Poster Presentations

Day 2

(March 23, 13:00 - 14:00)

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2P-055 – 2P-082	Neuron, Synapse (2)
2P-083 – 2P-105	Sensory Function (2)
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2P-001

Type II Na-Pi transporters mediate extracellular phosphate-induced potentiation of the voltage-gated H^+ channels in murine osteoclast-like cells

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Inorganic phosphate (Pi) is the major anionic component of the mineralized bone matrix, and is released into the extracellular compartment between the plasma membrane of osteoclasts and the bone surface during bone resorption. However, the actions of extracellular Pi on osteoclast functions are largely unknown. We previously found that additions of extracellular Pi facilitated the activation kinetics of the voltage-gated H^+ channels in osteoclast-like cells generated from a macrophage cell line, RAW-264.7. The facilitation was independent of the intracellular ATP and V-ATPase activities. In this study, we further investigated the mechanisms of the Pi-induced effects on the H^+ channels. The activation time constants were decreased by dialyzing the cell inside with Pi (5-40 mM) containing pipette solutions, while there were no significant changes in the steady-state current-densities. Furthermore, 1-5 mM phosphonoformic acid, an inhibitor for Na^+-dependent type II Pi-transporters, blocked the Pi-induced acceleration of the voltage-dependent activation of the H^+ channels. Staurosporine, a kinase inhibitor, did not affect the acceleration significantly. These data suggest that extracellular Pi is taken up by the type II Pi transporters in the plasma membrane of osteoclasts and that the resultant increases in intracellular Pi may contribute to the enhancement of the H^+ channel activities. (COI:No).

2P-002

Involvements of LRRC8A and amino acid transporter SLC proteins in the activity of volume-sensitive outwardly rectifying anion channel (VSOR)

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Molecular identity of the volume sensitive outwardly rectifying anion channel (VSOR) has long been discussed. Recently, LRRC8A has been shown as an essential component of VSOR. We showed that the expression level of mRNA of LRRC8A is not much different between VSOR-deficient KCP-4 cells and its parental VSOR-rich KB cells in the last PSJ Meeting. Here, we observed the protein expression level is also indistinguishable between KCP-4 and KB cells. These data suggest that some other component(s) is needed for the VSOR activity. We attempted to identify other VSOR components by random mutagenesis screening and microarray analyses. As the results, SLC7A5 (LAT1) and its binding partner SLC3A2 were identified as candidates of VSOR-related molecules. Knockdown of both of mRNAs by siRNAs reduced VSOR currents. However, there were no significant differences in VSOR currents among embryonic fibroblasts derived from LAT1-KO, hetero, and wild-type mice. Also, overexpression of LAT1 in HEK293 cells did not increase endogenous VSOR currents. These results showed that LAT1 is not the pore component but may be a regulatory component of VSOR. (COI:No)

2P-003

Conserved role of putative membrane interacting region in the phosphatase domain between voltage-sensing phosphatase (VSP) and PTEN

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Voltage-sensing phosphatase (VSP) has phosphatase activity toward phosphoinositides regulated by membrane potential (Murata et al. 2005). VSP consists of two major regions, transmembrane voltage sensor domain (VSD) and PTEN-like cytoplasmic region. The mechanism of the coupling between the two modules has been enigmatic so far. PTEN is a phosphatase which dephospholyrates PI(3,4,5)P3 and acts as a tumor suppressor through antagonizing with PI3K. The regulation of PTEN activity has been studied by many studies, pointing the phosphatase activity is mainly regulated by the membrane association.Comparison between PTEN and VSP will provide useful information for understanding the regulation of phosphoinositide phosphatase. Recent studies of PTEN by MD simulation and NMR suggested that N-terminal region of phosphatase domain (PD) of PTEN (NtPD), which is also conserved in the linker region between VSD and PD of VSP, is important for regulation of enzymatic activity and interfacial binding of phospholipid (Shenoy et al. 2012, Wei et al. 2015). In this study, we examined the roles of NtPD in regulation of the phosphatase activity in both of PTEN and VSP. We estimated phosphatase activity of PTEN with mutation expressed in E. coli. The results showed that PTEN with mutation at NtPD had weak or less activity. We are currently studying the role of NtPD in the coupling between VSD and PD of VSP mutants by electrophysiological analysis. (COI:No)

2P-005

Two voltage sensor domains of two-pore Na^+ channel 3 (TPC3) play different roles in voltage sensing.

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Two-pore Na^+ channels (TPCs) have 2-repeat of 6 transmembrane helices, each of which corresponds to a functional unit of tetrameric type of voltage-gated cation channels, composed of voltage sensor domain (VSD) and pore domain. Therefore, TPCs have two different VSDs in one subunit and function as dimer. We investigated the role of two VSDs of TPC3 derived from *Xenopus tropicalis* (XtTPC3) by two-electrode voltage-clamp technique using *Xenopus* oocytes expression system. We performed mutational analyses focusing on the 4th helix in two VSDs, which is assumed to play critical roles for voltage sensing. First, we introduced an additional Arg at the corresponding position of the helix S4 (repeat-1; Gln164) and S10 (repeat-2; Phe514), which is 3 residues upstream of the first Arg of the positively charged clusters. Whereas Gln164Arg did not show apparent differences from wild-type, Phe514Arg showed significantly slower deactivation. Second, we examined the role of a negatively charged amino acid residue in helix S10, Asp511, which is conserved among some TPC3 orthologues. In contrast, in helix S4, no such a residue exists at the corresponding position. Asp511Ala showed a positive shift of the current-voltage relationship, but Asp511Glu was not clearly different from wild-type. This result indicated that the negative charge in helix S10 has an important role for its voltage sensing. Taken together, it was shown that the roles of two VSDs for voltage sensing differ one another in XtTPC3. (COI:No)

2P-006

Two common ingredients in topical cream activate TRPV1 and TRPA1

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Although the topical agents are major dosage forms for skin diseases, some of them cause the smarting pain in barrier-disrupted skin. In topical therapy, this smarting pain is one reason for the reduction in adherence of topical agents. Therefore, it is thought that some ingredients contained in the topical agent evoke the smarting pain through the neural excitation in primary sensory neurons. In this study, we found that two chemical compounds could be involved in the smarting pain because these compounds induced calcium increases in HEK293T cells expressing human TRPV1 or human TRPA1. Interestingly, the nerve action potential in saphenous nerves of mice was markedly inhibited when we applied the topical cream without the two compounds to the barrier-disrupted skin of hind paw. These results suggest that the two compounds are the novel irritants. And, it is indicated that the identification of irritants in topical cream is a crucial way to develop topical therapy. (COI:No)

2P-007

Voltage sensor domain motions detected with Anap, an unnatural amino acid based fluorescence reporter

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The voltage sensor domain undergoes structural transitions in response to membrane potential changes and regulates its effectors, thereby playing a crucial role in amplifying and decoding membrane electrical signals. While recent crystallographic studies have revealed atomic scale snapshots of voltage sensor domains, dynamic aspects during the transitions have not been fully addressed. Through the voltage-clamp fluorometry using protein reporters, we have shown that not only the C-terminal region but also the N-terminal region of the voltage sensor domain exhibits significant structural changes upon the transitions (Tsutsui et al., Biophys. J, 2013). One drawback of protein reporters is their relatively large size. In this study, we probed motions of the voltage sensor domain derived from Ciona intestinalis voltage sensing phosphatase (Murata et al., Nature, 2005) using Anap, an unnatural amino acid based environment sensitive fluorescence reporter. This approach reconfirmed the N-terminal effect upon the transitions. The optical signals from Anap introduced in the transmembrane segments are also open to discussion. (COI:No)

2P-008

Clustering-dispersion behavior of the KcsA channels: high-speed AFM imaging and effects of the membrane lipids on the clustering

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The KcsA channel is a pH-dependent potassium channel, and mechanism underlying the gating conformational changes has been studied in the single-molecule level. We examined the membrane-embedded structure of the KcsA channel using the AFM, and found that the channels were clustered when the gate was closed at neutral pH, whereas they were dispersed when the gate was opened at acidic pH. High-speed AFM revealed the dynamic behavior of the KcsA channels at sub-molecular resolution. At acidic pH, the individual channels fluctuated and they showed repulsive motions when the channels encounter each other. At neutral pH, the cluster deformed their shape over time, and surprisingly we found that the channels crowded with restless motions. These loosely-packed channels deformed the cluster. To examine the effect of lipids on the clustering, the KcsA channels were embedded in various types of the membrane, having different physical property, transition temperature and charge. The results demonstrated that the charge of the lipids did not affect the clustering, but lowering the fluidity and associated increases in the membrane thickness remarkably facilitated the clustering. The lipid-induced clustering of the channels might modulate the channel function in membranes. (COI:No)

2P-009

Instantaneous change in lipid compositions in the bilayer membrane during current recordings using the contact bubble bilayer method

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Recently we have developed a novel technique for the electrophysiological measurements of ion channels named the contact bubble bilayer (CBB) method, which readily serves formation of the asymmetric lipid bilayer, the optical observation and the rapid perfusion. In this study we explored for establishing instantaneous lipid change method using the CBB. To this end, two strategies were examined. First, desired lipid molecules were delivered to the CBB as the liposomes. Suspended liposomes in the electrolyte solution were perfused at the vicinity of the CBB, and the liposomes would fuse spontaneously with the CBB, by which different phospholipids are incorporated into the CBB membrane. Second, desired lipid molecules were dissolved in the oil, which were blown from a glass pipette close to the CBB in the bulk oil phase. The lipids in the oil phase are spontaneously transferred to the CBB. The changes in the lipid composition of the CBB were monitored by measuring the channel activity that exhibits the lipid-dependent changes in the gating for the KcsA potassium channel and conductance for some pore-forming antibiotics. Applicability of the presented method to the ion channel study will be discussed. (COI:No)

2P-010

A serine protease inhibitor facilitates inactivation of Kv4 channel.

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Autism spectrum is a heterogenous neurological disorder characterized by the impaired social communication and interaction with environments. Studies with the fragile X mental retardation protein indicate that increased expression of Kv4 channel is in part responsible for the disease-associated changes in neuronal excitability. Genetic analysis with identical twins also identified a missense mutation in the channel gene that slows channel inactivation. These findings support the possibility that increased activity of Kv4 channels contributes to the autism spectrum symptoms. Here we report that a serine protease inhibitor (Drug X) induces rapid and marked changes in the gating of heterologously expressed Kv4.3 channel without affecting peak current density. Treatment with the drug results in faster inactivation of the channel with significantly smaller time constants at all pulse voltages. The drug causes ~14-mV negative shift in the steady state inactivation with a sharper slope, whereas it does not influence voltage dependence of the activation. These changes in inactivation occur at the drug concentration below 1 μM, lower than its reported effects on some serine proteases. These results suggest that Drug X covalently binds to a serine residue of Kv4.3 protein to convert the native channel to a faster-inactivating structure. Drug X and related chemicals may be useful tools to identify the molecular framework underlying channel inactivation. They may also act as lead compounds for the development of drugs targeted at autism spectrum disorders. (COI:No)

2P-011

Role of β subunit of L-type Ca^{2+} channels in proliferation of vascular smooth muscle cells

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Enhanced cell proliferation of synthetic vascular smooth muscle cells (VSMC) are due to constantly activated cell cycle progression. Entries of Ca^{2+} are necessary both for the progression of G1 to S phase and for the M phase. We aimed to identify Ca^{2+} channels, which were involved in the regulation of cell cycle. After treatment with nifedipine (1, 10, and 50 μM) for up to 96 h, cell proliferation was measured by adding WST-1 reagent. The cell proliferation of VSMC was significantly inhibited by treatment with nifedipine in a dose-dependent manner. However, exposure of VSMC to high KCl (90 mM) also significantly inhibited the cell proliferation. When β subunit of L-type Ca^{2+} channel was constitutively expressed under the control by tetracycline in the Flp-In™T-REx™-293 cell line (derived from 293 human embryonic kidney cells), the β subunit protein was only localized in the nucleus. Microarray analysis demonstrated that 35 genes were significantly upregulated in the β subunit-expressing cells. These results suggest that the regulation of cell proliferation of VSMC might be determined by the specific pathway of Ca^{2+} entry and that the β subunit in nucleus plays an important role by regulating many genes that involves cytosolic Ca^{2+} homeostasis. (COI:No)

2P-012

Role of S4-S5 linker in the RyR2 channel gating

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Type 2 ryanodine receptor (RyR2) is a Ca^{2+} release channel in the sarcoplasmic reticulum of cardiac muscle and plays an important role in excitation-contraction coupling. Although recent cryo-EM studies provided near-atomic structures of type 1 ryanodine receptor (RyR1), molecular mechanism of the channel opening remains largely unknown. The S4-S5 linker is an α-helical structure connecting the S4 and S5 transmembrane segments and mediates signal transmission in a wide variety of channels. To address the role of S4-S5 linker in the RyR2 channel gating, we systematically mutated residues in the S4-S5 linker (Thr4751-Asn4762) and neighboring transmembrane segments (S5 and S6). The mutant RyR2 was stably expressed in HEK293 cells, and the channel activity was investigated by ER luminal Ca^{2+} measurements and [^{3}H]ryanodine binding. Using high sequence identity between RyR1 and RyR2 (65% in the whole molecule and 93% in the core channel domain after S4), we constructed structural model of RyR2 based on the structures of RyR1, and the phenotypes of mutants were interpreted by the model. Plausible interactions between amino acid residues in the S4-S5 linker and neighboring domains that may regulate channel gating will be discussed. (COI:No).

2P-013

Characterization of Ca^{2+}-induced Ca^{2+} release via RyR2 mutants carrying various types of arrhythmogenic disorders

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The type 2 ryanodine receptor (RyR2) is the Ca^{2+} release channel on cardiac sarcoplasmic reticulum and plays a crucial role in cardiac E-C coupling. The mutations in RyR2 have been implicated in a number of arrhythmogenic disorders including catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular cardiomyopathy (ARVC), idiopathic ventricular fibrillation (I-VF) and atrial fibrillation (AF). In this study, we aimed to characterize Ca^{2+} release properties of RyR2s carrying the above 4 types of disease mutations. Wild type and mutant RyR2 channels were expressed in HEK293 cells and spontaneous Ca^{2+} oscillations were monitored by live-cell Ca^{2+} imaging using Ca^{2+} indicators for cytoplasm (fluo-4, GECOs) and ER (CEPIAs). In addition, the Ca^{2+}-induced Ca^{2+} release (CICR) activity were determined with [3H]ryanodine binding assay. There were good correlations between CICR activity, Ca^{2+} oscillation frequencies and ER Ca^{2+} levels among all of mutants examined. While all CPVT mutants showed enhanced CICR activity, I-VF mutants showed divergent CICR profiles. Our results demonstrate the importance of functional analyses of RyR2 mutations in vitro for therapies in patients with malignant arrhythmia. (COI:No)

2P-014

Functional reconstitution of the cardiac slow delayed rectifier I_{Ks} channel from zebrafish

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I_{Ks} channel underlies the slow delayed rectifier current in human hearts and contributes to the stable excitability. I_{Ks} channel is composed of at least two kinds of subunits, KCNQ1 and KCNE1. KCNQ1 is a voltage-gated potassium channel α subunit and KCNE1 is an auxiliary subunit for the KCNQ1 channel. When incorporated, KCNE1 drastically changes many biophysical properties of the channel, which is most notably exemplified by the extremely slow gating. *KCNQ1* genes have been identified widely, both in invertebrates and vertebrates. In contrast, *KCNE1* genes have been identified only in vertebrates. Therefore, fish *KCNE1* gene can potentially be one of the oldest forms of the KCNE protein and might be significant in the evolution of the heart. We isolated zebrafish orthologs of human *KCNQ1* and *KCNE1* genes (*zkcnq1* and *zkcne1*) from the zebrafish heart. Both genes were expressed in *Xenopus* oocytes at a level high enough for electrophysiological analysis. *zkcnq1* could be expressed by itself and produced KCNQ1-like potassium current with a relatively strong inactivation. Co-expression of *zkcnq1* and *zkcne1* produced I_{Ks}-like slowly-activating currents, similar to the complex of human KCNQ1 and KCNE1. We next examined whether the human KCNE1 could modulate the zKcnq1 channel, and vice versa. Cross-species combinations of KCNE1 and KCNQ1 displayed an interesting contrast: Human KCNE1 rendered zKcnq1 channel constitutively active, while zKcne1 failed to slow the activation kinetics of human KCNQ1. These results imply that interaction sites of KCNQ1 and KCNE1 have co-evolved. (COI:No)

2P-015

Function of amiloraide-blockable epithelial Na+ channel on hanging behavior in young tadpoles of Xenopus laevis and Bullfrog

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Tadpoles in several species of frog, including Xenopus laevis and bullfrog, spend most of its time hanging from surface of water or solid object like waterweed by its cement glands. Our previous studies showed epithelial sodium channel (ENaC) expressed specifically in embryonic cement glands in bullfrog. To examine function of ENaC in the hanging behavior, we studied behavioral change by addition of amiloraide, a blocker of ENaC, in tank water. Since Xenpus laevis can be breed in laboratory and its tadpole show the hanging behavior like bullfrog, we started our experiment using Xenopus laevis as subjects. Twenty Xenopus tadpoles in stage 37/38 were divided into two beakers with 200 ml water (using 100 mM NaCl in exp2). Each beaker was stirred and number of hanging subjects were counted 15 min after the stirring. This procedure was repeated after addition of amiloraide (0.1 mM in final volume) or same amount of water to each beaker. In H_2O condition, there was no difference between amiloraide and control group. In NaCl condition, NaCl itself reduced number of hanging subjects. Addition of amiloraide in NaCl recovered number of hanging subjects compared to control group. In experiments with bullfrog tadpole, while relatively longer time was needed to re-hanging, we got similar results as in Xenopus laevis. Under natural conditions, ENaC have no clear role in maintenance of hanging behavior. From the experiment in NaCl solution, however, it would appear that ENaC might be related with mechanisms of the hanging, since amiloride is specific blocker of ENaC. (COI:No)

2P-016

Contribution of TRPC3 channel to the hypotonicity-induced $[Ca^{2+}]_i$ elevation in the principal cells of rat CCDs.

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The principal cells of cortical collecting duct (CCD) are occasionally exposed to the hypotonic tubular fluid. We have previously demonstrated that the tubular hypotonicity induced $[Ca^{2+}]_i$ increase, which was inhibited by Nicardipine but insensitive to a TRPV4 channel inhibitor, Ruthenium Red. However, the molecular basis of the hypotonicity-induced Ca^{2+} entry pathway is not precisely unknown. The principal cells of CCDs have been reported to express TRPC3 channel, which is activated by mechanical stretch, in the apical membrane. Therefore in this study, we examined the effect of a selective TRPC3 channel inhibitor on the hypotoniciy-induced $[Ca^{2+}]_i$ increase in freshly isorated rat CCDs. In the experiments using fura-2AM, extracellular hypotonicity (245 mOSm) evoked transient increase in $[Ca^{2+}]_i$ of principle cells, which was largely reduced by removal of extracellular Ca^{2+} or application of a selective TRPC3 channel inhibitor, Pyr3 (10 μM). However, after more hypotonic (195 mOSm) stimulation, the guradual and prolonged $[Ca^{2+}]_i$ increase was observed even in the presence of Pyr3. The guradual $[Ca^{2+}]_i$ increase was not completely abolished by application of both Pyr3 and Nicardipine. These results suggest that Ca^{2+} entry via the TRPC3 channel would contribute at least in part to the increase in $[Ca^{2+}]_i$ in moderate hypotonic conditions in principal cells of rat CCDs. (COI:No)

2P-017

Critical $PI(4,5)P_2$ gating residues in DAG-activated TRPC channels

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3D architectures of TRP channels are getting revealed in recent studies (Cao 2013 *Nature*, Paulsen 2015 *Nature*), but its regulatory insights by small molecules largely remain unclear. In previous studies, we found that PIP_2 reduction upon activations of voltage sensing PIP_2 phosphatase (VSP) or PLC- coupled G-protein coupled receptors (GPCR) inhibits all diacylglycerol (DAG)-activated TRPC channels (Imai 2012 *J. Physiol*, Itsuki 2014 *J. Gen. Physiol.*). In present study, we determined a structural domain critical for the inhibition by PIP_2 depletion upon VSP activation using chimeric TRPC channels and extensive mutagenesis studies at positive amino acid residues localized in the inner leaflet/cytoplasmic side of TRPC6 channels. The kinetical assay of the entry to the inhibition (τ_{onset}) and recovery from its status followed by Danio rerio (Dr)VSP activation demonstrated that the several positive residues proximal to the transmembrane S1 segment (Pre-S1) are critical to the gating by PIP_2 in TRPC6 channels and other DAG-activated channels (C3 and C7). The mutants of the positive residues in the Pre-S1 also resulted in the significant reduction of receptor-operated TRPC currents and the effect was essentially consistent to the simulation result for the reduction of PIP_2 binding affinity on TRPC channels. These results strongly suggest that PIP_2 binding to the Pre-S1 is the key to limiting TRPC channel activity. (The authors declare that they have no conflicts of interest with the contents of this poster.) (COI:No)

2P-018

CatSper has a calcium-permeable voltage sensor domain.

