycol 3,350 mw), 1.46 N_aCl, 0.745 KCl, 1.68 N_aHCO_3, 5,698 $N_{a2}SO_4$ (in g/L). Drinking water or the laxative solution was given also in agar gel for 2 weeks after weaning.

Laxative treatment significantly ($p < 0.01$) improved the mean survival time of DF/DF mice from 41.1 days (n = 15) to 180.9 days (n = 16). Body weight increase of treated and non-treated DF/DF mice for 14 days after weaning was 2.4 ± 1.6 grams (mean ± SD, n = 9) and 1.9 ± 2.4 grams (n = 3), respectively. Only a small percentage of wild-type (wt/wt) and DF/wt mice died during observation.

In summary, the present data demonstrated that oral osmotic laxative improved survival of cystic fibrosis mice most likely due to resolution of ileus and better nutritional status. (COI:No)

AC-81

Flippase mediates the transmembranous transport of sphingosylphosphorylcholine, a spasmogen of human coronary and cerebral arteries

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Previously we identified sphingosylphosphorylcholine (SPC) as the causal molecule of abnormal vascular smooth muscle contraction leading to vasospasm, and demonstrated the SPC incorporation into human vascular smooth muscle cells (VSMCs). However, the molecular mechanism of the SPC incorporation has not been clarified. In this study, we examined the possible involvement of flippase, a transmembranous lipid transporter, in the SPC transport across plasma membrane.Since the well-documented characteristics of flippase is transmembranous transport of phosphatidylserine (PS), we used the PS labelled with fluorescent NBD (NBD-PS) to firstly prove the presence of flippase in human coronary artery VSMCs. We observed the incorporation of NBD-PS into VSMCs, which was inhibited by flippase inhibitors, such as N-ethylmaleimide and vanadate. Then, using the NBD-labelled SPC (NBD-SPC), we observed the incubation time-dependent SPC incorporation into the VSMCs, which was markedly diminished by vanadate, a flippase inhibitor at the concentration used for the inhibition of NBD-PS incorporation.Taken together, these results suggest that VSMC has flippase activity which mediates the SPC transport across plasma membrane, strongly suggesting the novel role of flippase as a new promising target molecule to prevent and remedy abnormal vascular smooth muscle contraction leading to vasospasm. (COI:No)

AC-82

Retrieval-induced forgetting in mice

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Retrieval-induced forgetting (RIF) is a phenomenon found in humans. The retrieval practice on a subset of target items leads to forgetting of non-target items. Here, we examined whether RIF occurs in mice using a modified spontaneous object recognition test. Mice were assigned to three experimental conditions (control, retrieval and interference), and experienced each condition in a random order. In the control condition, mice were allowed to explore a field in which two objects (A, B) were placed in the sample phase, and stayed in their cage during the reactivation phase. In the test phase, mice were allowed to explore two different objects (A, D), one of which was identical to that presented in the sample phase, and the other was novel one. In the retrieval condition, after the sample phase (C, D), mice explored two identical objects (C, C), which were the same as one of the objects presented in the sample phase, and tested two different objects (D, Y). In the interference condition, after the sample phase (E, F), mice explored two identical objects (G, G), which were novel for the animals, and tested two different objects (F, X). The time spent exploring each object was measured by experimenters. The discrimination ratios in the control and interference condition were significantly higher than the chance level (50%), while that in the retrieval condition was not significantly different from the chance level, and significantly lower than that in the control condition. These results demonstrated that RIF occurs in mice in a modified spontaneous object recognition test. (COI:No)

AC-83

Brain areas related with producing the bodily self-attribution: A rubber hand illusion

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The feeling of bodily self-attribution is critical for our daily interaction with the outside world. We have perceived touch sensations as arising from a rubber hand when the rubber hand and our own hand are repeatedly brushed in synchrony with the real hand hidden from view. This rubber hand illusion (RHI) provides a cue for studying the bodily self-attribution. In the present study, we investigated whether there were any brain areas related with producing the bodily self-attribution using fMRI. Right-handed healthy subjects participated in the imaging experiments. Visual and somatic RHI tests were performed: In visual RHI tests, the right (or left) rubber hand was aligned to the subject's own right (or left) hand and the experimenter synchronously brushed the rubber hand and the subject's hidden right (or left) hand. In somatic RHI tests, the subjects were blindfolded and the experimenter moved the subject's right (or left) hand holding a brush so that it brushed the left (or right) rubber hand and synchronously brushed the subject's left (or right) hand with another brush. We hypothesized that brain areas related with producing the bodily self-attribution would be activated during the illusion conditions. Such activation was detected in the left medial prefrontal cortex. The left medial prefrontal cortex may play a key role in the mechanism for the feeling of bodily self-attribution. (COI:No)