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The voltage sensor domain (VSD) of voltage-gated ion channels responds to the membrane potential changes, and the conformational change of VSD results in opening of the pore. Although the primary function of VSD is to sense membrane voltage, some reports showed ion permeation through VSD (Sasaki M, et al. 2006; Starace MD and Bezanilla F. 2004). CatSper channel, a cation channel expressed specifically in testis, is essential for fertilization in mice. It is believed that CatSper channel is formed by four distinct α subunits which are composed of VSD and pore domain. However, the detailed molecular characteristics of CatSper are still unclear since functional analysis using heterologous expression systems has been unsuccessful. Here, we report that one isoform of ascidian CatSper orthologues, Ci-CatSper3, has calcium-permeable VSD. Ca^{2+} influx was detected in *Xenopus* oocytes expressing the VSD of Ci-CatSper3 (CiCS3 VSD) upon hyperpolarization. Furthermore, by Ca^{2+} influx assay in liposome reconstitution system, it was demonstrated that CiCS3 VSD itself has Ca^{2+} permeability. This is, to our knowledge, the first study which reports calcium permeation through a voltage sensor domain. (COI:No)

2P-019

Involvement of TRPM7 in the intercellular junction formation in mouse urothelium

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Transient receptor potential melastatin 7 (TRPM7) is a Ca^{2+}-permeable cation channel with a kinase domain that is implicated in magnesium homeostasis. However, the physiological significance of TRPM7 *in vivo* remains unknown. We generated urothelium-specific TRPM7 knockout (KO) mice to reveal the function of TRPM7 *in vivo*. We observed that Mg^{2+}-inhibitable cation currents were significantly smaller in urothelial cells from TRPM7 KO mice compared to the cells from control mice. The assay for voiding behavior indicated a significantly smaller voided volume in TRPM7 knockout mice. Histological analysis showed partial but substantial edema in the submucosal layer of TRPM7 knockout mice, and expressions of pro-inflammatory cytokines, TNF-α and IL-1β were significantly higher in TRPM7 knockout bladder. In transmission electron microscopic analysis, immature intercellular junctions were observed in the superficial layer of TRPM7 KO urothelium but not in control mice. These results suggest that TRPM7 is involved in the formation of intercellular junctions in mouse urothelium. The immature intercellular junctions might lead to a disruption of barrier function resulting in an inflammation which may affect voiding behavior *in vivo*. (COI:No)

2P-020

Mechanosensor TRPV2 channel detects very weak mechanical stimulus during axon outgrowth; the molecular mechanism of stretch-dependent axonal outgrowth

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We previously reported that TRPV2 was a mechanosensor channel which contributed axonal outgrowth in membrane stretch dependent manner (Shibasaki et al., J. Neurosci. 2010). These results indicate that TRPV2 might be an important component for the responses against stretch, if TRPV2 can detect very weak mechanical stimulus. In this study, we examined whether TRPV2 can detect such very weak mechanical stimulus by a Ca^{2+}-imaging method, a whole-cell patch clamp recording and immunocytochemical analysis. We also examined whether the activation of TRPV2 by weak mechanical stimulus lead to the enhancement of axon outgrowth by a time-lapse imaging method. We visualized actin cytoskeletons in extending growth cones by ectopic expression of actin-EGFP fusion protein. Finally, we identified that TRPV2 had a potential to detect very weak mechanical stimulus, and the activation of TRPV2 promoted axon outgrowth through dynamic changes of actin cytoskeletons in Ca^{2+}-concentration dependent manner. Taken together, TRPV2 is a strong candidate molecule for the stretch-dependent axonal outgrowth. (COI:No)

2P-021

Hippocampal TRPV4 regulates neuronal excitability in brain temperature dependent manner

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Physiological brain temperature is an important determinant for neuronal functions, and it is well established that changes in temperature have dynamic influences on brain neuronal excitabilities. We previously revealed that a thermo-sensor TRPV4 (activated above 34°C) is activated by physiological temperature in cultured hippocampal neurons and thereby controls their excitability (J. Neurosci. 2007,BBRC 2013, JBC 2014, Shibasaki et al.). Therefore, if our brain temperature can dynamically change within a small range, a thermo-sensor TRPV4 can convert temperature information to electrical excitability in neurons. To confirm it, we prepared acute hippocampal slices from WT or TRPV4KO mice, and measured resting membrane potentials in the hippocampal granule cells from the slice preparations at 35°C, and found that TRPV4-positive neurons significantly depolarized the resting membrane potentials through the TRPV4 activation at the physiological temperature. The depolarization increased the spike numbers of action potentials depending on the enhancement of TRPV4 activation. Furthermore, the intracellular Ca^{2+} concentration was also significantly increased in the TRPV4-positive neurons. Taken together, we report that TRPV4 activation at the physiological temperature is important to regulate neuronal excitability in mammals. (COI: Properly Declared)

2P-022

Neuromodulatory action of nitric oxide on memory center neurons, called Kenyon cells in insect

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Nitric oxide (NO) has been implicated to play a role in the formation of long-term memory (LTM) during multiple trial conditioning in the cricket *Gryllus bimaculatus*. However, how NO modulates ion channels to affect neuronal excitability and leads to LTM formation is not well understood. The aim of this study is to elucidate the mechanisms underlying the modulatory effects of NO on the electrical activity of Kenyon cells, intrinsic neurons of the mushroom bodies of the cricket brain. Perforated whole-cell and on-cell patch clamp techniques were applied to freshly isolated Kenyon cells. Under the current clamp condition, application of NO donor, GSNO increases the frequency of spontaneous action potentials as well as the action potentials elicited by depolarizing current injection. To study the ionic channel basis of increased neuronal excitability by NO, I examined the modulation of major ion channels identified in cricket Kenyon cells. The results suggest that NO increases TTX-sensitive fast inactivating Na^+ currents, TTX-sensitie persistent Na^+ currents, L-type Ca^{2+} currents, and large conductance Ca^{2+} activated K^+ currents whereas decreases Na^+-activated $K+^+$ currents via classical cGMP/PKG signaling pathway. These results suggest that NO acts as an excitatory modulator on Kenyon cells by modulating a variety of currents and this increase in membrane excitability may participate in the initiation to start signal cascade to leads to LTM formation during multiple trial conditioning in cricket olfactory learning. (COI:No)

2P-023

Functional coupling between sodium-activated potassium channels and voltage-dependent persistent sodium currents in cricket Kenyon cells

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We examined the functional coupling between Na^+-activated potassium (K_{Na}) channels and Na^+-influx through voltage-dependent Na^+ channels in Kenyon cells isolated from the mushroom body of the cricket. Single channel activity of K_{Na} channels was recorded with the cell-attached patch configuration. The open probability (Po) of K_{Na} channels increased with increasing Na^+ concentration in a bath solution, whereas it decreased by the substitution of Na^+ with an equimolar concentration of Li^+. The Po of K_{Na} channels was found to be reduced by bath application of a high concentration of TTX (1 μM), whereas it was unaffected by a low concentration of TTX (10 nM), which selectively blocks I_{Naf}. Bath application of Cd^{2+} at a low concentration (50 μM), as an inhibitor of I_{NaP}, also decreased the Po of K_{Na} channels. Conversely, bath application of the inorganic Ca^{2+} channel blockers Co^{2+} and Ni^{2+} at high concentrations (500 μM) had little effect on the Po of K_{Na} channels. Perforated whole-cell clamp analysis using β-escin further indicated the presence of sustained outward currents whose amplitude was dependent on the amount of Na^+ influx. Taken together, these results indicate that K_{Na} channels could be activated by Na^+ influx passing through voltage-dependent persistent Na^+ channels. The functional significance of this coupling mechanism was discussed in relation to the membrane excitability of Kenyon cells and its possible role in the formation of long-term memory. (COI:No)

2P-024

A synergistic blocking effect of Mg^{2+} and spermine on the inward rectifier K^+ (Kir2.1) channel pore

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Inward rectifier K^+ channels (e.g. Kir2.1 channels) exhibit an intriguing rectifying feature in the current-voltage relationship. We have showed that the bundle-crossing region of the transmembrane domain constitutes the crucial pore segment responsible for the polyamine block. In this study, we demonstrated that the major blocking effect of intracellular Mg^{2+} on Kir2.1 channels is also closely correlated with K+ current flow, and the coupled movements of Mg^{2+} and K^+ happen in the same flux-coupling segment of the pore as polyamines. With a preponderant outward K^+ flow, intracellular Mg^{2+} would also be pushed to and thus stay at the outermost site of a flux-coupling segment in the bundle-crossing region of Kir2.1 channels to block the pore, despite that the apparent affinity of intracellular Mg^{2+} is much lower than spermine (SPM). On the other hand, in contrast to the evident possibilities of outward exit of SPM through the channel pore especially during strong membrane depolarization (i.e. a permeant blocker), intracellular Mg^{2+} does not seem to traverse the Kir2.1 channel pore in any case (i.e. an obligatory blocker). Intracellular Mg^{2+} and SPM therefore may have a synergistic action on the pore-blocking effect, presumably via prohibition of the outward exit of the higher-affinity blocking SPM by the lower-affinity Mg^{2+}. (COI:No)

2P-025

Modulation of NMDA channel activation and desensitization by Ca^{2+} and Cd^{2+} binding to the external pore mouth

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The NMDA receptor channel is characterized by high permeability of Ca^{2+}. Whether extracellular Ca^{2+} ion itself could directly bind to the NMDA receptor channel to have an effect on the key molecular behaviors of the channel has not been fully characterized. We found that extracellular Ca^{2+} and Cd^{2+}, an ion with nearly identical ionic radius as Ca^{2+}, could bind to the closed NMDA channel to affect channel gating as well as ion permeation. We also demonstrated that Cd^{2+} binds to the closed, open, and desensitized channel with apparent dissociation constants of ~5, ~2.5, and ~1.2 μM, respectively. DRPEER, a motif in the GluN1 subunit, is positioned just external to the externally located activation gate. The effect of Cd^{2+} on either the resting or the activated state is decreased correlatively to the number of charge-neutralization mutations in this motif. DRPEER motif therefore may constitute the binding site for Ca^{2+} or Cd^{2+}, and go through sequential conformational changes with channel gating. It is intriguing that the inhibitory effect of Cd^{2+} is also decreased by point mutation T647A located just inside the activation gate in the pore. Moreover, prominent "hook" current develops after wash-off of Cd^{2+} or Ca^{2+} in this case, suggesting preservation of the channel in the open state (prevention of entering the desensitized state). Desensitization thus very likely involves essential conformational changes in the vicinity of the activation gate in the NMDA channel. (COI:No)

2P-026

Properties of the maxi-anion channel in melanoma cells

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Melanoma is one of the most aggressive and lethal skin cancers due to its resistance to conventional antineoplastic therapy and escape from apoptosis. Expression levels and phenotype of the maxi-anion channel, a well-known player in volume-regulation and purinergic cell-to-cell signaling, have not been reported in melanomas. In the present experiments, we studied the phenotype of the maxi-anion channel in a cell line (KML, patent UZ IAP 02729), which was obtained by continuous culturing of excised primary lung tumors from mice intravenously injected with B16 melanoma. The cell-attached patches were silent, whereas patch excision resulted in activation of 10-15 single ion channels with unitary amplitude of 10.7&plusmn1.9 pA (n=7) and 9.9 &plusmn 1.8 pA (n=5) at +25 and -25 mV, respectively. The unitary I-V relationship was linear with a slope conductance of 392 &plusmn 11 pS. The channel exhibited a bell-shaped voltage dependency of open probability and was anion-selective with a permeability ratio of glutamate over chloride of 0.12 &plusmn 0.01. These properties, along with sensitivity to NPPB and SITS, suggest that KML melanoma cells express a high level of the maxi-anion channel with a phenotype similar to that reported for other cells types. The role of this channel in pathogenesis of melanoma remains to be elucidated. (COI:No)

2P-027

Functional characteristics of L1156F-CFTR associated with alcoholic chronic pancreatitis in Japanese

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Pourpose: Although cystic fibrosis is rare in Japanese, measurement of sweat Cl^- has suggested mild dysfunction of CFTR in some patients with chronic pancreatitis. In the present study, we have investigated the association of CFTR variants and alcoholic chronic pancreatitis (ACP) in Japanese and the functional characteristics of a Japanese-, and pancreatitis-specific CFTR variant, L1156F. **Methods:** Seventy patients with ACP and 180 normal subjects participated. All exons and their boundaries and promoter region of the CFTR gene were sequenced. HEK 293 cells and CFPAC-1 cells were transfected with 3 CFTR variants (M470V, L1156F, and M470V+L1156F). The protein expression was examined in HEK 293 cells. CFPAC-1 cells were loaded with BCECF and the activity of Cl^--HCO_3^- exchange was estimated from changes in intracellular pH upon removal of bath Cl^-. **Results:** Cystic fibrosis-causing mutations were not found. The allele frequency of L1156F in alcoholic chronic pancreatitis (5.0%) was significantly ($p<0.01$) higher than that in normal subjects (0.6%). L1156F was linked with M470V. Combination of M470V and L1156F significantly reduced CFTR expression to ~60% and impaired CFTR-coupled Cl^--HCO_3^- exchange activity to 20-30%. **Conclusion:** In summary, the present data suggest that L1156F causes mild dysfunction of CFTR and increases the risk of ACP in Japanese. (COI:No)

2P-028

Dihydropyridine-sensitive Na^+ current contributes to the diastolic depolarization in cardiac pacemaker cells

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We have recently found that L-type CaV1.3 Ca2+ channels are required for the generation of a dihydropyridine-sensitive Na+ current, previously described as the sustained inward current, Ist, in sinoatrial node (SAN) cells. This finding introduces a new concept in cardiac pacemaker activity, indicating that CaV1.3 channels serve a dual role in the pacemaker potential: as a source of Ca2+ entry and as a persistent inward Na+ current. Here we investigated the contribution of nifedipine-sensitive Na+ currents to the diastolic depolarization in spontaneously active SAN cells. To this purpose, we used the patch-clamp protocol comprised of a fast switching from the action potential (AP)-clamp to the current-clamp mode, which allowed us to monitor a free depolarization following the maximal diastolic potential. After establishment of the perforated patch-clamp configuration with amphotericin, the SAN cell was voltage-clamped using the AP waveform previously recorded in the same cell as the command voltage. Under the control condition, the spontaneous action potential were continuously evoked upon the switching from the AP-clamp to the current-clamp mode without changes in the diastolic depolarization rate (DDR) and rhythmicity. Removal of external Ca2+ by replacement with Mg2+ reduced the DDR slightly but eliminated the following AP. Subsequent application of nifedipine further decreased the DDR, which was accompanied by a negative shit in the equilibrium potential. These results suggest that Ist substantially contributes to the pacemaker depolarization in SAN cells. (COI:No)

2P-029

Extracellular ATP suppresses spontaneous activity of sinoatrial node pacemaker cells by activating P2X cation channels

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Intravenous administration of ATP is effective at suppressing the supraventricular tachyarrhythmias in clinical settings. This study was designed to examine the effects of extracellular ATP on spontaneous automaticity of guinea-pig sinoatrial (SA) node cells using whole-cell patch-clamp technique. In the current-clamp experiments, bath application of ATP at concentration of equal to or more than 1 μM depolarized the maximum diastolic potential (MDP), reduced the upstroke velocity of the action potential and eventually arrested the spontaneous action potentials of SA node cells in the presence of the P1-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) at 10 μM. In about 50% of SA node cells, irregular spontaneous action potentials with smaller amplitudes were restored despite the continued presence of ATP. Voltage-clamp experiments showed that extracellular ATP (5 μM) evoked a cationic conductance having a slight inward rectification and reversal potential of approximately 0 mV. This conductance also gradually decreased despite the presence of ATP. In contrast, bath application of 5 μM adenosine hyperpolarized MDP and arrested the spontaneous action potentials of SA node cells. These observations indicate that micromolar concentrations of extracellular ATP suppress the spontaneous automaticity of SA node cells presumably by activating an inward current through P2X cation channels and depolarizing the cell membrane, which is completely different from the ionic basis underlying the adenosine-induced suppression of SA node automaticity. (COI:No)

2P-030

Effects of Pitx2c overexpression on the heterogeneity of K^+ channels in HL-1 mouse atrial myocytes

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Changes in the K^+ channel expression are responsible for alterations in membrane excitability during cardiomyocyte differentiation and maturation. Pitx2c (paired-like homeodomain 2 transcription factor) is crucial for the development of the left atrium structure and function, but little is known about its roles in the regulation of ion channel expression. In the present study, we examined the effect of forced expression of Pitx2c on the expression pattern of K^+ channels in murine HL-1 atrial myocytes. In our patch-clamp recordings, HL-1 cells expressed predominantly the E4031-sensitive delayed rectifier K^+ current (I_{Kr}) but some fractions of cells displayed an overt expression of other voltage- and time-dependent K^+ currents, either the HMR1556-sensitive delayed rectifier current (I_{Ks}) or the 4-aminopyridine-sensitive transient outward current (I_{to}). The cellular fraction that exhibits I_{Kr} as the sole outward current was approximately 60% in GFP-positive control cells, which was considerably reduced by transient expression of Pitx2c (~40%). On the other hand, the fraction of cells that predominantly express I_{to} was greatly increased by Pitx2c. The fraction of I_{Ks}-expressing HL-1 cells was slightly reduced by Pitx2c. These changes in K^+ current expression pattern were accompanied by alterations in transcriptional levels of channel molecules. Taken together, our results suggest that Pitx2c contributes to the developmental changes in K^+ channel expression in mouse atrial myocytes. (COI:No)

2P-031

The role of NCX1 in T-tubule remodeling during progression of heart failure induced by pressure overload

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Cardiac Na+/Ca2+ exchanger (NCX1) is critical for [Ca2+]i control and localized to transverse tubule (T-tubule) membrane. T-tubules are invaginations of surface membrane with regular spacing in normal cardiomyocytes. Although T-tubule disruption is common features in failing myocytes, the role of NCX1 in T-tubule remodeling in failing heart (FH) have remain unclear. Here, we followed changes in the expression pattern of NCX1 during the progression of T-tubule remodeling induced by pressure overload after transverse aortic constriction (TAC)-surgery. At 16weeks (16w) post-TAC, the mice exhibited severe HF with significant chamber dilation and decrease in contractility. In the heart, T-tubule system was disrupted with loss of the regular striated pattern. To quantitatively evaluate the regularity of NCX1 localizations, the one-dimensional power spectra of immunofluorescent images of NCX1 were calculated as the magnitudes of fast Fourier transforms. Fourier analysis showed that the regularity of NCX1 signal was substantially disturbed at 12w post-TAC while L-type Ca2+ channels appeared as well-ordered patterns along T-tubule. At this time, NCX1 activity per cell significantly decreased compared to Pre-TAC. These results suggest that reduced activity and changes in the localization of NCX1 are involved in T-tubule remodeling in TAC-hearts. (COI:No)

2P-032

Impairment of Ca2+ signaling and dyastolic dysfunction in diabetic model mice

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Diabetes mellitus (DM) increases the risk of cardiac heart diseases such as heart failure. Diastolic dysfuncion of left ventricle is receiving attention as one of early cardiac changes in diabetic heart failure patients. Aiming at clarifying a mechanism linking DM and cardiac dysfunction, we examined the changes in cardiac function and expression levels of Ca^{2+} signaling proteins in atria and ventricle of DM mice. DM was induced by streptozotocin (STZ) in male mice. Four weeks or eight weeks after injection of STZ, hearts were excised and used for experiments. In right ventricular and left atrial free walls, the time required for 90% relaxation was significantly longer in STZ mice than those of control mice. The decay rate of Ca^{2+} transient was also significantly slower in ventricular myocytes of STZ mice. In atria and ventricle of STZ mice, mRNA levels of junctophilin-2, and RYR2 were significantly lower than control mice. In ventricle, mRNA level of SERCA2 was significantly down-regulated in STZ mice. In contrast, mRNA levels of β_1-adrenergic receptor, β_2-adrenergic receptor, NCX were not different between STZ and control mice. However, Ser^{16}-phosphorylation levels were lower in atria and ventricle of STZ mice as compared to those of control mice. These results suggest that the diabetes mellitus-induced myocardial diastolic dysfunction is, at least, partly caused by the impairment of Ca^{2+} signaling including reduction of SERCA2 function. (COI:No)

2P-033

Aberrant Ca^{2+} regulations of the ryanodine receptor channel by its arrhythmogenic mutations.

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A lot of gene mutations in the human cardiac ryanodine receptor (RyR2) channels have been found in not only European but also Japanese patients of catecholamine polymorphic ventricular tachycardia (CPVT). Polymorphic VT was induced by physiological exercise such as walking in the Japanese patients. We reproduced the Japanese CPVT-associated RyR2 mutations in the mouse RyR2 cDNA. Then we examined the dysfunction mechanisms of the recombinant RyR2 mutant (MT) channels compared with wild-type (WT) by measuring single-channel currents, [^{3}H]ryanodine binding, and cytosolic/luminal Ca^{2+} levels. Both cytosolic and luminal Ca^{2+} sensitivities were higher in MT than WT. ER Ca^{2+} level in HEK 293 cells heterogously expressing RyR2 proteins was remarkably decreased in MT than in WT, suggesting that the Ca^{2+} homeostasis in the cardiomyocytes carrying CPVT-associated RyR2 mutations is disrupted. Dramatically enhanced Ca^{2+} leak through the MT RyR2 from the sarcoplasmic reticulum lumen to the cytosol is likely to cause proarrythmic Ca^{2+} waves in cardiac myocytes. Our findings on the RyR2 MT have provided new structural and functional insights into the Ca^{2+}-dependent pore gating mechanisms. (COI:No)

2P-034

Construction of a mathematical model of mouse sinoatrial node cell considering structural crosstalk between mitochondria and sarcoplasmic reticulum

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We reported that there is a structural crosstalk between mitochondria and sarcoplasmic reticulum (SR) in mouse sinoatrial node cells. That is, electron microscopy experiments demonstrated that most of mitochondria co-localized with SR and ~40% of SR co-localized with mitochondria (Takeuchi et al., J Physiol Sci, 65, Supp1, S206, 2015). In the present study, in order to elucidate the roles of the structural crosstalk in functions of sinoatrial node cells, we constructed a mathematical model of a mouse sinoatrial node cell. The mouse sinoatrial node cell model reported by Kharche et al. (Am J Physiol Heart Circ Physiol, 301, H945-H963, 2011) was used as a base model and was newly incorporated with 2 mitochondria compartments, 4 SR compartments, and 2 SR-mitochondria subspace compartments, according to the electron microscopy data. Simulations with various expression levels of mitochondrial Ca carriers were then performed. It was shown that the smaller the expression level of mitochondrial Ca extruder NCLX, the smaller the Ca content in SR became, suggesting that there is a functional crosstalk between mitochondria and SR. It was also shown that the contribution of NCLX to generation of automaticity depends on how fast the Ca diffusion between subsarcolemmal space and cytosolic space is. (COI:No)

2P-035

A Mathematical Modeling of Phase-2 Reentry Attributable to Changes in Subcellular Expression of Nav1.5 Channel

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In an action potential (AP) initiation and AP propagation in the heart, cardiac voltage-gated sodium (Nav1.5) channel is of importance. Recent experimental studies have shown that changes in subcellular expression of Nav1.5 channel might be involved in Brugada syndrome (BrS). Our objective is to present a reproducible mathematical model of phase-2 reentry (P2R) seen in the BrS. We conducted computer simulations of AP propagation in a myofiber model, where myocytes were electrically coupled with both gap junctions and intercellular cleft conductor (electric field mechanism), and investigated relations between the spatial and subcellular distributions of Nav1.5 channels and P2R development. In the myofiber model with the spatial heterogeneity of Nav1.5 channels, P2R did not occur. However, in the same myofiber model but with the decrease in Nav1.5 channel expression from the lateral membrane of each myocyte, the P2R could be observed. These results showed that P2R required the alteration in Nav1.5 channel expression at the subcellular level as well as the spatially-heterogeneous Nav1.5 channel expression. Alterations in Nav1.5 channel expression within myocytes might be in part of responsible for the triggering of ventricular tachycardia/ventricular fibrillation in BrS. (COI:No)

2P-036

Blood pressure is not well maintained during 90 degrees head-up tilt for 30 min in anesthetized aging rats

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To investigate the changes of cardiovascular parameters during 90°head-up tilt(HUT) for 30 min in the aging animal, we measured systemic blood pressure (BP), blood flow toward head(BF) and heart rate (HR) in anesthetized aging rats(urethane 1.0-1.5 g/kg, i.p., n=8, 30-90 weeks) with analog-digital device(MP36: Biopac, USA). After onset of HUT both of BP and BF immediately decreased to 69.3±10.9 mmHg and 3.67±0.91 ml/min at 4.7±2.0 sec,respectively(mean±SD, $p<0.01$: paired t-test), and then they increased toward control value and steadied at 68.7±49.0 sec(plateau:BP,77.7±9.6 mmHg; BF,3.94±0.76 ml/min). Both of them decreased gradually during 30 min HUT.The HR increased after HUT and there were statistical differences at peak of plateau and 5 min compared with control value before HUT (by 6.9±4.6 beats/min at the plateau, by 5.0±4.7 beats/min at 5 min, $p<0.05$). These results indicated that the initial change in the BP just after HUT was caused by the hydrostatic pressure gradient the same as other aged rats, and the BP maintenance throughout HUT was similar to the result obtained from the experiment of sino-aortic denervation in our former study. It suggests that baroreflex in aging rats seems to be weaker,however other reasons must be considered. This work was supported by JSPS Grant # 26506024. (COI:No)

2P-037

The central amygdala regulates cardiovascular system without alteration of the baroreflex gain

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Under mental or emotional stress, it is well known that both blood pressure and heart rate increase via sympathoexitation, although the underlying mechanisms remain unknown. One of the major nuclei of the limbic system is the amygdala, which is known to induce emotional reactions. In this study the functional role of the central amygdala (CeA) on the baroreflex, a crucial cardiovascular reflex to maintain hemodynamic homeostasis, was investigated to understand brain mechanisms underlying stress-induced cardiovascular responses. The CeA was electrically stimulated(200 μA, 50 Hz, 30 sec)to induce pressor and tachycardiac responses, and the gain of the baroreflex induced by intravenous phenylephrine administration was measured before and during CeA stimulation. We found that the reflex gain was not altered by the electrical stimulation of the CeA, suggesting that the CeA increases both blood pressure and heart rate via resetting mechanisms of the baroreflex function. This may explain one of the central mechanisms of stress-induced cardiovascular responses. (COI:No)

2P-038

Changes of cardiovascular parameters after 90 degrees head-up tilt in anesthetized rat

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When the postural change is executed, the baroreceptor senses alterations of systemic blood pressure(BP) and its reflex occurs(BR). To examine the changes in the cardiovascular parameters after onset of supine position(SP) from 90°head-up tilt(HUT), we measured BP,common carotid arterial flow as blood supply toward head(BF),and heart rate(HR), before and after the postural change in anesthetized rats aged 13-19 weeks as adult or 31-90 weeks as aging(urethane,1.0-1.5 g/kg). After transition from HUT to SP, the fluctuation of BP was observed in each animal of both experimental groups;the BP in adult(n=12) were 76.2±14.7 mmHg under 30 min HUT just before postural change, 85.6±15.3 mmHg at 1.7±1.1 sec after transition, 77.2±15.3 mmHg at the 2nd change and 83.0±17.0 mmHg at the 3rd change, and the BP in aging rats(n=7) were 72.4±10.7 mmHg in 30 min HUT, 84.1±10.6 mmHg at 3.1±4.0 sec as the 1st, 78.6±10.3 mmHg and 81.4±10.0 mmHg, respectively. The BF after the postural change in each animal, basically changed according to the BP fluctuation. The HR decreased slightly during the BP fluctuation in each expt. group, and the differential changes of HR were not clear. These results indicate that the 2nd change in the BP fluctuation is caused by BR in response to the elevated BP due to hydrostatic pressure change after transition from HUT to SP, suggesting that the BR response is weaker in aging rats.This work was partly supported by JSPS KAKENHI Grant 26506024. (COI:No)

2P-039

Differential cardiovascular regulation of the amygdala

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Under physical and mental stress such as performing vigorous exercises and encountering the enemy, predominant sympathetic nerve excitation is induced. Although the amygdala has been known as a negative emotion center processing fear conditioning and defensive behavior, the functional roles of the amygdala for cardiovascular autonomic control remain unclear. To answer the question, in this study, we examined whether microstimulation of the amygdala has effects on cardiovascular responses, and if does, which brain areas would input to the effective sites of the amygdala. As results, electrical stimulation (200 μA, 50 Hz, 30 sec) for unilateral amygdala of urethane-anesthetized wister rats (n = 10) evoked location-specific responses. Stimulation of central nucleus of the amygdala (CeA) exhibited phasic pressure increase and tachycardia, while that of basolateral amygdala showed pressure decrease. In addition, we injected retrograde tracer (fluorogold, 50 nL) into the CeA (n = 7) and observed projections from several areas including monoaminergic neurons, substantia nigra (dopamine), pedunculo-pontine tegmental nucleus (acetylcholine) and dorsal raphe (serotonin). These results suggest that the amygdala has possible to bidirectional control (facilitation or inhibition to cardiovascular responses) through its own local circuits and various neuromodulator inputs. (COI:No)

2P-040

The tracts of neural reflex for the teeth clenching-induced pressor response in rats

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We have previously reported a new reflex response of teeth clenching-induced pressor response. The reflex tracts of its response are, however, unknown. To elucidate the receptors, the afferent pathway and the efferent pathway of the pressor response, we examined the effects of local anesthesia of the molar regions, the entrapment of gadolinium (the mechanoreceptor blocker of the group3 muscle afferent) in the masticatory muscles, bilateral trigeminal ganglion block, and the intravenous administration of hexamethonium in urethane-anesthetized rats. Teeth clenching was induced with electrical stimulation of the bilateral masseter muscles. Bilateral local anesthesia of the molar regions significantly reduced the pressor response to 25.8±24.5% of the non-local anesthesia. The entrapment of gadolinium in the masticatory muscles also significantly reduced the pressor response to 62.4±6.8% of the non-gadolinium entrapped state. Furthermore, bilateral trigeminal ganglion block or the intravenous administration of hexamethonium completely abolished the pressor response. These results indicated that the receptors may be the periodontal membrane and the mechanoreceptor in the masticatory muscles, and that an afferent pathway may be the trigeminal afferent nerves, and an efferent pathway may be the autonomic nerves, respectively. (COI:No)

2P-041

Dynamical Mechanisms of Phase 2 Early Afterdepolarizations in Human Ventricular Myocyte Models

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Aim: Early afterdepolarizations (EADs) are known as the major cause of lethal ventricular arrhythmias in long QT syndromes. We tested human ventricular myocyte models for capability to reproduce phase-2 EADs and examined dynamical mechanisms of EAD generation in terms of nonlinear dynamics. Methods: Numerical simulations and bifurcation analyses were applied to human ventricular myocyte models developed by Kurata et al (2005), ten Tusscher and Panfilov (2006), Grandi and Bers (2010), and O'Hara et al (2011). Bifurcation diagrams were constructed as functions of model parameters. Effects of modulating the delayed-rectifier K^+ channel currents (I_{Kr}, I_{Ks}), L-type Ca^{2+} channel current (I_{CaL}), Na^+/Ca^{2+} exchanger current (I_{NCX}) and intracellular Ca^{2+} dynamics were examined.Results and Discussion: 1) EADs were reproduced by reducing I_{Ks} conductance (g_{Ks}) in Kurata and ten Tusscher models, but not in other models. 2) Decreasing I_{Kr} conductance (g_{Kr}) induced EADs in Kurata and O'Hara models. 3) During g_{Kr} decreases, Kurata model reproduced two types of EADs, I_{Ks} activation-dependent and I_{CaL} inactivation-dependent ones. 4) In O'Hara model, EAD generation depended strongly on slow I_{CaL} inactivation. 5) Effects of I_{NCX} and Ca^{2+} dynamics were different in Kurata and O'Hara models. Thus, capability and dynamical mechanisms of EAD generation are model dependent; further refinements of model cells are required for more profound understanding of the mechanisms of EAD generation. (COI:No)

2P-042

Correlation of heart rate variability during otolith stimulation with tone bursts and changes in arterial pressure upon head-up tilt

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Sound pressure using tone bursts (TBs) has been used to test otolith function. In the present study, the R-R interval variability (RRIV) in the supine position without and with TBs, changes in mean arterial pressure (MAP) at the onset of 60 degrees head-up tilt (HUT) without TBs, and their relationship were analyzed. The mean decrease in MAP was 7 mmHg. At the onset of HUT, MAP increased or decreased by <7 mmHg in 16 subjects (UP). However, MAP decreased by >7 mmHg in 13 subjects (DOWN). Application of TBs of 105 dB at a random frequency from 2.5 to 7.5 Hz did not change the mean R-R interval or MAP. The high-frequency component (HF) of RRIV, an index of cardiac parasympathetic nerve activity, decreased in UP subjects but increased in DOWN subjects. The percent changes in both the low-frequency component (LF) and HF with TBs were significantly correlated with changes in MAP at the onset of HUT. Thus, the autonomic nervous system responses to TBs are variable, but TBs possibly control parasympathetic nerves. The degree of excitation or depression in parasympathetic nerves during otolith stimulation is considered to be important for controlling MAP at the onset of HUT. (COI:No)

2P-043

Role of pulmonary stretch receptors in regulating heart rate and sympathetic nerve activity during obstructive sleep apnea in conscious rats.

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Obstructive sleep is associated with a marked increase in sympathetic nerve activity and bradycardia, while mechanisms underlying these responses remain unknown. The present study assessed a potential role of pulmonary stretch receptors in regulating heart rate and sympathetic nerve activity during obstructive sleep apnea in conscious rats. Wistar male rats were chronically instrumented with electrodes for measurements of renal and lumbar sympathetic nerve activity, and electroencephalogram, electromyogram, and electrocardiogram and with catheter for measurement of systemic arterial pressure and with a tracheal balloon for induction of apnea. The tracheal balloon was inflated for 40 seconds during non-rapid eye movement sleep. Injection of lidocaine around the tracheal bifurcation and bilateral vagotomy were carried out to assess a potential contribution of afferent information originated from pulmonary stretch receptors via the vagal nerve. The lidocaine injection and bilateral vagotomy diminished the bradycardia response while these maneuvers exerted minor effect on renal and lumbar sympathetic nerve activity. These results suggest that pulmonary stretch receptors plays a significant role in inducing bradycardia during obstructive sleep apnea, which may protect cardiac muscle against sever hypoxia due to apnea. (COI:No)

2P-044

Novel xanthine oxidase inhibitor attenuates hypoxia/reoxygenation injury in isolated rat heart

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Allopurinol (Allo), a traditional xanthine oxidase (XO) inhibitor, is known to protect the heart against ischemia/reperfusion injury by inhibiting production of reactive oxygen species. In this study, we aimed to determine the effect of novel XO inhibitor, febuxostat (Feb), on cardiac tolerance to hypoxia/reoxygenation injury using Langendorff perfused rat heart model. Rats (300-350g) were randomly divided into three groups: control, Allo and Feb groups. After decapitation, the hearts of rat were excised for Langendorff perfusion, and exposed to 30-min hypoxia/30-min reoxygenation. During hypoxia, the hearts in Allo and Feb groups were perfused with hypoxic solution containing 30 μM Allo and 30 μM Feb, respectively. The treatment of both Allo and Feb significantly improved recovery of cardiac function in comparison with control group during reoxygenation ($p<0.05$). In addtion, cardiac function during 20-30 min after reoxygenation was significantly higher in Feb group than that in Allo group ($p<0.05$). The treatment of Feb resulted in the complete recovery of heart rate after reoxygenation, but not in control and Allo groups. At the end of hypoxia, ATP content and the inhibition of XO activity in heart of Feb group was significantly higher than that in Allo group ($p<0.05$). Our data suggest that the treatment of febuxostat during hypoxia enhance cardiac tolerance against hypoxia/reoxygenation injury, which may be due to, at least in part, the decrease in energy consumption during hypoxia and the increase in inhibition of XO activity. (COI:No)

2P-045

Magnesium deficiency inhibits both voltage-gated and receptor-operated vascular smooth muscle contraction

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Magnesium (Mg) deficiency has been reported to be associated with the development of various cardiovascular diseases, however, effects of Mg deficiency on vascular responses have not been fully elucidated. The purpose of the present study is to evaluate vascular contractile and relaxant response in dietary-induced Mg deficient rat model. Male Sprague-Dawley rats were fed an Mg-deficient diet (Mg content; 4.5 mg/100 g diet) and deionized water for eight weeks, and then the thoracic aorta were excised to measure isometric tension in organ chambers. Both high K^+-induced contraction and α_1-adrenergic agonist phenylephrine-induced contraction were significantly reduced in Mg deficient group (Mg-Def) compared to control group, whereas high-K^+-induced maximal force development at 60 mM K^+ was not different in both groups. Supplemental $CaCl_2$-induced contraction of 25 mM K^+-depolarized vessels under Ca^{2+}-free condition were markedly reduced in Mg-Def. Both endothelium-dependent and -independent vascular relaxations elicited by either acetylcholine or sodium nitroprusside were significantly enhanced in Mg-Def in vessels precontracted to equal level of contraction with 60 mM K^+. Thus, these results suggest that Mg deficiency inhibits both voltage-gated and receptor-operated vascular smooth muscle contraction without affecting maximal force development of contractile apparatus in vascular smooth muscle. (COI:No)

2P-046

Influence of mechanical stimulus on chick heart cell aggregates

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The numerous studies have indicated the electrical-mechanical interactions between cells in order to regulate the heartbeat. The aggregations of cardiac cells, the heart constitution, spontaneously beat and synchronized on a petri dish and periodic electric signals can trigger beats as a heart pacemaker works to regulate the heart pace. However, mechanical stimulus, for example periodical tapping, has not applied to test the capability of triggering the beat although the influence the stretching on inter beat intervals (IBI's) and their action potential profiles was investigated. Here, we will present the results of an artificial mechanical stimulus on single aggregates to excite their beats. The heart cell aggregates of embryonic chick heart were prepared based on traditional protocols. Tungsten probes were used to apply the mechanical stimulus cycles on aggregates. Generally, the mechanical stimulus changed the beat activity. We have carefully investigated the parameters of mechanical stimulus vs. the IBI and its stability. Since there is a variation of IBI stability of the aggregations on a dish, it is noteworthy to observer the stability changes before and after the stimulus. (COI:No)

2P-047

A novel pathway for up-regulation of microRNA expression in cardiomyocytes exposed to angiotensin II

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We have previously shown that microRNA-X (miR-X) is up-regulated in cardiomyocytes with persistent atrial fibrillation (AF), in response to cellular Ca^{2+}-overload. It is well-known that abnormal Ca^{2+} dynamics may lead to the development of atrial fibrillation (AF), however, mechanisms for miR-X up-regulation in AF cardiomyocytes have not been elucidated. To investigate whether Ca^{2+}-overload regulates miR-X expression in adult or neonatal rat cardiomyocytes, we infused angiotensin II (Ang II; 1.68 mg/kg/day) or noradrenaline (NA; 5.4 mg/kg/day) for 2 weeks via osmotic minipump into adult Wistar rats, because Ang II and NA are well-known drivers for onset of AF. Ang II infusion, but not NA, markedly increased miR-X expression in atrium accompanied by an increase in BNP production. Also Ang II rapidly increased miR-X expression as a short-term effect in cardiomyoyctes in *in vitro* application (3 h) and in the myocardium in *in vivo* application (6 h), both of which were sustained for up to 24 h. Furthermore, exposure of cardiomyocytes to BNP, a down-stream molecules of Ang II signaling, significantly increased expression of miR-X in dose- and time-dependent manner. Our data provide first evidence that acute- or long-term stimulation of cardiomyocytes by Ang II induces elevation of miR-X expression independently of the changes in blood pressure, which suggests that miR-X could be a tool for predicting the risk of AF. (COI:No)