AC-84

Optogenetic stimulation of the raphe nucleus modulated the neuronal activity of the ventral hippocampus

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Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in multiple brain functions ranging from motor to autonomic functions. Optogenetics is an elegant tool to control neurotransmitter release with millisecond precision and cell type-specific resolution. We previously succeeded in generating transgenic mice that expressed a light-sensitive channelrhodopsin-2 variant ChR2 (C128S) in serotonergic neurons. The ventral hippocampus (vHP) is known to be a major target region of raphe nucleus serotonergic neurons, and highly expresses several types of serotonin receptors (Htrs) including Htr1a, 2a, 2c, and 7. The recent report demonstrated that the optogenetic activation of raphe nucleus serotonergic neurons caused the elevation of extracellular serotonin level at the vHP and transiently enhanced anxiety-like behavior in mice (Ohmura et al., Int. J. Neuropsychopharmacol. 2014). However, it remains unclear how serotonin modulates the activities of neurons in the vHP. To clarify the effect of the serotonergic modulation on the neuronal activities in the vHP with high temporal and spatial resolution, we recorded local field potential (LFP) by a 16-channel silicon probe before and after illumination. Our preliminary results showed that the activation of serotonergic neurons by optogenetics reduced theta, gamma and high frequency band powers of LFP in the vHP. (COI:No)

AC-85

Correlation between pupil size and subjective passage of time in monkeys

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Our daily experience of the passage of time is strongly influenced by internal states, such as mood, arousal, and attention. Although noradrenaline (NA) has been shown to have a close relation with them, its role in temporal processing remains largely unknown. To investigate this, we trained monkeys to perform the time production task and assessed correlation between subjective elapsed time and pupil size, which has been reported to reflect neuronal activity in the locus coeruleus (LC)-NA system (Aston-Jones & Cohen, 2005).
In the self-timed saccade task, a visual cue was presented during fixation. The animals had to report the passage of 1 s by making a self-initiated saccade to the cue location to obtain reward. Pupil size was measured using the infrared eye tracking system. We found negative correlation between pupil size just before the cue onset (500 ms in duration) and self-timed saccade latency. When blocks of large/small reward were alternated every 10 trials, pupil size and saccade latency altered in the opposite directions, showing a significant negative correlation (rs = -0.38 ± 0.23, n = 12, p &lt 0.001). Furthermore, sessions with greater modulation of pupil size exhibited larger latency modulation (rs = -0.80, n = 12, p &lt 0.01).
Our results showed that when the activity of LC-NA system was supposed to be higher, the subjects reported shorter interval. Since NA is thought to facilitate neurons in the cerebral cortex, thalamus, and cerebellum, the data suggest that NA might increase neuronal activity that keeps track of time, thereby speeding up the subjective passage of time. (COI:No)

AC-86

Visual pathways for response of dopamine neurons to reward predictors in blindsight monkeys

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Midbrain DA neurons are initially activated by an unpredicted reward, however, when there is an association between a sensory cue and reward, response of DA neurons shifts from the reward to the cue. Although these properties of DA neurons are well known, it is still unclear how DA neurons are activated by the reward-predicting visual information. The direct projection from the superior colliculus (SC) to DA neurons in the substantia nigra pars compacta (SNc) was found (Comoli E, et al, 2003). Because the response of DA neurons was elicited at short latency by visual predicting cue (CS), it is possible that the visual information via SC plays a key role in activating DA neurons. To test this hypothesis, we used monkeys with unilateral primary visual cortex (V1) lesions (an animal model of "blindsight" in humans). When the visual CSs were presented in the lesion-affected visual field, clear short latency phasic responses were observed in DA neurons, and larger responses were elicited in large reward trials compared with the responses in small reward trials. Furthermore, to investigate the contribution of visual information via SC, we injected muscimol, a $GABA_A$ receptor agonist, into ipsi-lesional SC. Then, the response of DA neurons was completely diminished.These results indicate that visual information via SC can activate the reward-predicting responses in DA neurons. (COI:No)

AC-87

Night eating syndrome's feeding pattern affects the circadian and metabolic system using by a model mice with night eating syndrome