2P-048

Effect of carvedilol, aβ-blocker on Na^+/Ca^{2+} exchange current in heart

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Carvedilol has α1, β1 and β2-adrenergic blocking effects together with the effects such as vasodilating, anti-ischemic, anti-oxidant, anti-apoptotic, anti-proliferate and anti-inflammatory activities. Carvedilol inhibited I_{Kr}, I_{Ks}, $I_{Ca\text{-}L}$ and I_{to} in rabbit cardiac ventricular myocytes. Carvedilol and its analogues inhibited delayed afterdepolarizations (DADs) in Langendorff-perfused rabbit hearts. It is well known that DADs are caused by the Na^+/Ca^{2+} exchange current (I_{NCX}). We examined the effects of carvedilol and metoprolol, two aβ-blockers, on I_{NCX} by using the whole-cell voltage-clamp method in isolated guinea pig ventricles and CCL39 fibroblast cells expressing dog cardiac Na^+/Ca^{2+} exchanger (NCX1). Carvedilol suppressed I_{NCX} in a concentration-dependent manner but metoprolol did not in isolated guinea pig cardiac ventricular myocytes. IC_{50} values for the Ca2+ influx (outward) and efflux (inward) components of I_{NCX} were 69.7 μM and 61.5 μM, respectively. Carvedilol at 100 μM inhibited I_{NCX} in CCL39 cells expressing wild type NCX1 similarly to mutant NCX1 without the intracellular regulatory loop. Carvedilol at 30 μM abolished ouabain-induced DADs in isolated cardiac ventricular myocytes. Carvedilol suppressed bi-directional I_{NCX} in a concentration-dependent manner with the IC_{50} value of approximately 65 μM. We conclude that carvedilol inhibits NCX1 at supratherapeutic concentrations. (COI:No)

2P-049

Production of calcineurin B homologous protein 3 knockout mice and analysis of cardiac function

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Pathological cardiac hypertrophy is an adaptive response caused by various stimuli, and predicted to lead to chronic heart failure. Although previous studies suggested importance of Ca^{2+} signaling in this process, pathological significance of many Ca^{2+}-handling proteins is still unknown. Calcineurin B homologous protein 3 (CHP3) is an EF-hand Ca^{2+}-binding protein with unknown function, which is highly expressed in the heart. In vitro knockdown study suggested that CHP3 negatively regulates cardiomyocyte hypertrophy via inhibition of glycogen synthase kinase 3 phosphorylation (Kobayashi, et al., JMCC, 2015). In this study, we produced the global knockout (KO) mice of CHP3, analyzed the cardiac function and histological changes. We detected the increases in left ventricular mass and plasma level of a hypertrophic marker atrial natriuretic peptide (ANP) in KO mice (6 weeks-old), indicating a sign of cardiac remodeling caused by genetic ablation of CHP3. However, echocardiographic analysis suggested that cardiac function of CHP3-KO mice with 6 weeks-old were nearly normal, although age-dependent change was not yet analyzed. Using produced CHP3-KO mice, we are now studying the age-dependency and response of KO mice to stressors such as transverse aortic constriction or exposure to hypoxia. (COI:No)

2P-050

Increased contractile effects of thrombin in pulmonary artery of monocrotaline-induced pulmonary hypertension

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Thrombin induces contraction of vascular smooth muscle via its receptor PAR_1. However, the contractile effects of thrombin in the systemic circulation are observed only in the limited types of artery under physiological conditions. In contrast, it is augmented in the vascular lesions under pathological situations. The contractile effects of thrombin in the pulmonary circulation remain elusive. The present study addressed this question, by examining the contractile effects of PAR_1 agonists in the isolated pulmonary artery and the isolated perfused lung preparations of normal animals and the monocrotaline-induced pulmonary hypertension rats. Thrombin induced a sustained contraction in the normal porcine pulmonary artery. Both Ca^{2+}-dependent canonical and Ca^{2+}-independent non-canonical mechanisms were involved in this contraction. In the perfused lung experiments, PAR1 agonist peptide induced a modest pressor effect in the normal rats. This effect was significantly augmented with concomitant upregulation of $PARPAR_1$ expression in the monocrotaline-induced pulmonary hypertension. In conclusion, the pulmonary artery has a unique responsiveness to thrombin. The increased contractile effect of thrombin may contribute to pathophysiology of pulmonary hypertension associated with increased coagulability. (COI:No)

2P-051

Remote ischemic preconditioning with a specialized protocol activates the non-neuronal cardiac cholinergic system and increases ATP content in the heart

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We previously proposed a concept of a non-neuronal cardiac cholinergic system (NNCCS) and revealed that the locally produced ACh played pleiotropic roles in regulating cell-cell communication and cellular energy metabolism. Heart-specific choline acetyltransferase transgenic mice, known as a final model for enhanced NNCCS, disclosed that the heart was exclusively resistant to global ischemia. Ischemic preconditioning (IPC) renders the targeted organ resistant to prolonged ischemic insults. For searching NNCCS activation modalities, we reported that remote ischemic preconditioning (RIPC) activated NNCCS to accelerate de novo cardiac ACh synthesis. Therefore, we aimed to optimize a specific protocol to most efficiently activate NNCCS using RIPC. In this study, we elucidated that the protocol with 3 min of ischemia repeated three times in a murine lateral hindlimb increased cardiac ChAT protein expression (139.2 ± 0.4%; $P<0.05$) as well as cardiac ACh contents (14.2 ± 2.0 ×10-8 M; $P<0.05$) and cardiac ATP contents (2.13 ± 0.19 μmol/g tissue; $P<0.05$) within 24 h following RIPC. Moreover, in the specific protocol, several characteristic responses against energy starvation and for obtaining adequate energy were observed, e.g., increased Sirt6 and decreased PDK4. This suggests that RIPC induces a fasting signal to the heart to be adapted for an energy deficient condition. Therefore, it is suggested that RIPC evokes a robust response by the heart to activate NNCCS through the modification of energy metabolism related pathway. (COI:No)

2P-052

Influences of nitro-arginine and indomethacin on membrane potential responses of vascular endothelial cells

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Acetylcholine (ACh) induces the endothelium-dependent relaxation of vascular smooth muscle via releasing the endothelium-derived relaxing factors such as nitric oxide (NO) and prostacyclin (PGI_2), and also via the endothelium-dependent hyperpolarization (EDH). Thus vascular dilation due to EDH is usually isolated by using nitro-arginine and indomethacin to block syntheses of NO and PGI_2, respectively. However influences of such blockers on the membrane potential responses of vascular endothelial cells have not been examined. In the present experiments ACh-induced membrane responses were observed in the acutely isolated endothelial layer preparations from the guinea-pig mesenteric arteries and effects of nitro-arginine and indomethacin were examined. ACh-induced membrane responses were not a simple hyperpolarization but biphasic hyperpolarizations and once ACh was washed out a transient depolarization and another hyperpolarization having slow time-course were followed. In the presence of nitro-arginine or indomethacin the second phase of hyperpolarization appeared to be suppressed. Voltage-clamp experiments revealed that ACh activated both K+ current and Cl^- current which were responsible to the membrane hyperpolarization and depolarization, respectively. Nitro-arginine or indomethacin seemed to increase the Cl^- current masking the second phase of hyperpolarization. Endothelium-derived NO and prostanoids may enhance the endothelial cell hyperpolarization by decreasing the membrane anion conductance. (COI:No)

2P-053

Sympathetic hyper-excitation in obesity and pulmonary hypertension; relevance to the Obesity Paradox

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Sympathetic nerve activity (SNA) plays an important role in facilitating pulmonary vasodilation. Since SNA is elevated in obesity, we aimed to assess the impact of enhanced SNA on pulmonary vascular homeostasis in obesity, and the severity of pulmonary hypertension (PH); the 'obesity paradox' phenomenon. Zucker obese and lean rats were exposed to normoxia or chronic hypoxia (CH-10% O_2) for two weeks. Pulmonary SNA was recorded (electrophysiology), or the pulmonary microcirculation was visualized using Synchrotron microangiography. Acute hypoxic pulmonary vasoconstriction (HPV) was assessed before and after blockade of B_1-adrenergic receptors (AR) and B_1+B_2-adrenergic. Pulmonary SNA of normoxic obese rats was higher than lean counterparts (2.4 uV.s and 0.5 uV.s, respectively). SNA was enhanced following the development of PH in lean rats, but more so in obese rats (1.7 uV.s and 6.8 uV.s, respectively). The magnitude of HPV was similar for all groups. Although B-blockade did not modify HPV in lean rats, it significantly augmented the HPV in normoxic obese rats (B_1 and B_2 blockade), and more so in obese rats with PH (B_2-blockade alone). This study suggests that sympathetic hyper-excitation in obesity may play an important role in constraining the severity of PH and, thus, contribute in part to the 'obesity paradox' in PH. (COI:No)

2P-054

Cryopreservation method of isolated cardiac myocytes of adult rat

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Cardiomyocytes in a physiologic condition are useful model for cardiac physiological research. However, there are inevitable problems to isolate cell; 1) need to sacrifice the animal, 2) need to find the appropriate experimental conditions and adequate enzymes, and 3) difficult to maintain the quality of the isolated myocytes in each isolation. Especially, these problems are the biggest obstacles for isolating human cardiomyocytes. To resolve the above problems, cryopreservation method should be established. In this study, we tried to find the cryopreservation method that can store and recover the cardiomyocytes in a physiologic condition. We tested the several cryoprotective agents, the freeze-thawing conditions, the used solution compositions, and the other agents. We found DMSO was the best cryoprotective agents than the others. 15 % DMSO condition produced the best cell viability. Cell viability is about 7 percent higher when the rat serum(RS) was used instead of FBS. Pretreatment of BDM or Blebbistatin definitely improved the cell shape and the survival rate. In conclusion, DMSO reduced cell damage from the procedure of freezing or thawing. Using rat serum helped the cell survival rate. From the above results, we could obtain more than 75% survival rate after cryopreservation compared to the survival rate of the initial isolation by optimizing the cryopreservation conditions. This work supported by MSIP/COMPA (NO. 2015K000247) and NRF (NO. 2014M3A9D7034366 and NO. 2015M3A9B6028310) (COI:No)

2P-055

The molecular machinery underlying neurotransmitter receptor immobilization at postsynaptic sites is poorly understood

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The molecular machinery underlying neurotransmitter receptor immobilization at postsynaptic sites is poorly understood. The NMDA receptor subunit NR1 can form clusters in heterologous cells via a mechanism dependent on the alternatively spliced C1 exon cassette in its intracellular C-terminal tail, suggesting a functional interaction between NR1 and the cytoskeleton. The yeast two-hybrid screen was used here to identify yotiao, a novel coiled coil protein that interacts with NR1 in a C1 exon-dependent manner. Yotiao mRNA (11 kb) is present modestly in brain and abundantly in skeletal muscle and pancreas. On Western blots, yotiao appears as an kDa band that is present in cerebral cortex, hippocampus, and cerebellum. Biochemical studies reveal that yotiao fractionates with cytoskeleton-associated proteins and with the postsynaptic density. With regard to immunohistochemistry, two anti-yotiao antibodies display a somatodendritic staining pattern similar to each other and to the staining pattern of NR1. Yotiao was colocalized by double-label immunocytochemistry with NR1 in rat brain and could be coimmunoprecipitated with NR1 from heterologous cells. Thus yotiao is an NR1-binding protein potentially involved in cytoskeletal attachment of NMDA receptors. Consistent with a general involvement in postsynaptic structure, yotiao was also found to be specifically concentrated at the neuromuscular junction in skeletal muscle. (COI:No)

2P-056

Differences in the spatial pattern of the rhythmic motor activity between the thoracic and lumbar segmental networks in the α-chimaerin knockout mouse spinal cord.

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Neuronal networks in the spinal cord generate the basic rhythmic motor output for walking in mammals. The neurons involved in hindlimb locomotion are widely distributed in the thoracic and lumbar spinal cord in rodents. In the α-chimaerin knockout (Chn1-KO) mouse, the Rac-GAP chimerin crucial for the downstream signaling of EphA4 is disrupted, resulting in aberrant midline-crossing of ipsilateral-projecting axons in the spinal cord. This leads to abnormal hopping gait of this mutant mouse (Iwasato et al. 2007, Borgius et al. 2014). However, the neuronal organization of the local networks responsible for the hopping gait remains unclear. In this study, we analyzed the spatial pattern of rhythmic motor activity in the left and right sides of a single spinal segment preparation from the thoracic (T13) and the lumbar (L1) cord. In T13 preparations taken from wildtype (WT) and Chn1-KO mice, alternating left-right rhythmic activity was induced by bath-application of NMDA and 5-HT. By contrast, a synchronous left-right pattern was observed in the majority of L1 preparations taken from Chn1-KO but not in ones taken from WT mice . These results indicate that the network elements generating the hopping gait in Chn1-KO mouse might be localized mainly in the lumbar spinal cord. Supported by KAKENHI No. 15K06695. (COI:No)

2P-057

Weak electric fields direct retinal ganglion cell axons *in vitro*

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Growing axons are directed by an extracellular electric field, known as galvanotropism. The electric field is a predominant guidance cue directing retinal ganglion cell (RGC) axons to the future optic disc during embryonic development. Specifically, the axons of newborn RGCs grow along the extracellular voltage gradient that exists endogenously in the embryonic retina (Yamashita, 2013). To investigate the molecular mechanisms underlying galvanotropic behavior, the quantification of the electric effect on axon orientation must be examined. In the present study, a culture system was built to apply a constant, uniform direct current (DC) electric field by supplying an electrical current to the culture medium, and this system also continuously recorded the voltage difference between the two points in the medium. A negative feedback circuit was designed to regulate the supplied current to maintain the voltage difference at the desired value. A chick embryo retinal strip was placed between the two points and cultured for 24 hours in an electric field in the opposite direction to the endogenous field, and growing axons were fluorescently labeled for live cell imaging (calcein-AM). The strength of the exogenous field varied from 0.0005 mV/mm to 10.0 mV/mm. The results showed that RGC axons grew in the reverse direction towards the cathode at voltage gradients of > 0.0005 mV/mm, and straightforward extensions were found in fields of > 0.2-0.5 mV/mm, which were far weaker than the endogenous voltage gradient (15 mV/mm). These findings suggest that the endogenous electric field is sufficient to guide RGC axons *in vivo*. (COI:No)

2P-058

Simulated roles of astrocyte in maintenance of ionic and volume homeostasis

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In response to perturbation of extracellular environment in the brain, astrocyte transport ions and water to maintain proper environment for neural activity. Extracellular K^+ concentration ($[K^+]_{out}$) in the brain increases in response to ischaemia, hypoxia, hypoglycaemia, seizures, and spreading depression and may lead to significant problems including cell death in the brain. High $[K^+]_{out}$ also induces swelling in astrocytes, leading to cytotoxic edema and cell death. Failure of pH regulation results in further cell death. Astrocytes are thought to maintain $[K^+]_{out}$, extracellular volume and pH homeostasis to prevent cell death. Contrary to neurons, astrocytes exhibit on depolarisation and/or increase of extracellular K^+ (as a result of neuronal activity) an alkaline shift called as depolarization-induced intracellular alkalinization (DIA). Previously, we constructed astrocyte models by incorporating various mechanisms such as intra/extracellular ion concentrations and models of ion channels and transporters, and showed a simulation analysis of $[K^+]_{out}$ and volume homeostasis. In the present study we incorporated new models of pH related transporters (NBCe1-C, NHE1 and V-ATPase) into the previous models. The present model reproduced astrocytic pH regulation, such as DIA under high $[K^+]_{out}$ and revealed controversial mechanisms of astrocytic pH regulation. (COI:No)

2P-059

Evoked inward currents in trigeminal ganglion neurons caused by intercellular transmitters, ATP, released from mechanically stimulated odontoblasts

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Various stimuli applied to the dentin surface elicit membrane deformation in odontoblasts (OBs) via dentinal fluid movement, which activates ATP release via pannexin-1 channels in response to activation of mechano-sensitive TRP channels. The released ATP functions as a neurotransmitter and activates $P2X_3$ receptors in pulpal-trigeminal ganglion (TG) neurons to establish intercellular OB-TG neuron communication, which mediates the transduction of sensory signals from dentin. In the present study, we investigated the detailed electrophysiological properties of OB-TG neuron sensory transduction by recording inward currents in TG neurons caused by intercellular transmitters released from mechanically stimulated OBs. The OB and TG neuron were isolated from neonatal rats, primary cultured, and subjected to OB-TG neuron co-culture system. Mechanical stimuli were applied using a glass pipette filled with standard extracellular solution. Evoked inward currents in TG neurons were recorded by whole-cell patch-clamp method. When mechanical stimuli were applied to OBs, evoked transient inward currents in both large diameter (>25 μm) and small diameter (<25 μm) TG neurons were recorded. These inward currents were inhibited by NF110, a $P2X_3$ receptor antagonist. We conclude that OBs establish neurotransmission with large TG neurons (i.e. A neurons) via ATP to mediate sensory signal transduction in dentin. (COI:No)

2P-060

Optogenetic dissection of multiple signals in single cerebellar granules cells in vivo

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Tactile signals from the orofacial area are delivered to the cerebellar cortex directly from the trigeminal nuclei as well as indirectly from the pontine nuclei that relay signals from the cerebral somatosensory cortex. Although both of these pathways form mossy fibers that project onto granule cells, it is not known whether two pathways make converging inputs to the same population of granules cells or parallel inputs to separate population of granule cells. Here, using transgenic mice (VGAT-ChR2 mice, 3-8 weeks-old) expressing modified channelrhodopsin-2 (hChR2-H134R) fused with YFP in GABAergic interneurons, we optogenetically inactivated the cerebral cortex in vivo in a temporally specific manner while recording from single cerebellar granule cells with a whole-cell patch-clamp method. We found that, in a significant proportion of granule cells in the crus II area, tactile stimulation evoked both fast and slow responses and that only the slower response was suppressed by the optogenetic inactivation of the somatosensory cortex, indicating that the slow component was the cerebro-pontine inputs while the fast component was the trigeminal ones. Accordingly we also found that the magnitude of slow component was affected by spontaneous fluctuation of the cerebrocortical activity. These results indicate that the direct and the indirect pathways converge onto individual granule cells. Such circuit structure may have important significance in physiological function of the cerebellum, including motor control. (COI:No)

2P-061

All-optical approach to study signal integration in the somatosensory cortex of mouse

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Spatiotemporally patterned inputs in layer IV are integrated to generate complex sense of form, size, movement or texture in the somatosensory cortex. To understand how neurons in other layers are involved in the integration and modification of the primary inputs, we developed an all-optical system which enables one to apply patterned stimulation optogenetically and to image Ca^{2+} signals from multiple neurons distributing across layers *in vivo*. To make this system, we solved the following three issues. First, the neurons should express either channelrhodopsin or Ca^{2+} sensor. We used a transgenic mouse expressing G-CaMP7 in which ChRFR gene was introduced in layer IV neurons using *in utero* electroporation. Second, the neurons should be imaged *in vivo* through multiple layers. The neurons were visualized across the layers using a microprism under two-photon microscopy (Andermann et al., 2013). Third, the ChRFR-expressing neurons should be irradiated with a certain spatiotemporal pattern. We fabricated new optical system which focuses a patterned signal of light on the imaging plane through objective lens. It is suggested that our system would facilitate the all-optical investigation of the neural computation in the cortex. All animal experiments were approved by the Tohoku University Committee for Animal Experiments. Y.H. is supported in part by Takeda Pharmaceutical Co. Ltd. and Fujitsu Laboratories. (COI:Properly Declared)

2P-062

Analysis of novel functions of an *atonal* homolog in aversive behavior and gap junction formation

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atonal (*atoh1* or *math1*) is a widely conserved transcription factor, and it plays important roles in the developing brain. Human homolog ATOH1 is expressed in the nociceptive pathway, such as the spinal cord, the cerebellum, the frontal cortex, the cingulate cortex, and the temporal cortex, suggesting its involvement in nociception and aversive behavior. Such phenotypes, however, have never been characterized, because null mutation causes death in mammals. Then, we analyzed *atonal* homolog *lin-32* of *C. elegans* as a model. Their neural circuit is most thoroughly described, and null mutants can survive. So far, we found that *lin-32* is involved in aversive behavior through regulating expression of the gap junction channel in the central nervous systems. Since both *atoh1* and gap junctions concern to hair cell functions in mammals, such regulation may be conserved. Moreover, *lin-32* mutants showed a delayed calcium response in the downstream motor neurons, and mimics of the delay using optogenetics could reproduce the aversive behavior defects. The motor neurons project neck muscles, which contract to return. We also found reduced calcium concentration of the muscles in the mutants, suggesting the defect in contracting to return. Our results suggest that gap junctions determine the timing of motor neuron response and contraction of muscle strength. Our finding will clarify the prototype circuit of molecules, neurons, and muscles of aversive behavior. (COI:No)

2P-063

Neuropsin dependent behavioral tagging

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Synaptic plasticity is widely accepted to provide a cellular basis for learning and memory. Synaptic associativity could be involved in activity-dependent synaptic plasticity, because it distinguishes between local mechanisms of synaptic tags and cell-wide mechanisms that are responsible for the synthesis of plasticity-related proteins. An attractive hypothesis for synapse specificity of long-term memory (LTM) is synaptic tagging: synaptic activity generates a tag, which captures the plasticity-related proteins derived outside of synapses. Previously we have been reported that neuropsin, a plasticity-related extracellular protease, was involved in synaptic tag setting. In the present study, we tested the hypothesis that neuropsin was engaged in behavioral tagging for LTM in vivo. Behaviorally, weak training, which induces short-term memory (STM) but not LTM, can be consolidated into LTM by exposing animals to novel but not familiar environment 1 h before training. We found that neuropsin deficient mouse impaired such transformation short-term into long-term memory. These results suggest neuropsin as a tagging in vivo. (COI:No)

2P-064

Cell division in neurons isolated from the adult rat cerebral cortex

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Since Ramon y Cajal (1928) concluded that there is no possibility for regeneration of neurons in the adult brain and spinal cord, it has long been recognized that neurons in mammals complete cell division during embryonic and neonatal life and thereafter they differentiate and do not divide any more. While neural stem or progenitor cells are currently known to exist in the postnatal brain and produce neurons *de novo*, the differentiated neurons are still believed not to divide. Contrary to this belief, the present time-lapse imaging showed that adult rat neurons, isolated from the cerebral cortex and cultured in neurobasal medium supplemented with B-27 and L-glutamine, are dividing. Quantitative analysis revealed that 17.6 &plusmn 1.4% (n=233 time-lapse samples) of neurons divided during 12 h and that the mean division interval was 21.9 &plusmn 0.7 h (n=90 dividing cells). These data were similar to those obtained from cultured fetal or newborn rat neurons derived from various central nervous regions. The divided cells were identified as neurons, since they were positive for neuronal markers (MAP2 [microtubule-associated protein 2] or Tuj1 [Neuron-specific class III beta-tubulin]) but not for the astrocyte marker (GFAP [glial fibrillary acidic protein]) or the oligodendrocyte marker (O4). These results suggest that adult rat cerebral cortical neurons have the ability of cell division under physiological culture conditions. (COI:No)

2P-065

Bilateral spinal potentiation induced by NO after hemilateral and transient ischemia applied to the mouse hindpaw

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We have reported that spinal and cortical responses, observed using flavoprotein fluorescence imaging and elicited by vibratory hindpaw stimulation, were bilaterally potentiated during and after the ischemic treatment applied only to the left hindpaw in mice. In the present study, we investigated a possible role of nitric oxide (NO), one of the diffusible mediators that may induce neuropathic pain in the spinal cord. We applied L-NAME, an inhibitor of NO synthase (NOS), intrathecally and the spinal or cortical potentiation during ischemia was clearly suppressed. The spinal or cortical potentiation was not observed in neural NOS knockout mice. Furthermore, application of NOR3 alone potentiated cortical responses. Mechanical allodynia was also observed after NOR3 application. We also observed diffuse NO formation during ischemia covering both sides of the spinal cord using DAF-FM, a fluorescent NO indicator. Taken together, these results indicate that NO induces bilateral spinal potentiation and neuropathic pain after ischemic treatment. The significant effects of NO were clearly observed in mice, possibly because the small size of the mouse spinal cord of mice may facilitate the effects of diffusible substances. (COI:No)

2P-066

Activity-dependent modulation of GABAergic synaptic currents in neonatal rat hippocampus.

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$GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) modulate excitatory synaptic transmission by altering the excitability of principal neurons. Although one group reported that in neonatal hippocampus, repetitive depolarization of postsynaptic neurons facilitates or suppresses inhibitory synaptic transmission, polarity and mechanism of the plasticity have not been fully established. In the present experiments, effect of the short repetitive postsynaptic depolarization on spontaneous GABAergic currents was determined in acute slices of neonatal rat hippocampus. Pharmacologically isolated spontaneous IPSCs were recorded by whole-cell patch clamp method. Depolarization of postsynaptic neurons alone caused marked alteration of neither frequency nor amplitude of spontaneous IPSCs. Simultaneous activation of presynaptic and postsynaptic neurons transiently inhibited the frequency of the spontaneous IPSCs. This inhibition was prolonged in the absence of an antagonist of NMDA receptor. Antagonists of metabotropic glutamate receptors reduced the inhibition of the IPSCs, though exogenous application of agonist of these receptors could not mimic the effect of presynaptic activation on the IPSCs. An antagonist of CB1 receptor was also effective to prevent the inhibition of the IPSCs. These results suggest that pre- and post-synaptic activation transiently inhibits the spontaneous IPSCs and that metabotropic glutamate receptors and CB1 receptor probably participate in this inhibition. (COI:No)

2P-067

STN-HFS induced the D_1-receptor activation dependent IPSC-LTP at Substantia Nigra pars reticulata (SNr) GABA neurons in the slices from reserpinized rat.

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A deep brain stimulation (DBS) are known as an effective therapeutics for a progressed Parkinson"s disease patient. Instead of DBS, a high frequency electrical stimulation onto a subthalamic nucleus (STN-HFS) was applied in the slices including the midbrain. It induced the IPSC-LTP at SNr GABA neuron in the half of neurons tested. In the slices from reserpinized rats (an acute animal model of Parkinson disease), no IPSC-LTP was induced in the solution with 100 μM α-methyl-L-tyrosine, a tyrosine hydroxylase inhibitor. The normalized amplitude of IPSC at 120 min after STN-HFS was 0.889 ± 0.099. This value was not significantly different from the control before STN-HFS ($n = 10$, $p = 0.305$). In the solution with 5 μM SKF38393, a D_1-dopamine receptor agonist, STN-HFS induced the IPSC-LTP in 6 out of 9 neurons. At 120 min after HFS, the IPSC was significantly increased in its amplitude. The normalized amplitude was 1.4588 ± 0.11066 ($n = 6$, $p = 0.00895$). This increase in the amplitude of IPSC was accompanied with the increase in sIPSC frequency at 120 min after STN-HFS, indicating that the presynaptic mechanism induces the IPSC-LTP. This and reported results might suggest that IPSC-LTP and -LTD induced by STN-HFS are dependent the activations of D_1- and D_2-dopamine receptor, respectively. (COI:No)

2P-068

N-glycosylation of AMPA receptor play a key role in synaptic plasticity

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The intracellular molecular mechanisms underlying the regulation of the AMPA receptor have been dramatically elucidated in the past few decades. In contrast, the regulation of the extracellular domain remains unclear. Here, we focused on N-glycosylation of the AMPA receptor in the extracellular domain and tried to clarify their functions by combining molecular biological and electrophysiological techniques. We previously reported that 401 asparagine residues (N401), putative N-glycosylation site, in GluA1 subunit might be a responsive site for drastic changes of glutamate responses of AMPA receptor from desensitization to re-sensitization. In the present study, we will report a physiological role of N-glycosylation in AMPA receptor-mediated synaptic transmission. For this aim, we attempted to express N401Q-substituted GluA1 in hippocampus CA1 region of GluA1 knockout (KO) mice by lentivirus infection. In the hippocampal slice, excitatory post-synaptic currents (EPSCs) elicited by electrical stimulation of Schaffer collateral did not show the re-sensitization, and moreover, miniature EPSC analysis revealed no significant differences in some physiological parameters including the decay time between wild-type and N401Q-substituted GluA1. However, long-term potentiation (LTP) induction in N401Q-substituted GluA1 was not possible to maintain its potentiation. These results are suggested that N-glycosylation of AMPA receptor play a key role in synaptic plasticity. (COI:No)

2P-069

Perineuronal nets regulate GABAergic synaptic transmission in the deep cerebellar nuclei

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Perineuronal nets (PNNs), composed mainly of chondroitin sulfate proteoglycans (CSPGs), are the extracellular matrix that surrounds cell bodies, proximal dendrites, and axon initial segments of adult CNS neurons. PNNs are known to regulate neuronal plasticity, although their physiological roles have not been fully elucidated. Here, we examined whether PNNs play a role in regulating GABAergic synaptic transmission of Purkinje cells onto deep cerebellar nuclei (DCN) glutamatergic neurons enwrapped by PNNs. We depleted CSPGs in acute cerebellar slices from 18 to 25 days old mice by incubating with chondroitinase ABC (chABC) for more than 3 hours. We then recorded IPSCs from DCN neurons by whole-cell voltage-clamp recordings. Depletion of CSPGs increased the amplitude and rise time constant of evoked IPSCs, and reduced the paired-pulse ratio. ChABC-treatment also robustly facilitated spontaneous IPSCs, and increased the frequency of miniature IPSCs without changing the amplitude. These results suggest that depletion of CSPGs in the DCN enhances GABA release from presynaptic terminals of Purkinje cells. Therefore, PNNs of DCN glutamatergic neurons suppress GABAergic synaptic transmission and may restrict neuronal plasticity in the DCN. We will also present data for roles of PNNs in the cerebellar interpositus nucleus in delay eyeblink conditioning. (COI:No)

2P-070

Wave-like propagation of the infra-slow oscillation over the rat cortex

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Distinct rhythmic oscillations of the brain activity at various frequency ranges are recognized, and neural basis of many of these oscillations have been well characterized. However, the nature and origin of the infra-slow oscillation (about 0.1Hz) is not well understood. It has been suggested that the infra-slow oscillation reflects slow blood-flow dynamics in the cortex. Here, we report that an oscillation at that slow range was detected by the intrinsic signal imaging technique, which propagated like a stable wave over the anesthetized rat cortex. The rat was anesthetized with isoflurane, and the intrinsic signal was detected through a large cranial window covering both hemispheres. The intrinsic signal showed a broad wave-like pattern with 1 - 2 peak and trough cycle(s) over the cortex, which propagated mostly along the rostral-caudal direction at 6 - 7 sec/cycle. The intrinsic signal likely reflects the dynamics of O_2hemoglobin, because the signal modulation was clearly detected with the light of 620nm or longer. Consistent with the idea that the signal reflects the local oxygen demand by the brain tissue, high-frequency whisker stimulation caused a temporal increase of the signal over the barrel cortex. Repeating the whisker stimulation with the interval of 6 sec could successfully phase-lock the wave-like propagation of the signal. Importantly, stimulation with shorter or longer intervals failed to entrain the wave propagation. The fact may reflect biological constraint(s) of the wave-generating mechanism, e.g., fixed time-constants of the dilation of the blood vessels and limited blood flow volume. (COI:No)

2P-071

Rapid synaptic plasticity within 5 min: experienced episode increases diversity of excitatory and inhibitory synapses in the hippocampal CA1

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The hippocampus plays a central role in encoding of memory. Since the memory of a strong episode strengthens both excitatory and inhibitory CA1 synapses, each CA1 neuron shows high diversity of post-synaptic currents (Mitsushima et al., Nature Commun, 2013). In the present study, to examine dynamic change of these synapses after experience of the episode, we examined a miniature excitatory and inhibitory post-synaptic current (mEPSC and mIPSC) and a paired pulse ratio (PPR) at the 0, 5, 10, 20 and 30 min after presentation of episode. As a learning model, we employed inhibitory avoidance (IA) task, and acute brain slices were prepared for patch clamp analysis. Untrained rats showed relatively small mEPSC and mIPSC amplitudes with low diversity of post-synaptic currents. Episode of IA training significantly increased mEPSC and mIPSC amplitudes at the 5, 10, 20 and 30 min after experience. Moreover, both mIPSC frequency and PPR of evoked IPSC increased immediately after the episode (at 0 min), while neither mEPSC frequency nor PPR of evoked EPSC changed. Bath treatment of CNQX (an AMPA receptor antagonist, 10 μM) or bicuculline methiodide (a $GABA_A$ receptor antagonist, 10 μM) consistently blocked the mEPSC or mIPSC responses, respectively. These results suggest that rapid decrease in presynaptic GABA release and following post synaptic strengthens of $GABA_A$ receptor- and AMPA receptor-mediated synapses after the IA episode. (COI:No)

2P-072

GABA signaling during degeneration and regeneration of mouse sciatic nerve.

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In the adult central nervous system (CNS), GABA is an inhibitory neurotransmitter, whereas GABA acts as an excitatory transmitter in the immature CNS. In the present study, to investigate GABAegic involvement during the axonal degeneration and regeneration, we morphologically examined changes in GABAergic signaling in the spinal cord of sciatic nerve ligation animal model. We performed immunohistochemistry for glutamic acid decarboxylase (GAD) as the marker for GABA neurons, vesicular GABA transporter (VGAT) as a marker for GABAergic terminal, and K^+ -Cl^- cotransporter (KCC2) as a marker for GABAergic inhibition. Sciatic Function Index (SFI) was also used as a limb motor function evaluation. We found that (1) SFI was significantly decreased 3days after operation of sciatic nerve, (2) SFI was recovered at day 28, (3) KCC2 expression was decreased in the ventral horn of the sutured side at Day3, and gradually increased on Day 21 and 28. (4) In the dorsal horn, the level of KCC2 was decreased between Day3 and Day7. After that, KCC2 expression was increased. These results suggested that GABAergic action might change in both motor and sensory system after sciatic nerve injury. (COI:No)

2P-073

Visualization of HCN4-expressing neurons with GFP using *Tet*-expression system

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Hyperpolarization-activated cyclic nucleotide-gated (HCN1-4) channels are widely expressed in the central nervous system (CNS). Among 4 types of HCN channels, HCN1 and HCN2 in CNS are reported to participate in synaptic integration, epilepsy, and nociception. Although HCN4 is a major subtype expressed in cardiac pacemaker cells, physiological function of HCN4 in CNS is not well understood. In order to explore this question, we tried to visualize the expression pattern of HCN4 in living brain. For this purpose, we generated a knock-in (KI) mouse, incorporating the tetracycline-controlled trans-activator (tTA) gene and its responsive element (TRE) between the translation initiation site of HCN4 gene and its upstream promoter region ($HCN4^{+/tTA\_TRE}$; Nakashima et al., J.Physiol.,591, 1749-69, 2013). By crossing this KI mouse with another transgenic mouse, which carries the TRE-GFP cassette, we have successfully visualized GFP-positive, HCN4 expressing neurons in thalamic sensory pathways, motor systems of basal ganglia, cerebellum, and limbic system. We concluded that $HCN4^{+/tTA\_TRE}$ is a useful tool to study the physiological role of HCN4 in brain. (COI:No)

2P-074

Mapping of neurons that send direct synaptic input to histaminergic neurons in the mouse brain

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The histaminergic system in the mammalian brain is involved in multiple homeostatic functions including the regulation of sleep/wake cycle and body temperature. The diverse effects are exerted by widespread projection of the histaminergic neurons located in the tuberomamillary nucleus (TMN). In order to understand how the system modulates and integrates the physiological functions, it is essential to characterize the neuronal circuits controlling the histaminergic system. To identify the input neurons that are mono-synaptically connected with the histaminergic neurons, we employed a newly-developed retrograde tracing method based on genetically modified rabies viruses that are trans-synaptically and retrogradely transported and express the fluorescence protein, EGFP. Using this method, we could find the EGFP-labeled neurons distributed widely in the mouse brain. In the vicinity of the TMN, preoptic area, anterior/posterior hypothalamus and mammillary body are the main regions possessing the dense population of the labeled neurons. In addition, other neurons projecting over a long distance are also found in several areas. To characterize the labeled neurons, we conducted histological and electrophysiological studies. As one example, in the ventrolateral preoptic nucleus, we identified the GABAergic neurons that were hyperpolarized by 5-HT and NA. Thus, these approaches uncovering the properties of input layers would help to clarify the brain functions that the histaminergic system controls. (COI:No)

2P-075

Characterization of the Neuronal Circuits in the Spinal Dorsal Horn of Peripheral Nerve-injured Mice

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Peripheral nerve injury induces permanent changes in local neural circuits in the spinal dorsal horn, which might be associated with neuropathic pain. In the present experiment, we characterized the synaptic circuits in the spinal dorsal horn of peripheral nerve-injured mice by using a cross-correlation analysis of the spike trains. Experiments were performed on 6- to 8-week-old male ICR mice. Spinal cord slices were prepared 10 days after the sciatic nerve was partially ligated under halothane anesthesia. The slices were placed into a recording chamber with the MEA (Multi Channel Systems, Germany). Electrodes were arranged 30-200 μm apart. Single-unit spike trains were sorted from the recordings, and cross-correlograms between spontaneous spike trains were constructed using DataView (Heitler, 2009). The occurrence of a central peak in the cross-correlogram, which is suggestive of common excitatory inputs to the two neurons in the pair, was significantly higher in sciatic nerve-ligated mice than in sham-operated control mice. Additionally, the occurrence of a lagged trough in the cross-correlogram, which is suggestive of monosynaptic inhibitory action from one neuron to the other neuron in the pair, was decreased in sciatic nerve-ligated mice. The observed changes in the occurrence of these shapes of cross-correlogram might provide additional evidence for the synaptic rearrangement in the spinal dorsal horn following peripheral nerve injury, and might be associated with the maintenance of peripheral nerve injury-induced neuropathic pain. (COI:No)

2P-076

Refractory period of somatosensory evoked potential dynamically changes under anesthesia

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Evoked potentials have been used for many studies as an indicator of the neural activity to the sensory input under both conscious and anesthetic conditions. When we apply shorter inter-stimulus interval, we could obtain more evoked responses. It may however cause inaccuracy of the observations due to the refractory period of the sensory system. To find the optimum inter-stimulus interval of somatosensory evoked potential under anesthesia -that should be longer than the refractory period-, we measured the evoked potential by using double consecutive stimulation with three different inter-stimulus interval (300, 600 and 900 ms) under sevoflurane anesthesia in SD rat. We also confirmed the stability of the optimum inter-stimulus interval under two different anesthetic depths (1 and 1.5 mean alveolar concentration. Small screw electrodes were placed for evoked potential measurement in male rat skull under sevoflurane anesthesia. Two consecutive electrical stimulations were applied to the upper limb, and first negative peak amplitude ratio of the first somatosensory evoked response (R1) and second response (R2) were calculated (R2/R1). While the inter-stimulus interval is 900 ms, R2/R1 was stable regardless of the anesthetic depth. On the other hand, when the inter-stimulus interval is 300 ms, R2/R1 dynamically changed in response to the depth of anesthesia. These results suggest that the refractory period of the somatosensory system dynamically changes with the anesthetic depth and we may need to set the inter-stimulus interval of somatosensory evoked potential as longer than 1 s under anesthesia. (COI:No)

2P-077

Systemic energy states regulate NMDA receptor-mediated synaptic plasticity onto oxytocin neurons in the hypothalamic paraventricular nucleus.

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It has been shown that synaptic transmission onto the neurons in arcuate nucleus is modulated by peripheral feeding regulatory hormones, and that this modulation of synaptic plasticity regulates feeding. Recent studies have shown that oxytocin (Oxt) neurons in the paraventricular nucleus (PVN) of the hypothalamus serve as 2nd order neurons to integrate peripheral and central signals and thereby induce satiety. However, it remains unclear whether metabolic energy states regulate the synaptic transmission on Oxt neurons in PVN. We investigated the synaptic transmission onto Oxt neurons under fasted/fed states. The excitatory postsynaptic currents (EPSCs) mediated by AMPA type and NMDA type glutamate receptors were increased under fed, compared to fasted states. AMPA/NMDA current ratio of evoked EPSC was significantly smaller in fed than fasted states. In Oxt neurons, dynein light chain 2 (DYNLL2), a protein implicated in the NMDA receptor trafficking to the postsynapses, was increased under fed, compared to fasted states. The present results suggest that fed condition increases excitatory synaptic input on Oxt neurons via mechanisms involving DYNLL2 upregulation and NMDA receptor-mediated synaptic reorganization, a process possibly implicated in production of satiety. (COI:No)

2P-078

Large-scale somatotopic reorganization with afferent fiber remodeling in the mice whisker sensory thalamus after peripheral sensory nerve injury

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Peripheral sensory nerve injury causes large-scale functional reorganization in the brain. However, neural circuit mechanisms underlying the reorganization have not been sufficiently explained yet. Using a transgenic mouse line in which the whisker sensory principle trigeminal nucleus(PrV2)-origin lemniscal fibers are specifically labeled, we found that transection of the whisker sensory nerve largely reorganized somatotopic information along with remodeling of lemniscal fibers: PrV2-origin lemniscal fibers were retracted and non-PrV2-origin ones invade the V2 VPM. Origins of the non-PrV2-origin lemniscal fibers included the mandibular (V3) subregions of trigeminal nuclei and the dorsal column nuclei, which normally represent body parts other than whiskers. The transection also induced ectopic receptive fields of V2 VPM neurons and mechanical hypersensitivity on the V3 region on the face. The lemniscal fiber remodeling, ectopic receptive fields, and mechanical hypersensitivity all concomitantly developed within a week and lasted over three months. This spatial and temporal consistency strongly suggest a close link between the lemniscal fiber remodeling and the in vivo functions. Threfore, our results shed light on understanding of the large-scale reorganization beyond somatotopic border after peripheral nerve injury. (COI:No, This work is supported by JSPS KAKENHI Grant Number 26290010,15H01667.)