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The circadian clock regulates the feeding rhythm, and food intake in turn affects the peripheral clock phase, suggesting a close interaction between the circadian clock and the feeding. Although human eating disorders, such as night eating syndrome (NES) and sleep-related eating disorder, may be caused by an abnormality of the circadian clock, this particular disorder of the circadian clock system has not been investigated yet. In addition, the relationship between NES and obesity has not been revealed yet. Hence, we tried to elucidate these relationships, after we prepared a model mouse of NES. We fed mice with a normal diet (ND), used ad lib-fed mice as control, and restricted feeding with a high-fat diet for 5-30 min during the inactive period under ND ad lib-fed mice as NES model. Body weight of the NES model group increased and the respiratory exchange ratio of the NES model group did not show decrease during the inactive period compared with the control group. Next, metabolic genes expressions were examined in liver. In consequence, lipid synthesis genes expressions in the NES model group were increased compared with the control group. These results indicated that the NES feeding pattern impaired not only circadian rhythm but also lipid metabolic rhythm. This work was partially supported by the Council for Science, Technology and Innovation, SIP, Technologies for creating next-generation agriculture, forestry and fisheries (S.S.). (COI:No)

AC-88

Changes of temporal pattern of licking behavior in mice showing binge-like overconsumption: inhibitory effect of anorexic gut hormone, peptide YY

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Our previous study suggests that binge-like overconsumption is partly due to the dysfunction of postprandial feeding inhibition. The dysfunction may prolong duration of ingestion in binge-like behavior. However, the temporal pattern of ingestive behavior remains unknown in mice showing binge-like overconsumption of sucrose. In the present study, we examined the licking pattern and the effect of peripheral administration of anorexic dose of peptide YY (PYY) on the licking pattern in mice bingeing on sucrose. Mice received limited access to sucrose and normal chow during daytime under nocturnal food deprivation for 10 days. On day 11, after re-feeding, mice received an intraperitoneal injection of PYY (25 nmol/kg BW) or saline followed by the sucrose access. The licking pattern for sucrose was recorded. On day 10, the number of licking for the first hour was significantly increased relative to that on day 1. On day 11, the number of licking in mice with PYY injection was significantly smaller than that in saline-injected animals. No significant changes in interlick interval on days 1, 10 and 11 were found. The administration of PYY did not affect licking for water in water-deprived mice. These results suggest that mice bingeing on sucrose maintain hedonic motivation for the sugar for a long period of time. PYY is likely to suppress the hedonic-driven sugar consumption without deficiency of lickomotor performance and homeostatic motivation for water. (COI:No)

AC-89

Examination of the effects of theobromine feeding on motor learning and adaptive behavior of mice by using three-lever operant task

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Activation of mammalian target of rapamycin (mTOR) is involved in various physiological and pathological processes including learning, aging, and diseases. We previously reported that mTOR signaling was suppressed by theobromine, a methylxanthine derivative. In the present study, we examined effects of theobromine feeding on motor learning and adaptive behavior by analyzing the performance on the three-lever operant task. Before training, mice were provided normal food (control mice) or theobromine-containing food (TB-mice) for a month. In the operant box, three levers (A-C) were positioned 2 cm (right and left levers) and 4 cm (center lever) above the floor. One training session lasting 60 min was given once a day and five times a week. The mice were trained to press any one of the active levers for a food reward as shaping (1-lever task), and then trained to press three levers in a given sequence (ABC) (3-lever task). After the mice showed good performance of the three-lever task, the order was reversed to CBA (reverse 3-lever task). In 1-lever task, the rate of inactive lever press just after inactivation of the lever was lower in TB-mice than in control mice. In 3-lever task, success rate was higher in TB-mice than in control mice. In reverse 3-lever task, the shift of lever press pattern from ABC to CBA was faster in TB-mice than in control mice. These results strongly suggest that theobromine ingestion promotes motor learning and adaptive behavior in mice. (COI:No)

AC-90

Reduction of plasma estradiol level in female rats may change heat tolerance due to greater metabolism rate in the heat

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Purpose We assessed the effect of plasma estradiol (E_2) on thermoregulatory responses to heat. ***Methods*** Female Wistar rats (n=15, age of 9 wk) were sham operated (n=5, Sham) or bilaterally ovariectomized. In the ovariectomized rats, two silicon tubes containing 17-beta estradiol were subcutaneously placed in one group (n=5, E_2 (+)), and empty tubes in the other (n=5, E_2 (-)). Core temperature (T_{core}) and tail temperature (T_{tail}) were measured with thermistor probes in the abdominal cavity and the proximal part in the subcutaneous tissue of the tail. Oxygen consumption rate was measured by indirect open-circuit calorimetry. On 8th day after the surgery, the baseline values were obtained. Nine days after the surgery, rats were sequentially exposed to the environment of 28°C, 31°C, and 34°C for each 1h. ***Results*** At 25°C, there were no differences in T_{core} and T_{tail} between the E_2 (-) and E_2 (+) groups. At 28°C, T_{tail} was higher during first 30min in the E_2 (-) than the E_2 (+) group. At 31°C and 34°C, T_{core}, T_{tail}, and oxygen consumption rate were higher in the E_2 (-) than the E_2 (+) group during the entire periods. ***Conclusion*** A reduction of plasma estradiol level may be associated with decrease in heat tolerance. Heat dissipation from the tail may not be the involved mechanism; however, greater metabolic in heat could decrease the tolerance. (COI:No)