2P-079

Effects of biogenic amines on the spontaneous neuronal activity of in vitro synchronous oscillatory network in the slug

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Biogenic amines play important roles for memory acquisition in mammalian olfaction and regulation of synchronous oscillatory activity in the brain laminar structure. Synchronous oscillatory activity in a laminar structure is common in the olfactory system of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC) and its frequency changes are suggested to encode the olfactory information and memory. Biogenic amines are known to change the oscillatory frequency in the PC, and are the candidates of the neurotransmitters or neuromodulators that are involved in such higher cognitive functions. We recently found that oscillatory neuronal network was formed from dispersed cell culture of PC neurons. In the present study, we thus examined whether histaminergic and octopaminergic system are present in cultured PC neuronal network or not. First, increases in neurite arborization and neurite connection were observed after a week in culture. Second, using calcium imaging for each PC neurons, histamine or octopamine were bath-applied to examine whether the number of spontaneous calcium transients and its synchronous oscillatory activity are changed. These results may suggest that biogenic amines can function as a transmitter/modulator in cultured PC neuron network, and furthermore, amines play common roles for synchronous oscillation in the olfactory neuron network of both vertebarates and invertebrates. (COI:No)

2P-080

Generation of animal models of spinocerebellar ataxia by AAV9-mediated gene delivery

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Spinocerebellar ataxia (SCA) is hereditary disease showing progressive neurodegeneration. For past 2 decades, generation of various SCA model mice and their examination lead to extensive understanding of the pathology and potential therapies. As yet, no therapy has proceeded to the clinic. One major reason may be absence of non-human primate (NHP) model of SCA, by which scientists can assess the effectiveness of possible therapies for future clinical use. In this study, we aimed to generate marmoset models of SCAs by virally expressing pathogenic gene casing SCAs.Using adeno-associated virus serotype 9 (AAV9) vectors, we first produced SCA1 model mice, since assessment of behavioral phenotypes has been already established. The ATXN1 and ATXN3 genes having an expanded CAG repeat (ATXN1[Q123] and ATXN3[Q89]) were used as the pathogenic genes for SCA1 and SCA3, respectively. Mice injected with AAV9 vectors expressing ATXN1[Q123] and those expressing ATXN3[Q89] showed progressive ataxia and extensive neuronal cell death in deep cerebellar nuclei, whereas mice treated with AAV9 expressing ATXN3[Q15] are almost indistinguishable from PBS-injected control mice. Then, we proceeded to generate marmoset models by cerebellar injection of the AAV9. The marmosets virally expressing ATXN1[Q123] or ATXN3[Q89] showed significantly poorer performance in two different behavioral tests, compared with a marmoset that expressed ATXN3[Q15] or naive marmosets. These results suggest that NHP models of SCA can be successfully generated by AAV vector-mediated gene delivery. (COI:No)

2P-081

Protein synthesis-dependent morphological plasticity and maternal memory

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The behavior of mother animals to increase the survival probability of their pups is collectively called maternal behavior. The maternal behavior of rodents includes active one such as the retrieval of scattered pups to the nest and passive one such as taking crouching posture to nurse the pups. Such behavior is not seen immediately in virgin females but quickly acquired after parturition and maintained long, even if contact with pups is suspended. Hence, the maintenance of maternal behavior is regarded as a typical form of long-term memory and called maternal memory. In general, the long-term memory is assumed to depend on protein synthesis and structural changes of neural circuit. To confirm that the maternal memory depends on protein synthesis, we applied intravenricularly a protein synthesis inhibitor anisomycin to dams immediately after parturition. The application on 3 consecutive days strongly suppressed the dams' pup-retrieving behavior. Single applications on postparturition day 1, 2 or 3 also suppressed the behavior partially. To confirm that the maternal memory is coupled with structural plasticity, we examined the density of dendritic spines in the nucleus accumbens (NAc) and the basolateral amygdala that are supposed to be relevant to the release of maternal behavior. At the postparturition day 15, the spine density was increased in the core and shell of NAc. The examination of structural changes in the ventral tegmental area and the hippocampus, the regions having contact with NAc, is presently under way. (COI:No)

2P-082

Congenital nystagmus gene FRMD7 is necessary for establishing a neuronal circuit asymmetry for direction selectivity

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Neuronal circuit asymmetries are important components of brain circuits, but the molecular pathways leading to their establishment remain unknown. Here we found that the mutation of FRMD7, a gene which is defective in human congenital nystagmus, leads to the selective loss of the horizontal optokinetic reflex in mice, as it does in humans. This is accompanied by the selective loss of horizontal direction selectivity in retinal ganglion cells and the transition from asymmetric to symmetric inhibitory input to horizontal direction-selective ganglion cells. In wild type retinas, we found FRMD7 specifically expressed in starburst amacrine cells, the interneuron type that provides asymmetric inhibition to direction-selective retinal ganglion cells. This work identifies FRMD7 as a key regulator in establishing a neuronal circuit asymmetry and suggests the involvement of a specific inhibitory neuron type in the pathophysiology of a neurological disease. (COI:No)

2P-083

The role of inhibitory neurons in the primary somatosensory cortex in chronic pain

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Inhibitory neurons play critical roles in maintenance of brain function. Abnormality of these neuronal activities contributes to several diseases. In the primary somatosensory cortex (S1), which code intensity and location of pain, inhibitory GABAergic neurons in the S1 project to S1 excitatory neurons and can attenuate excessive excitation of excitatory neurons. Under chronic pain conditions, excitatory neuronal activities in the S1 increase and cause chronic pain. However, little is known about how inhibitory neurons in the S1 modulate excitatory neuronal activities and pain behavior under chronic pain conditions. Using two-photon calcium imaging and electrophysiological methods, we found that inhibitory neuronal activities increased in the S1 in inflammatory chronic pain. K-Cl cotransporter expression decreased in the S1 excitatory neurons and this reduction resulted in inhibition being less efficacious. Thus, although there is net increase in inhibition within S1 cortical circuit, it is not enough to balance the enhanced excitatory neuronal activities and prevent chronic pain behavior. (COI:No)

2P-084

Functional and morphological differentiation of retinal ganglion cells from human iPS cells

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We generated self-induced retinal ganglion cells (RGCs) with functional axons from human iPS cells. RGCs induced from ES cells and iPS cells in previous studies carried markers characteristic of RGCs, but were not accompanied by long axons. In this study, after development of the optic vesicle from the induced stem cell embryoid body in three-dimensional culture, conversion to two-dimensional culture, achieved by supplementation with BDNF, resulted in differentiation of RGCs at a rate of nearly 90% as indicated by a marginal subregion of an extruded clump of cells, suggesting the formation of an optic vesicle. RGCs observed had long, prominent axons expressing NFs, Tuj1, and tau and growing in straight lines on the culture plate. Quantitative PCR and immunohistochemistry results showed a gene expression profile and protein markers characteristic of RGCs, including Brn3b, Math5, Islet1, Sncg, and Tuj1. The physiological function of the axons was demonstrated by sodium-dependent action potentials using patch-clamp techniques and observation of axonal flow. Anterograde axonal transport was identified by induction of both NTRK1- and mitochondria-specific vital stains and cholera toxin B into cell bodies. (COI:No)

2P-085

Spatial population dynamics of lateral interaction in superficial superior colliculus

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The superior colliculus (SC) is a brainstem center which plays a key role in mediating the signal for sensory-motor translation. It is hypothesized that spatial integration of visual information in its superficial layer (sSC) is important for controlling spatial attention; however, the way how it is implemented in neural circuits remains elusive especially at the neuronal population level. Therefore, we applied an *in vivo* two-photon Ca^{2+} imaging to the mouse sSC, and analyzed spatial dynamics of visually evoked neuronal population activity. Two-point experiment showed mutual facilitation by smaller separation between small two stimuli (< 3°, 6°). On the other hand inhibitory effect was observed when the two stimuli were presented with large separation (> 9°).We also tested surround suppression by using five different stimulus sizes (0.5°, 1°, 3°, 5°, 10° in diameter). Significant response decrease around the response center was observed by large stimulus (> 5°). Both excitatory and inhibitory neurons showed quite similar spatial response pattern of surround suppression. Our results suggest that remote long-range inhibitory connection is appropriate to account for the circuit mechanism of surround suppression in the sSC, rather than local inhibitory interaction, which was shown in mouse visual cortex. (COI:No)

2P-086

Neural mechanisms of long-term potentiation maintenance: in the case of synapses in the mouse accessory olfactory bulb

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Some urinary pheromones of an unfamiliar male mouse block pregnancy of the female exposed within 24 hours after mating. However, those of the mating male do not induce pregnancy block. The inability of the mating male to disrupt the pregnancy depends on the memory of his pheromones formed by the female. Previous reports have shown that the pheromonal memory depends on new protein synthesis and can last for 30-50 days. The pheromonal memory is based on the neural changes in the accessory olfactory bulb (AOB), the first relay in the vomeronasal system; the thickening of the excitatory synapse is observed in the pregnant female. Microcircuits in the AOB include the prominent reciprocal dendrodendritic synapse between mitral cells, a single class of projection neurons, and granule cell interneurons. At the AOB synapse, long-term potentiation (LTP) is expected to underlie the pheromonal memory. To elucidate the neural mechanisms, we focused on the possible mechanisms to maintain LTP at the AOB synapse. In the previous presentations, we have suggested that both new protein synthesis and actin polymerization underlie LTP maintenance at the AOB synapse. By adding the electrophysiological and pharmacological data on the crosstalk, we discuss the neural mechanisms to maintain long-term potentiation in the mouse accessory olfactory bulb. (COI:No)

2P-087

Cingulate presynaptic long term plasticity contributes to chronic pain induced anxiety

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Patients with chronic pain often suffer from affective disorders such as anxiety. Among several cortical regions, the anterior cingulate cortex (ACC) has been demonstrated to play important roles in sensory perception and emotional responses. Neurons in the ACC are activated by noxious sensory stimuli, and inhibiting central plasticity in the ACC produces analgesic effects in animal models of chronic pain. Interestingly, the ACC has also been implicated in anxiety. Human imaging studies observed increased ACC activity in patients with anxiety disorders and surgical lesions or chemical inactivation in the ACC produced anxiolytic effects in humans and animals. However, the molecular and cellular basis of anxiety and its interaction with chronic pain is not known.Here we characterized two forms of long-term potentiation (LTP), which is the major form of activity-dependent plasticity in the ACC; a presynaptic form (pre-LTP) that requires kainate receptors and a postsynaptic form (post-LTP) that requires N-methyl-D-aspartate receptors. Pre-LTP also involves adenylyl cyclase and protein kinase A and is expressed via a mechanism involving hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Interestingly, chronic pain and anxiety both result in selective occlusion of pre-LTP. Significantly, microinjection of the HCN blocker into the ACC produces both anxiolytic and analgesic effects. Our results provide a mechanism by which two forms of LTP in the ACC may converge to mediate the interaction between anxiety and chronic pain. (COI:No)

2P-088

Acid-sensing ion channel 3 is involved in muscular mechanical hyperalgesia after lengthening contractions in rats

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We previously reported that intramuscular injection of amiloride ameliorated muscular mechanical hyperalgesia induced by lengthening contractions (LC). This means that acid-sensing ion channels (ASICs) contribute to this type of pain, but a subtype of the channels remains to be identified. In the current study we investigated a subtype, ASIC3. In behavioral tests, mechanical withdrawal threshold of the muscle underwent LC was decreased as we previously reported. The decreased withdrawal threshold was significantly increased by intramuscular injection of APETx2, a selective blocker of ASIC3 channels (2.2 μM, 50 μl) 30, 60, and 120 min after injection, while APETx2 0.22 μM and its vehicle had no effects on the threshold. Single-fiber electrophysiological recordings were performed using the extensor digitorum longus muscle-peroneal nerve preparations in vitro. Mechanical response threshold of muscle C-fiber nociceptors was significantly increased 15, 30, and 60 min after APETx2. The response magnitude was significantly decreased when compared with the vehicle control. Intramuscular injection of APETx2 into the muscle without LC had no effect on the mechanical response of C-fibers. These results indicate that ASIC3 is involved in mechanical hyperalgesia after LC. (COI:No)

2P-089

Facilitated mechanical response of muscular nociceptors in an animal model of fibromyalgia induced by repeated cold stress

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Fibromyalgia (FM) is characterized by chronic widespread pain with a variety of symptoms such as sleep disorder, irritable bowel syndrome, depression, anxiety, disturbed higher brain function, etc. Physical and mental stress is assumed to be related to the onset and maintenance of FM. Central mechanisms have been strongly suggested in the etiology, however, peripheral components have never been intensively studied. Here we examined general characteristics and response properties of muscular nociceptors using an animal model of FM induced by loading repeated cold stress. One to two weeks after the stress exposure when behavioral hyperalgesia was obviously established, ex vivo single-fiber electrophysiological recordings were made using extensor digitorum longus muscle-common peroneal nerve preparations taken from rats euthanized by inhalation of CO2. Muscular C-fiber nociceptor was identified by the teased fiber technique. Mechanical response threshold was significantly decreased, and the response magnitude was significantly increased in a FM model. The responsiveness to cold and heat stimuli remained unchanged. No change was detected in the general characteristics (conduction velocity, spontaneous activity, and receptive field). These results suggest that augmentation in the mechanical response of muscular C-nociceptors contribute to mechanical hyperalgesia in a FM model. (COI:No)

2P-090

Oxytocin alleviates orofacial hypersensitivity following infraorbital nerve injury in rats.

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Oxytocin (OT) is a nine amino acid neuropeptide, which is synthesized in the hypothalamus and released into the bloodstream via pituitary gland. Recently, it has been reported that OT could modulate nociception and its underlying mechanism has not been elucidated. In this study, we examined the effect of OT on trigeminal neuropathic pain associated with partial ligation of the infraorbital nerve (IoN-PNL) in rats. The head-withdrawal threshold to mechanical stimulation (MHWT) of the maxillary whisker pad skin on the side ipsilateral to IoN-PNL was measured using von Frey filaments. OT (1 mM, 0.5 μl) was directly administrated to the trigeminal ganglion (TG) once after MHWT measurement on day 6 or 7 when MHWT significantly decreased. The significant recovery of MHWT was observed at 2 and 5 hrs after the OT administration compared with that of vehicle (PBS), while OT administration to sham rats did not show any significant changes in MHWTs. We also examined the effect of OT on the excitability of TG neurons acutely isolated from IoN-PNL rats. Ten μM of OT was applied to the culture medium 2-6 hrs before whole-cell patch-clamp recording. The resting membrane potentials of OT-treated TG neurons were significantly decreased. Threshold currents in OT-treated neurons for spike generation during current injection were also significantly greater than that of PBS. Present findings suggest that OT could be at least partially effective on the suppression of hyperexcitability of TG neurons and exert analgesia on orofacial neuropathic pain. (COI:No)

2P-091

Histamine affects voltage-gated outward currents in amacrine cells in the mouse retina.

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Mammalian retinae express histamine receptors. Recent immunohistochemical studies showed that histamine H1 receptor (HR1) was expressed in the mammalian amacrine cells. Here we investigated the effect of histamine on mouse amacrine cells, using the whole-cell version of the patch-clamp technique. Mouse retinae were sliced at 200 μm in thickness. The slice patch-clamp recording was performed at the inner nuclear layer of the retina. The amacrine cells were identified by the locations of the soma in the retinal layer and by the shapes of the fluorescence with injected Lucifer yellow. Under voltage-clamp conditions, the amplitude of voltage-gated outward currents was enhanced by the application of 100 μM histamine in 63% of amacrine cells (25.2 ± 6.2%; mean ± SEM, n = 21/33). Although these recorded cells were classified into seven types of amacrine cells, the narrow-diffuse type of the amacrine cells was significantly responded to histamine. Next, we identified subtypes of histamine receptors in the amacrine cells. We applied 100 μM ranitidine or clobenpropit, each antagonist of the histamine H2 or H3 receptor (HR2, HR3), to histamine-sensitive amacrine cells. These inhibitors reduced the effect of histamine. These results indicate that functional HR2 and HR3 exist in retinal amacrine cells, and HR1 is not the only receptor involved in the intracellular signaling of histamine in retinal cells. The processes of narrow-diffuse amacrine cell span all sublamina of inner plexiform layer in the retina. Therefore, histamine may play robust and important modulation in the visual processing. (COI:No)

2P-092

The breakdown of the symmetric signal processing by pathway-dependent synaptic inputs to cholinergic amacrine cells in the retina.

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The direction selectivity, a representative circuit of dendritic computation, is an attractive target in the retina. Currently, GABAergic inputs from the cholinergic amacrine cells (CA cells) to the direction selective ganglion cells are thought to be an essential factor for direction selectivity. However, there is no available data how the GABA release from the CA cells are regulated as the membrane properties of the CA cells have not been elucidated. In this study, we examined how CA cells are driven by the neurotransmitters such as ATP, glycine, or glutamate in ON-and OFF pathways. The OFF-CA cells were driven by ATP and glutamate but not by glycine. The ON-CA cells were driven by glycine and glutamate but hardly by ATP. Purinergic inputs in the OFF-CA cells were mediated by P2X2-purinoceptors and glycinergic inputs in the ON-CA cells were mediated by glycine receptors, presumably assembled by α4 subunits. Glutamatergic inputs were mediated by AMPA/KA and NMDA receptors. Thus, the OFF-CA cells and the ON-CA cells received synaptic inputs in a pathway-specific manner. We have reported that P2-purinoceptors modulate the firing activity of ON- and OFF-ganglion cells in the pathway-specific manner. The retina is asked to shift the threshold of ON- or OFF-pathway independently to adjust its visual activity to the wide range of light intensity. The presence of pathway-specific inputs to CA cells might favor for the pathway-specific adjustment of dynamic range to achieve the best visual acuity in the mouse retina. (COI:No)

2P-093

Functional evaluation of electrode allay for STS-type retinal prosthesis by single-unit activity

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Suprachoroidal Transretinal Stimulation (STS) is a novel retinal prosthetic system we have been developed, in which stimulating current is provided from scleral electrodes. We previously confirmed that, with single-type STS electrode, the retinal area activated by STS is limited near the electrode by single-unit recording study from relay cells in cat lateral geniculate nucleus. Here we evaluated the stimulated retinal area from the electrodes, which had the same dimension as used for clinical trials and formed allay arrangement, by single-unit activity in LGN relay neurons as previously.

STS electrode allay was implanted into the scleral pocket on the posterior of cat eyeball (n=4). The size of single electrode is 0.5mm in diameter and 0.3mm in height. More than 10 days after surgery, the cat was anesthetized and the single unit activities were recorded with biphasic stimulation of 100-1000μA and 0.5ms/phase. The receptive fields of recorded units were mapped on tangent screen and the positions of STS electrodes were overlaid using optical coherence tomography.

We recorded the burstic activities of relay neurons by STS. As the receptive field was near the STS electrode, the responses became more prominent. With the stimulation of 500μA, the area with more than 50% of response probability was limited within 1 mm from the STS electrode.