AC-91

Neurogenesis in the brain may contribute to increased heat tolerance due to long-term spontaneous exercise

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Aim Exercise training augments heat tolerance. In the present study, we tested the hypothesis that neurogenesis in hypothalamus, the center of thermoregulatory is involved in the mechanism. ***Methods*** Telemetry devices for the measurements of core temperature and spontaneous activity were placed in the abdominal cavity of male C57BL/6J mice (n = 21). Mice were individually housed at 25°C, and 11 mice among 21 mice were supplied with a running wheel in a cage 4weeks. In the middle 2 weeks, mice had water with BrdU (1mg/ml). At the end of the experiment, mice were exposed 39.5°C for 3h, and the brain was removed immediately after the exposure. Immunohistochemistry of cFos and BrdU was performed. ***Results*** During heat exposure, an increase in body temperature was suppressed in the mice with a running wheel. Greater expression of BrdU was observed the mice with a running wheel. Moreover, co-expression of cFos was observed in a part overlap. ***Conclusion*** Several mechanisms are involved in heat tolerance. The present results may suggest that central thermoregulatory system or thermosensitivity is partly involved in the mechanism. (COI:No)

AC-92

Hypothalamic and hippocampal temperature and oxidative stress during hyperthermia in rats

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Aim We tested the hypothesis that excessive heat injures the hypothalamus due to increase in oxidative stress. Moreover, thermal gradient in the brain may partly be involved in the mechanism. ***Methods*** A telemetry device for body temperature measurement was placed in abdominal cavity of male Wistar rats. In addition, two thin thermocouples were placed in the middle part of the hypothalamus and the CA3 of the hippocampus. Ten days after the surgery, mice were exposed to 39°C heat or remained at 25°C for 3.5 hours. After the exposure, the brain was excised. Immunohistochemistry of superoxide dismutase (SOD) 2 and 8-hydroxyguanosine (8-OHdG) was conducted. ***Results*** Abdominal temperature in the 39°C group was significantly higher than that in the 25°C group. During the 39°C heat, the brain temperatures were higher than the abdominal temperature. The temperature at the hippocampus was lower than that at the hypothalamus. The expression of SOD2 decreased in hippocampus, and that of 8-OHdG increased in hypothalamus after the heat. ***Discussion*** There was a difference in brain temperature among the regions. The hypothalamus may be the region which easily have oxidative damage due to heat. (COI:No)

AC-93

Blunted glucose elevation sustains high palatability of sucrose in mice with intermittent access to sucrose

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The taste reactivity test is a method that can be used to assess the palatability of tastants in animals by examining behavioral responses to intraorally infused solutions. Intermittent sucrose access under food deprivation induces sucrose overconsumption in mice. We previously found the following two points: 1) the intermittent access to sucrose delayed the onset of aversive reactions to sucrose in the taste reactivity test; 2) the feeding procedure blunted glucose elevation and this was rescued by an injection of an orexin-1 receptor antagonist SB334867 (SB). We hypothesized that blunted glucose elevation might contribute to sustain high palatability of sucrose in the mice. To address this hypothesis, we employed the taste reactivity test to examine whether the decline in the palatability of sucrose occurred early when blunted glucose elevation was rescued by SB administration. Mice under 20-h food deprivation had given 4-h access to chow and 0.5 M sucrose solution for 10 days. Over the feeding procedure the intake of sucrose increased successively but not that of normal chow. On day 11, the mice were pretreated with SB or vehicle followed by an intraoral infusion of 0.5 M sucrose solution. The latency to express aversive reactions to sucrose was shorter in SB-injected mice than vehicle-injected mice. The finding suggests that blunted blood glucose elevation in mice under the feeding regimen contributes to maintain high palatability of sucrose, resulting in an increase of the amount and duration of sucrose consumption. (COI:No)

AC-94

Application of dehydroepiandrosterone improved the remodeling of somatotopic area in intact hemisphere after focal infarction in somatosensory cortex