In conclusion, the activated area by STS from the electrode allay was limited as we previously studied for single-type electrode. (COI:Properly Declared)

2P-094

In vivo imaging of cortical map remodeling in a mouse model for peripheral nerve injury

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Peripheral nerve injury remodels the primary sensory pathway. However, little is known about how sensory peripheral nerve injury affects the cortical topographical map. To address this issue, we recorded intrinsic optical signal from mouse primary somatosensory cortex evoked by the tactile stimulation, following ligation of the whisker sensory nerve with absorbable surgical thread for several weeks. The tactile stimulation was applied onto whiskers and/or the mandibular part. The signal in the barrel area was mostly diminished by the whisker stimulation on post-operative day 7. On the other hand, the signal did not change when the mandibular part was stimulated. Interestingly, when both whiskers and mandibular part were simultaneously stimulated, a strong signal appeared in the dysgranular area surrounding the barrel cortex. This result is consistent with the c-Fos expression pattern in somatosensory cortex of the injured animals; c-Fos positive cells decreased in the barrel cortex, whereas increased in the dysgranular area. After losing retention strength of the thread, the signal in the barrel cortex reappeared and the strong signal in the dysgranular area restored to the normal level. As the dysgranular area is a part of the paralemniscal pathway, receiving input from the posterior thalamic nucleus, this result suggests that the injury of whisker sensory nerve can induce hyperactivity of the paralemniscal pathway when whiskers and the mandibular part are simultaneously stimulated. (COI:No, This work is supported by JSPS KAKENHI Grant No.15H01667, 15K21387.)

2P-096

Effect of different phase spectra on neuronal activities in the primary auditory cortex

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A time-domain of sound signal can be transformed into the amplitude spectrum and the phase spectrum. Many studies have demonstrated that the amplitude spectrum is a primary acoustic cue for timbre perception and the neural mechanism for processing the sound amplitude has been examined. While psychoacoustic studies revealed that the difference of phase spectrum could be perceived as difference of sound timbre, the cortical substrate for encoding the phase spectrum has seldom been reported. In the present study we prepared periodic sound sets in which each sound has the same amplitude spectrum but different phase spectra and investigated effects of the phase difference on neuronal activities in the primary auditory cortex (A1) of an awake cat. The phase spectrum was systematically changed by morphing between Schroeder and minimum phases. The fundamental frequency (F0) was also changed systematically from 25 to 400 Hz. The averaged driven rates of single A1 neurons to 5 different phase stimuli were compared in 5 different F0 conditions. Most of neurons showed phase-preferences and the sensitivities were larger in lower F0 conditions. It is consistent with the result of phase effect on the timbre of complex tones in human experiments. These results suggest that A1 neurons might play an important role for detecting the timbre difference caused by the phase difference. (COI:No)

2P-097

Emotional odour modulates response selection process-An ERP study

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The function to control and select behavioral responses is the cornerstone of adaptive human behavior. Recently, an increasing number of studies have shown the modulation of this function by emotional states. However, majority of the previous studies used visual or auditory stimuli for emotion induction, and little is known about the influences of emotional stimuli in other sensory modalities. The present study investigated the influence of emotional odorant (citral and valeric acid) on event-related potentials (ERPs) in executing Flanker-task, a well-established behavioral paradigm to measure ones executive function.The results have shown that exposure to unpleasant odour increased parietal P3b amplitude when the load of executive control was low. No effect of odour pleasantness on P3b was observed when high cognitive load was imposed. Because P3b amplitude is supposed to indicate the readiness for response execution, the present results seem to indicate that exposure to unpleasant odour potentiates response selection and execution. (COI:No)

2P-098

Tumor Necrosis Factor-Alpha antagonist improves functional recovery following olfactory system injury by suppressing local inflammation

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We previously reported that recovery in the olfactory system depends on the severity of injury and that treatment with anti-inflammatory drug as steroid is effective in improving recovery outcome. It is known that tumor necrosis factor-alpha (TNF-alpha) plays an important role in inflammatory reaction. To investigate if blockade of TNF-alpha is useful for neural regeneration and recovery, we transected the olfactory nerves along the surface of the olfactory bulb using a stainless steel blade and injected TNF-alpha antagonist (etanercept) which is used for clinical treatment of refractory inflammation as rheumatoid arthritis. Histological assessment of recovery within the olfactory bulb was made at 5, 14, 42 and 70 days after injury. We used X-gal staining to label the degenerating and regenerating olfactory nerve fibers and immunohistochemical staining to detect the presence of reactive astrocytes and macrophages. Etanercept-injected animals showed significant smaller areas of injury-associated tissue, less astrocytes and macrophages, and an increase in regenerated nerve fibers in a dose-dependent manner. Behavioral study using avoidance conditioning and electrophysiological study showed better functional recovery of olfactory system in etanercept-injected mice than in control animals. These findings suggest that etanercept can be useful as a therapeutic drug for olfactory dysfunction by head injury. (COI:No)

2P-099

Transition of the responsible area for abnormal behavior from peripheral nerve to CNS following sciatic nerve injury in rats

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The aim of this study is to clarify the period twhen CNS neuron has advantages for development of abnormal behavior following peripheral nerve injury (CCI). Abnormal behavior (flinching) was automatically detected and objectively evaluated using a MicroAct apparatus (Neuroscience, Tokyo, Japan). It was increased number of flinching after CCI. We recorded from WDR neurons in GN (gracile neucleus) following CCI of sciatic nerve. The lidocaine was applied to the dorsal column tract which is the bundle of the central axons of the dorsal root ganglion cell in order to block the inputs from the primary afferent sensory fibers. An antidromical spike of WDR neuron in GN was recorded following VPL stimulation and monitored while blocking the dorsal column tract. It could not find a difference of the reduction rate of neuronal activity between in rats at 3 days, 7 days after CCI and in naive rat. The reduction rate of WDR neuronal activity in rat 14days and 35 days after CCI were significantly decreased when compared to that in naive rat. At 35 days after CCI, the reduction rate became over 50% following a block of primary afferent input. It is suggested that GN neuron may take advantage for neuropathic abnormal sensation from 35 days after CCI. (COI:No)

2P-100

In vivo extracellular and patch-clamp analyses of sensory neuronal activities in the anterior cingulate cortex

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Recent studies suggest that anterior cingulate cortex (ACC) has an important role on emotional and motivational aspects of pain. However, how the detail neuronal mechanism of this pain processing is not fully understood. In this study, we investigated ACC neuronal activities in response to cutaneous sensory stimulation by using in vivo extracellular and patch-clamp recordings. Rats were deeply anesthetized with urethane and isoflurane under artificial ventilation, and then a small hole was opened in the skull to insert extracellular recording electrodes or patch pipettes above the ACC according to the stereotaxic coordinates. AAC neurons exhibited spontaneous action potentials. The frequency of the action potential is changed by the anesthetic depth. In the whole-cell recordings, ACC neurons exhibited up- and down-state membrane potentials and fired action potentials during up-states. Cutaneous mechanical pressure but not brush stimulation elicited the bursts of up-states, and increased the frequency of action potentials. The mechanical pressure responses were evoked mostly in intrinsic bursting type neurons which were classified by based on their firing patterns in response to current injections from the recording pipette. The present results suggest that a subgroup of ACC neurons respond to mechanical pressure but not innocuous stimulation, and this may have an important role on the emotional or motivational ACC functions. (COI:No)

2P-101

Development of the recording method of specific neuronal activity in conscious mice using in vivo fiber photometry

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Recording the activity of specific types of neurons in conscious animals is important to show correlation between neuronal activity and animal behavior. Here, we set out to record the activity of orexin neurons in conscious mice by using a fiber photometry system we developed that utilizes a single silica fiber to provide excitation light and to detect fluorescence simultaneously. In order to express genetically encoded Ca2+ indicators (for example GCaMP6), the tTA/TetO gene expression system was used. Orexin-tTA mice in which orexin neurons express tTA were bilaterally injected with an adeno-associated virus (AAV) that drives expression of GCaMP6 in the presence of tTA. An immunohistochemical study showed that GCaMP6 was exclusively expressed in orexin neurons. Patch clamp recording and calcium imaging using acute brain slices confirmed that fluorescence increase was associated with frequency of action potentials. As a result, we succeeded in recording the activity of orexin neurons in vivo using fiber photometry. We are expecting to clarify a new role for orexin neurons by continuing to utilize this new method in conscious animals. (COI:No)

2P-102

In vivo nociceptive responses in the marginal layer of the spinal cord induced by intravesical capsaicin administration during micturition

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Coordinated movement of the bladder and urethra in the lower urinary tract is essential for micturition. The multiple afferent sensory signals from the organs to the spinal cord have an important role in the precise feedback micturition regulation. We have shown that sensory synaptic inputs from the bladder to spinal parasympathetic preganglionic nucleus effectively control micturition cycle. It is not clear, however, how afferent nociceptive information arising from the bladder and urethra is conveyed to the spinal dorsal horn. When fast-DiI (DiI) and Fluoro-Gold (FG) were injected into the bladder wall and urethral sphincter, respectively, small to medium sized neurons in the lumbosacral dorsal root ganglion were labeled with either DiI or FG. Intravesical capsaicin induced c-fos expression in the spinal dorsal horn. In particular, c-fos was expressed in the marginal layer (lamina I) by capsaicin urethra infusion. In vivo spinal recordings showed that neurons in the marginal layer were excited when intravesical pressure was almost at the maximum. Intravesical capsaicin increased the firing frequency of the spinal marginal neurons. These findings suggest that afferent inputs from the urethra to marginal layer of the spinal cord may induce urodynia. (COI:No)

2P-103

An analysis of inhibitory inputs to vertical cells in lamina II of the mouse spinal dorsal horn

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Lamina II of the spinal dorsal horn contains excitatory and inhibitory interneurons, which have axons that travel locally and remain within the spinal cord. While these neurons have excitatory inputs from primary afferents, they also have excitatory and inhibitory inputs from local interneurons and form various local circuits. Among the interneurons, a major subset of excitatory interneurons consists of vertical cells. Unique and important morphological features of these cells are dendritic trees expanding into deeper laminae and axonal arbors terminating onto lamina I projection neurons. Recently, we have observed contacts between vertical dendrites and vesicular glutamate transporter 1 (VGLUT1)-immunoreactive terminals. Since myelinated low-threshold mechanoreceptors (A-LTMR) express VGLUT1 in their central terminals within inner lamina II and deeper laminae, vertical cells could provide a route through which A-LTMR inputs polysynaptically activate lamina I projection neurons. These results suggest that vertical cells may have a crucial role in the development of pathological pain and then to identify sources of inhibitory inputs to vertical cells and/or VGLUT1-immunoreactive terminals would be important to understand physiological sensation. In this study, contacts among dendrites of vertical cells, VGLUT1-immunoreactive terminals and terminals from glycinergic inhibitory interneurons were investigated, since it has been reported that vertical cells were known to have glycinergic inhibitory inputs and that tactile allodynia was induced by targeted ablation of glycinergic interneurons. (COI:No)

2P-104

Involvement of the rostral ventromedial medulla cells in motor cotex stimuli-induced modulation of pain in chronic pain model (spared nerve injury) rat

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Motor cortex stimuli provide anti-nociceptive effects in chronic pain model rats by spinal cord inhibition, however, the precise mechanism remains to be unknown. The purpose of this study is to examine whether the RVM is involved in this cortex-stimuli-induced anti-nociception. We tested the responses of rostral ventromedial medulla (RVM) cell spontaneous activity to motor cortex stimuli in chronic pain model (spared nerve injury (SNI) model) rats. Single unit activity of the RVM cells was recorded with tungsten microelectrodes under general anesthesia and was classified into 3 groups, ON, OFF, and Neutral cells, based on their responses to nociceptive pinch stimuli. Stimulating electrode was placed in cerebral motor cortex, where the location of the tip of electrode was identified by the presence of single unit activity recorded before stimuli and stimuli-induced movements of extremities. To confirm synaptic contact between cortex neuron and the RVM cell, field potential was also recorded with the same recording electrode as for unit activity in the RVM. We found that in SNI rats OFF cells increased their spontaneous activity, and ON cells decreased their spontaneous activity, for at least 30 min after cortex stimuli. These results suggest that motor cortex activation induces spinal cord inhibition through the RVM. (COI:No)

2P-105

Repeated forced swim stress enhances CFA-evoked mechanical hypersensitivity and affects the expression of delta-FosB and acetylation of histone H3 in the rostral ventromedial medulla

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Exposure to stressors causes substantial effects on the perception and response to pain. And it has been reported that chronic stress produces lasting hyperalgesia in several animal models. The rostral ventromedial medulla (RVM) receives projections from the periaqueductal gray matter (PAG) and is a major source of descending pathways to the spinal cord. The PAG and RVM with its spinal projections constitute neural circuits of descending pain modulatory system. In the present study we examined the expression of delta-FosB and acetylation of histone H3 in the RVM after forced swim stress (FS) and CFA injection to clarify changes in descending pain modulatory system in the rats with stress-induced hyperalgesia. FS (day 1, 10min; days 2-3, 20min), but not CFA injection into the hindpaw, induced a significant increase in the expression of delta-FosB and acetylation of histone H3 in the RVM. FS prior to CFA injection showed significant enhancement of CFA-evoked mechanical hypersensitivity and potentiated the expression of delta-FosB and acetylation of histone H3 in the RVM. Quantitative image analysis showed that the number of delta-FosB-IR cell in the RVM was significantly higher in the FS + CFA group (38.0±7.8) than that in the CFA group (7.0±3.7, $p< 0.05$). These findings suggest neuroplastic and epigenetic changes in the RVM after forced swim stress, which may be involved in the enhancement of CFA-induced mechanical hypersensitivity through dysfunction of descending pain modulatory system. (COI:No)

2P-106

The effects of maternal separation during or after SHRP on the fear-related behavior in the adult mice

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Several maternal separation paradigms are used to study the mediating mechanisms of early-life stress. However newborn rodent experience a so-called stress hyporesponsive period (SHRP), when HPA axis shows a rapid regression from PND 2-14 in the mice. SHRP is hypothesized to be neuroprotective against stress-induced excessive release of corticosterone during postnatal development. In the present study, we investigated the effects of maternal deprivation (MD) during or after SHRP on the fear-related behavior. Pups per dam were separated from their dam and littermates for 3 hr per day from PND1-14 (MD14), as well as from PND 15-21 (MD21). Young adult mice underwent a battery of behavior tests for fear; an open field test, an acoustic startle test and fear conditioning test (FCT). Except FCT, there were no statistically significant differences between MD14/MD21 and control mice. FCTis a Pavlovian associative learning. In conditioning training, conditioning stimulus (context/cue) was given pairing with an aversive unconditioning stimulus: US. Contextual test was performed without US in same context as training chamber, and cued test was performed in altered context without cued period followed by with cued period. MD14/MD21 mice froze lesser on contextual and cued test compared to control. Especially in MD14 mice, freezing level during cued stimulus was significant attenuated. In MD21 mice, freezing level both at conditioning training and at altered context was also significant attenuated. We will discuss about MD related-neurogenesis. (COI:No)

2P-107

Altered regulation of mood-related behaviors and serotonergic pathways in *Rev-erbα* knockout mice

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A defect in behavioral rhythms is a key feature of mood disorders such as depression, seasonal affective disorder, and bipolar disorder. The behavioral rhythms are generated by the circadian clock system in which a core set of clock genes play a major role. Recent studies have proposed *Rev-erbα* as a key clock gene which links behavioral rhythms to mood regulation because stability of REV-ERBα protein relies on glycogen synthase kinase 3, whose activity is inhibited by lithium, a commonly used drug for bipolar disorder. However, how the *Rev-erbα* gene participates in the regulation of mood remains poorly understood. Here we show that *Rev-erbα* knockout (KO) mice develop emotional instability, which is often observed in human bipolar disorder subjects. In the elevated plus-maze test, KO mice spent more times in open arms when compared to wild-type (WT) animals, indicating a decrease in anxiety-like behavior in KO mice. However, in the open field test, exploration time in the center of the field is shorter in KO than in WT animals, which suggests an increase in anxiety-like behavior in KO mice. These contrary results between the two different behavioral tests indicate the existence of instability in KO mice. Importantly, gene expression analyses using several nuclei essential for mood regulation reveal dysregulation of the serotonergic pathway in the prefrontal cortex of KO animals. Together, our findings suggest that *Rev-erbα* plays an important role in controlling the serotonergic pathway, and thereby regulate mood and behaviors. (COI:No)

2P-108

Regulatory role of AMPK in PVH-CRH neurons in social stress-induced alteration of food selection behavior

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Corticotropin releasing hormone (CRH) secreting neurons in the paraventricular hypothalamus (PVH) have important roles for the stress responses. Recently, we revealed that activation of AMP-activated protein kinase (AMPK) in the CRH neurons increased carbohydrate selection. However, it has still been unclear whether AMPK in PVH-CRH neurons regulates feeding behavior in response to stress. We subjected C57BL/6J mice to social defeat stress and examined the alteration of food selection behavior. We found that social stress increased carbohydrate selection. Preferential expression of shRNA for AMPK in PVH-CRH neurons by using lentivirus and CRH neuron-specific Cre-recombinase expressing mice, completely suppressed the stress-induced carbohydrate selection. In contrast, the expression of shRNA for AMPK did not inhibit increase in CRH mRNA level in the PVH or plasma corticosterone level after social defeat. Preferential expression of constitutively active AMPK (CA-AMPK) in the CRH neurons mimicked stress-induced alteration of food selection, but did not affect plasma corticosterone level. Suppression of CRH expression in the PVH or its neuronal activity blunted the change in food selection as well as increase in plasma glucocorticoid level. These results indicate a physiological importance of AMPK in PVH-CRH neurons in the stress-induced alteration of food selection behavior but not in the activation of hypothalamic-pituitary-adrenal axis. (COI:No)

2P-109

Serotonergic neurons of the dorsal raphe mediate anti-cataplectic action of orexin neurons by suppressing amygdala

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The loss of orexin neurons in humans is associated with the sleep disorder narcolepsy, which is characterized by excessive daytime sleepiness and cataplexy. Mice lacking orexin peptides, orexin neurons, or orexin receptors recapitulate human narcolepsy phenotypes, further highlighting a critical role for orexin signaling in the maintenance of wakefulness. However, the precise neural mechanisms downstream of these neurons remain unknown. By means of focal restorations of orexin receptor expression in orexin receptor-deficient mice, we previously found that orexin neurons inhibit cataplexy-like episodes and the pathological fragmentation of wakefulness (i.e., sleepiness) via serotonergic neurons of the dorsal raphe (DR) and noradrenergic neurons of the locus coeruleus (LC), respectively. Here, we aimed to further identify the target area of anti-cataplectic effects by DR serotonergic neurons. Channelrhodopsin 2 was expressed using a Cre-dependent AAV expression vector specifically in DR serotonergic neurons of orexin neuron-ablated (orexin/ataxin-3) mice harboring a SERT-Cre allele. Optogenetic activation of DR serotonergic neurons, as well as optogenetic activation of their projections to the amygdala, efficiently inhibited cataplexy-like episodes. Furthermore, pharmacogenetic suppression of amygdala activity by inhibitory DREADD hM4Di also suppressed cataplexy-like episodes in orexin/ataxin-3 mice. Thus, upon orexinergic activations, DR serotonergic neurons may inhibit cataplexy by imposing a limitation on the emotional outflow of the amygdala. (COI:No)

2P-110

Synchronized saccades to isochronously alternating visual stimuli in monkeys

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Humans spontaneously generate synchronized tapping, but monkeys do not (Zarco et al., 2009). It has been proposed that only "vocal learners" are capable of synchronizing with external rhythms (Patel et al., 2009). However, accumulating evidence shows that monkeys can monitor the passage of time and can generate predictive movements. In this study, we attempted to train monkeys to make synchronized eye movements. Three Japanese monkeys generated sequential saccades in response to alternately presented visual stimuli. During the training sessions, stimulus onset asynchrony (SOA) for each trial was chosen randomly from a set of 300, 400, 800 and 900 ms. To facilitate predictive saccades, water reward was given for every saccade around the time of target onset (within ± 20% of SOA). While the latency of the 1st and 2nd saccades in the sequence averaged 204-263 ms, the means of the 7-8th saccades ranged from -88 to 75 ms. When the target was presented at the time of predictive saccade in the middle of the sequence, the animals kept track of the preceding rhythm, indicating that they were able to entrain internal rhythms. Sequential motor learning was unlikely attributable because synchronization was also found for novel SOAs (500, 600 and 700 ms) in the test sessions. Importantly, the predictive saccades disappeared during the second training sessions in which the reward was given only for reactive saccades. These results suggest that monkeys do have the ability of synchronization, but unlike vocal learners, they may not be motivated to do so by the synchronized movements themselves. (COI:No)