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After focal ischemia, contralateral hemisphere of infarction plays an important role for functional compensation in the somatosensory cortex (SSC) (Takatsuru et al., 2009). Recent studies indicate that infarction of the unilateral SSC changes the somatotopic area in the intact contralateral SSC (Takatsuru et al., 2013). We examined the changes of somatotopic area in the contralateral (left) SSC during recovery from stroke using electrophysiological technique. Sensory responses by right paw stimulation are recorded only in the left SSC. When the right SSC is infarcted, however, sensory responses by left paw stimulation are also recorded in the left SSC (Takatsuru et al., 2009). Furthermore, the size of the receptive field responded by right and left limb somatosensory stimulation at 4 weeks after stroke significantly increased compared with those at 2 weeks after stroke, resulting in the increase of receptive field, responding to all 4-limbs stimulation. This change was modified by repeated application of dehydroepiandrosterone (DHEA), which is known to have a neuroprotective effects, during 2 to 3 weeks after stroke. Interestingly, application of DHEA during 1 to 2 weeks after stroke did not show such effect. We next determined the concentration of neurotransmitters such as glutamate, glutamine, glycine, GABA, and dopamine by using in vivo microdialysis. These results also shown in this presentation. (COI:No)

AC-95

Role of tRNA modifications by Ftsj1 in cancer progression

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Transfer RNA modification is one of the key elements in accurate protein synthesis, and its dysregulation is observed in various diseases such as diabetes and cardiac muscle failure. In cancer biology, up-regulated protein synthesis is observed and it is crucial for the progression, but the involvement of tRNA modifications in this state is not fully understood. Here we report that Ftsj1, which catalyzes 2-O-methylation in cytosolic tRNA, is required for cancer expansion. The expression level of Ftsj1 mRNA is significantly higher in cancer than normal tissues. Both in gastric and breast cancer cell lines, knockdown of Ftsj1 inhibited cell growth and attenuated sphere-forming capacity, indicating loss of stemness. On the other hand, overexpression of Ftsj1 drives sphere-forming capacity of these cell lines. Intriguingly, these phenotypic outcomes depended on the Hippo pathway transducers YAP/TAZ activities. YAP/TAZ is recently identified as a determinant of cancer stem cell related-traits. Our results demonstrated the novel mechanism of cancer progression from the point of view of tRNA modifications. (COI:No)

AC-96

Function of adenosine deaminase in the central nervous system

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Adenosine deaminase (ADA) is a ubiquitous enzyme that catabolizes adenosine and deoxyadenosine. During cerebral ischemia, extracellular adenosine levels rapidly increase and ADA catabolizes the increased levels of adenosine. Since adenosine is a known neuroprotective agent, ADA was thought to have a negative effect during ischemia. In this study, however, we demonstrate that ADA has substantial neuroprotective effects in the striatum, which is especially vulnerable during cerebral ischemia. We used temporary oxygen/glucose deprivation (OGD) to simulate ischemia in rat corticostriatal brain slices. We newly introduced optogenetically evoked DC-like field potentials (optFP) as the reproducible index of the degree of neuronal damage. We used transgenic rats expressing channelrhodopsin-2, which depolarizes neurons in response to blue light. Time courses of optFP were recorded during and after OGD. The levels of optFP decreased after 10 min of OGD. Bath-application of 10 μg/ml ADA during OGD significantly attenuated the OGD-induced reduction in levels of optFP. The number of injured cells decreased significantly, and western blot analysis indicated a significant decrease of autophagic signaling in the ADA-treated OGD slices. These results indicate that ADA has protective effects in the striatum. (COI:No)

AC-97

In vivo two-photon laser ablation by improvement of focusing property in living mouse brains

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In vivo two-photon microscopy (TPM) is a powerful tool for observing deep regions of living mouse brains. Penetration depth of the imaging could be improved by changing beam diameter of excitation laser in our previous work. However, the real values of the focal spot size (FSS) that relates the spatial resolution of TPM in deep regions remained unclear. Here, we evaluated the resolution of *in vivo* TPM from single fluorescent beads imaging that were injected under the living condition at various depths. Next, we estimate the FSS depends on the beam diameter, the refractive index of the immersion liquid, or the depth from the surface. The results indicated that the FSS at a narrower beam with a higher power was larger than that under the full-filled condition. However, the FSS did not degrade remarkably in the cortical region. Moreover, by reducing the index mismatch between the immersion liquids and the objects, we achieved a smaller FSS at deeper regions of the cerebral cortex. These results suggested that the laser power density at the focal spot could be maximized by adjusting the optical parameters. Next, we applied this combination of the optical parameters to the laser ablation technique that requires high power density of the focal spot. In general, *in vivo* two-photon laser ablation has been limited in superficial cortical regions. Here, we successfully achieved laser ablation of single dendrites of neurons in cerebral cortex over 500 μm from the surface by applying the combination. (COI:No)