2P-111

Animal model of spatial neglect in macaque monkeys

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Spatial neglect is a characteristic failure to explore the side of space contralateral to a brain lesion. It cannot be explained by primary sensory or motor disorders. The purpose of this study is to establish monkey model of spatial neglect. The spatial neglect of the human involved dorsal attention network and ventral attention network. The homologous region of the ventral attention network in the human includes Superior Temporal Gyrus (STG) in monkeys. Therefore, we made a lesion in the right STG of two monkeys. We evaluated behavior of the two monkeys after the lesion. To evaluate behavior in the cage, we used food choice task. A piece of apple was hidden in a well under the cover with striped pattern. If the monkey select the well with striped pattern, the monkey get the reward. Reaction time to the contralesional side was longer than that to the ipsilesional side for more than 2 weeks after the lesion. To evaluate behavior on the chair, we used target select task. The visual stimuli is displayed by a display of the touch panel. The visual stimuli consist of a target and nine distractors. If the monkey touches the target within 2 sec, the monkey get the reward. The percentage of correct answers to the contralesional side was lower than that to the ipsilesional side for 2 weeks after the lesion. Motor deficit and sensory deficit were not detected in the behavior on the chair. These results suggest that STG lesion induced spatial neglect in the monkeys. (COI:No)

2P-112

Psychosomatic conditions and daily activity in university students may be more affected by biological rhythm

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We have been studying about the biological rhythm related to hormone secretion, morning psychosomatic conditions and daytime sleepiness in total 195 healthy Japanese university students (2008-2015). To examine effects of biological rhythm on the psychosomatic conditions, academic performance and daytime sleepiness, we investigated relationships between sleeping pattern (time go to bed, duration of sleeping and secretion pattern of hormones) and questionnaires include self-rate conditions and grade point average scores (GPA). To analyze their biological rhythm, salivary melatonin, growth hormone and cortisol were measured by enzyme-linked immunosorbent assay (ELISA). Salivary samples were taken from each subjects 5 times a day. Results of our study revealed that 1) group with irregular circadian rhythm of melatonin, growth hormone showed more psychosomatic complaints than regular group. This hormone secretion pattern can be altered by basic lifestyle habit even in healthy students. 2) sleep-wake patterns, especially later time into bed group produced lower academic performance (GPA). (COI:No)

2P-113

Zinc deficiency with reduced mastication impairs spatial memory in young adult mice

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Sufficient oral microelements such as zinc and fully chewing of foods are required to maintain cognitive function despite aging. No knowledge exists about the combination of factors such as zinc deficiency and reduced mastication on learning and memory. Here we show that tooth extraction only in 8-week-old mice did not change the density of glial fibrillary acidic protein-labeled astrocytes in the hippocampus or spatial memory parameters. However, tooth extraction followed by zinc deprivation strongly impaired spatial memory and led to an increase in astrocytic density in the hippocampal CA1 region. The impaired spatial performance in the zinc-deficient only (ZD) mice also coincided well with the increase in the astrocytic density in the hippocampal CA1 region. After switching both zinc-deficient groups to a normal diet with sufficient zinc, spatial memory recovered. Interestingly, we found no differences in astrocytic density in the CA1 region among all groups at 22 weeks of age. Our data showed that zinc-deficient feeding during a young age impairs spatial memory performance and leads to an increase in astrocytic density in the hippocampal CA1 region and that zinc-sufficient feeding is followed by recovery of the impaired spatial memory along with changes in astrocytic density. The combination of the two factors, zinc deficiency and reduced mastication may inhibit recovery of impaired spatial learning. (COI:No)

2P-114

Effects of melatonin on learning and memory in mice.

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We investigated the effects of melatonin on learning and memory in mice. Some previous studies have demonstrated that melatonin treatment impairs learning and memory performance. Others, however, demonstrated that melatonin facilitated the performance. Among these studies, there are a large variety of experimental designs which differ in animal species, tasks used to evaluate learning and memory, and timing and dose of melatonin administration, which may contribute to conflicting results. In the present study, our experiments were designed to investigate the effects of acute melatonin administration with different timing and doses on learning and memory by using novel object recognition task, which consists of three stages: habituation, acquisition and retention. Nine-week-old male ICR mice were given a single i.p. injection of melatonin either 30 min before an acquisition trial, 5 min after an acquisition trial, or 30 min before a retention trial. We have found that low-dose melatonin facilitated learning and memory while high-dose melatonin impaired it, when administered 5 min after an acquisition trial. Ongoing studies are investigating the effects of different doses of melatonin at the other injection timings. (COI:No)

2P-115

Behavioral study of relationship between impulsive behavior and GABA-A receptor in mice

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Stress manifests itself in various characteristic responses including emotions such as fear, anxiety and impulsivity. Animals under stress take adaptive actions that may lead to various types of behavioral disinhibition. Such behavioral disinhibition, when expressed excessively and impulsively, can result in harm in individuals and cause a problem in our society. In the present study, we firstly confirmed the effect of three benzodiazepine receptor agents, diazepam, flumazenil or FG7142, on the anxiety-related behaviors including with impulsive or disinhibited behaviors using several behavioral tests to clarify the suitable evaluation of anxiety-related behaviors in mice. FG7142 shortened light area spent time in a dose-dependent manner in the light / dark box test. In addition, FG7142 also increased the locomotor activity in a dose-dependent manner in the open field test. In the cliff avoidance test, FG7142 shortened open area spent time, but diazepam chanceled the FG7142-induced effect. On the other hand, diazepam significantly prolonged open area spent time in the cliff avoidance test. Therefore, we could confirme the possibility of cliff avoidance test to determine the impulsivity in mice. (COI:No).

2P-116

Reward-related activity in parvalbumin-positive interneurons in hippocampal CA1 region

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Inhibitory interneurons inhibit surrounding neurons, thereby shaping the activity pattern of local synaptic circuit and ultimately the output. However the contribution of various subclass of interneurons to cell assembly are not well-known. To elucidate this, the activity of parvalbumin positive interneurons (PV-INs) was imaged using Cre-loxP system and Ca^{2+} sensor fluorescent protein, GCaMP. Mice head-fixed under a two-photon microscope performed a reward-seeking task in virtual reality. When mice enter the reward zone in a virtual linear track, they can receive ten drops of water as reward at 1 sec interval. PV-INs fired during running, consistent with previous results. While receiving rewards, PV-INs activity was increased between rewards. In addition, after final rewards, PV-INs activity strongly increased. When interval was increased to 2 sec, the timing of this activity shifted. To elucidate the relation between excitatory and inhibitory neurons activity in this task, transgenic mice which express GCaMP in excitatory neurons were imaged. The activity of excitatory neurons increased between each rewards and also after the final rewards. The timing of after-rewards activity was also shifted interval-dependently similarly to PV-INs. Interestingly, when reward number was randomized, the reward end activity of excitatory neurons decreased but not that of PV-INs. These results suggest that reward-interval information is regulated by PV-INs and reward end activity in excitatory neurons encodes the number of reward. (COI:Properly Declared)

2P-117

Opposite-sex odor induced dopamine release in the nucleus accumbens of male and female rats

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Rodents are attracted to odors containing pheromones from opposite sex conspecifics. The dopaminergic projections to the nucleus accumbens (NAcc) are considered to be important in mediating the effects of natural rewards. Our previous study revealed that male rats showed female-directed odor preference and female odor-induced c-Fos expression in the NAcc only after sexual experience. Estrous female rats, on the other hand, showed male-directed odor preference without showing male odor-induced c-Fos expression in the NAcc irrespective of presence or absence of sexual experience, suggesting that opposite-sex odors are rewarding stimuli for male rats, but not for female rats. The present in vivo microdialysis study was conducted to investigate this possibility by examining dopamine (DA) release in the NAcc during exposure to opposite-sex odors in male and female rats both with and without sexually experience. The male rats which had experienced six ejaculations showed DA increase in the NAcc in response to female odors, although neither the sexually naive nor less experienced (three ejaculations) male rats showed such an increase in DA release. In the female rats both with and without prior sexually experience, however, male odors induced a significant increase in DA release. These data suggests that in male rats the degree of sexual experience appears to contribute to female odor-induced DA release, and that in female rats sexual experience dose not contribute to the male odor-induced DA release, probably because male odors are intrinsically rewarding for them. (COI:No)

2P-118

Fear conditioning induced protein kinase A activation in sleep

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One of the gene cascades associated with plasticity and long-term memory is protein kinase A (PKA). PKA activation occurs during a critical period of approx. 3-4h, following fear conditioning (FC) (Bourtchouladze, et al, 1998). Further, inhibition of PKA during FC produced fear memory deficits (Schafe, et al, 1999). Sleep deprivation during similar time windows (0-4h) post learning also produce fear memory deficits (Graves, et al, 2003). Interestingly, an enhancement in PKA activation has been reported in sleep (Luo, et al, 2013). We have also shown that intra-hippocampal administration of the PKA inhibitor Rp-cAMPs during sleep (0-4h) following FC produced a long-term memory deficit, while administration of the PKA activator Sp-cAMPs produced normal memory, in sleep deprived animals (Cho, et al, 2015). Here we examined whether FC induces PKA expression/phosphorylation specifically during the critical time window of sleep for the consolidation of fear memory. Adult, male rats were trained in a contextual FC paradigm or used as non-conditioned controls. They were then placed in a recording chamber and allowed either to sleep during a 0-4h or 4-8h period. Control animals were kept awake during this period. After reaching a continuous 30+ min sleep criterion, or at similar times for control animals, they were sacrificed and various brain regions including hippocampus, amygdala and prefrontal cortex were dissected and frozen for determining PKA/pPKA levels, using Western blot (WB) analysis. We are currently analyzing the WB data. Supported by KAKENHI #26285161H to CP. (COI:No)

2P-119

Toward Understanding of the Development of Seep/Wake Architecture: Functional Development of Hypothalamic Orexin Neurons from Embryonic to Neonatal Mice

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The amount, quality, and diurnal pattern of sleep/wake change greatly during development and the sleep/wake architecture matures for three weeks after birth in rodents. To unveil the neuronal mechanism underlying the development of sleep/wake architecture, we focused on the lateral hypothalamic orexin neurons, which play an important role for maintenance of wakefulness (e.g., lack of neuropeptide orexin released from orexin neurons causes the fragmentation of wakefulness). We first investigated the anatomical development of orexin neurons from embryonic to postnatal stages in mice. The activity of prepro-orexin promoter was detected in E12.0, and its peptide was detected after E14.0 in Orexin-EGFP transgenic mice. Using patch-clamp techniques, we next analyzed electrophysiological properties of orexin neurons in developing mice such as the membrane capacitance, resting membrane potential, spontaneous firing, miniature excitatory/inhibitory postsynaptic currents, and reactivity to serotonin. These properties in postnatal stages were equivalent to that in matured stages. Taken together with anatomical data, the expression of orexin precedes the maturation of electrophysiological properties of orexin neurons, and orexin is possibly released in perinatal stages. Therefore, the fragmentation of wakefulness in early postnatal stage is not due to immaturity of individual orexin neurons, but might be caused by immaturity of the connection between wake-regulating neurons. (COI:No)

2P-120

Relationship between feeding rhythm and sleep pressure

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Sleep quality is influenced by various circadian factors including feeding rhythm. Recent study reported that quality of sleep was damaged by feeding of high caloric food in the night. But the mechanism how night eating affects sleep regulation has not elucidated. Our study investigated the effect of feeding pattern in active dark phase on sleep/wake regulation especially sleep pressure in mice. We separated male C57BL/6J mice to 3 groups, which fed different feeding schedule in dark phase for 2 weeks respectively (Ad-lib group; ZT12-24, Morning group; ZT12-18, Evening group; ZT18-24). Phases of wake, rapid eye movement (REM) and non-REM (NREM) sleep were distinguished by the electroencephalogram (EEG) and electromyography activities. In this study, power density of the EEG delta (ratio of delta to theta) during NREM sleep was used as a parameter of sleep pressure. There is no difference at the amount of NREM, REM and wake among 3 groups. Evening group showed lower sleep pressure than other 2 groups. Cerebral cortex in Evening group had higher concentrations of dopamine and its metabolite, and increased mRNA expression of orexin in hypothalamus, which activate wake system. Additionally, Evening group showed higher AMPK phosphorylation which is activated by sleep deprivation in hypothalamus. These results suggest that Evening-type feeding schedule may reduce sleep pressure with increase of AMPK activity. (COI:No)

2P-121

Role of mesopontine tegmentum and amygdala in induction of blood pressure fluctuation during REM sleep

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During REM sleep, animals display large fluctuations of autonomic signs such as blood pressure, heart rate or respiration. We have shown that the amygdala is deeply involved in blood pressure fluctuation during REM sleep. REM sleep center in the mesopontine tegmentum regulates various components of REM sleep, including EEG desynchronization, muscular atonia, rapid eye movement or PGO wave. So, it is highly probable that the REM sleep center is involved also in inducing blood pressure fluctuation during REM sleep. Using male SD rats, effects on blood pressure of electrical stimulation to the mesopontine tegmental area were examined. Blood pressure fluctuation was induced by the stimulation of the areas including laterodorsal tegmental/pedunculopontine tegmental nuclei (LDT/PPT) or parabrachial nucleus. In some areas, the threshold for inducing blood pressure fluctuation was lower during REM sleep than other states (waking or slow wave sleep). Single neuronal recording from the LDT revealed that about 41% (7/17) of the LDT neurons showed firing correlated with blood pressure fluctuation during REM sleep. Of 7 neurons correlated with blood pressure, 6 were cholinergic, judging from the shape of action potential. In most cases, the increase in firing occurred prior to the blood pressure increase, indicating that the cholinergic neurons in the mesopontine tegmentum drive blood pressure fluctuation during REM sleep. Relation of the amygdala and LDT for the regulation of blood pressure during REM sleep has been examined. (COI:No)

2P-122

Mapping of neurons that send direct input to lateral hypothalamic orexin neurons

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Orexin A and orexin B are critical regulators of sleep/wakefulness states. Orexin-producing neurons (orexin neurons) are localized exclusively in the lateral hypothalamic area (LHA) and send abundant axonal projections to the broad areas in the brain, with particular dense projections to monoaminergic/cholinergic neurons in the hypothalamus and brainstem regions. Recent evidences have suggested that orexins are involved in motivated behaviours, including feeding, emotional behaviour and reward seeking. To know the neuronal pathways through which orexin neurons are activated is essential to understand the mechanisms by which orexin signaling is regulated. To address this, it is essential to identify the direct synaptic inputs that control orexin neurons. Using a recombinant rabies virus (SAD δG vectors)-mediated trans-synaptic retrograde tracing, we identified neurons that make monosynaptic inputs to orexin neurons in the entire brain. Many positive neurons were found in the septal regions, BST, shell of the nucleus accumbens, ventral pallidum, preoptic area (POA), anterior hypothalamus, LHA, basal hypothalamic regions, posterior hypothalamus, and periaqueductal gray of the midbrain and pons. GABAergic neurons in the POA, which are thought to play an important role in initiation and maintenance of sleep, were revealed to send prominent projections to orexin neurons. We also found many neurons in the paraventricular hypothalamic nucleus, including CRH and AVP-positive neurons, send direct input to orexin neurons. These inputs might play roles in regulating orexin neurons. (COI:No)

2P-123

Subregion-specific striatal pathway control of Pavlovian associative learning

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The striatum is a key neural substrate for the control of associative learning processes that guide appropriate behavioral responses to maximize reward or minimize harm. Hypothesized to facilitate this process are the activity of two distinct striatal output pathways comprised of striatonigral (direct) or striatopallidal (indirect) projection neurons (Macpherson et al, 2014). Here we explore the role direct and indirect pathways originating from three striatal subregions, the nucleus accumbens (NAc), dorsomedial striatum (DMS) and dorsolateral striatum (DLS), in mediating Pavlovian approach behaviour in an autoshaping task. Using a reversible neurotransmission blocking (RNB) technique (as described, Hikida et al, 2010) to separately inhibit activity in each pathway, we revealed a specific role of NAc direct pathway neurons in controlling Pavlovian approach behaviour to an environmental cue associated with a natural food reward. These findings provide insight into the neural circuits underlying Pavlovian conditioning, and may be important in the identification of therapeutic targets for the treatment of disorders associated with a loss of impulse control, including addiction. (COI:No)

2P-124

Search for the evolutionary origin of the brain

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How brain appeared during evolution is an issue of hot debate. A widely accepted scenario is that evolution of body plan from radial symmetry to bilateral symmetry provoked the concentration of neurons along the symmetry axis forming ganglia and this structural concentration was a trigger for the appearance of brain functions. This implies that ganglia appeared first and brain function followed. Here we present evidence against this scenario by demonstrating that some basic functions of the brain e.g. perception of environmental stimulus and motor control can already be identified in the nervous system of Hydra that has radially symmetrical body plan and has no ganglia. We find that tearing basal disc off the substrate enhances the adhesiveness of tentacles to the substrate as a response and that this leads to the rise of locomotory activity of the animal. We also find that the function of the enhancement is restricted to the neural population in the aboral part of Hydra termed peduncle. As to perception, we find that Hydra has negative geotaxis in its behavior and the gravity sensing tissue is located in the head of the animal. It is therefore likely that the information about gravity is transmitted to the peduncle finally resulting in the locomotory activity opposite to gravity. From these observations, we propose that the peduncle nervous system of Hydra is furnished with functions that are basically similar to the functions of the brain in a very primitive form even without the concentration of nervous system forming ganglia. (COI:No)

2P-125

Effect of Bisphenol A on AVP-releasing rhythm of SCN in culture

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In mammals, circadian rhythms are driven by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN shows clear circadian rhythm of arginine vasopressin (AVP) release in culture. When Bisphenol A (BPA) was applied to the culture, AVP release was markedly inhibited. However, BPA did not inhibit AVP release from the supraoptic nucleus (SON). The same effect was observed by the application of estradiol and the reagents had effect on both male and female SCN. Furthermore, a pulse application of BPA induced phase-dependent phase shift of AVP rhythm. Phase advance of the rhythm was observed after the application during early subjective day. These results suggest that BPA or its derivatives might modulate AVP rhythm in the SCN. (COI:No)

2P-126

Circadian properties of a solitary single suprachiasmatic nucleus neuron in mice

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A master circadian clock in mammals is located in the hypothalamic suprachiasmatic nucleus (SCN) which is composed of multiple oscillator cells in the circadian range. Recent studies have demonstrated that SCN neurons are heterogeneous not only in their cellular compositions but also in their oscillatory properties, and the network consisting of these cells is critical for the clock works. It is important to examine the circadian properties of a solitary single neuron to understand the functions of the neural network. However, a long-term monitoring of the circadian rhythms has been technically difficult. In this study, we examined circadian properties of solitary single SCN neurons cultured on spatially isolated collagen-microislands which were made by splaying collagen solution with an atomizer on agarose glass surface in the bottom of a 35 mm petri dish. SCN neurons were derived from transgenic mice carrying a bioluminescent reporter for *Per1*-expression (*Per1*-luc) or PER2::LUC knock-in mice. Bioluminescence was measured by an EMCCD camera at every one hour. We succeeded to demonstrate circadian rhythms of *Per1* or PER2 expression in spatially dissociated solitary SCN neurons for at least 5 days. The formal properties were analyzed. Interestingly, instability which was attributable to the pacemaker was smaller for PER2 than that for *Per1*. These results indicate that a single SCN neuron is capable to oscillate robustly without any neural interaction. (COI:No)

2P-127

Developmental changes of Transport Response after maternal separation in mouse pups

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In many altricial mammals a mother carries her young. During maternal carrying, the transported young calms. This calming response is called Transport Response and composed of various components including trunk pliability, limb flexion, immobilization, and other physiological changes. Here, we investigated whether the Transport Response altered after maternal separation stress using C57BL/6 mouse. Pups were exposed to one of three conditions and then examined the immobilization time: (1) undisturbed condition, (2) maternal separation in the home-cage, where pups stayed in the home-cage only with littermates for 30 min and, (3) maternal separation in a novel cage, where a pup stayed in a novel cage without mother and littermates for 30min. Until day 12, pups showed no difference in the immobilization time among the three conditions. After day 13, the immobilization time was reduced in pups exposed to either type of maternal separation compared to undisturbed pups. Next, we compared c-fos mRNA expression in various brain areas among the three conditions using day 10 and 16 pups. c-fos mRNA expression in the anterior cingulate cortex was increased in both types of maternal separations at day 16 but not at day 10. Other brain areas did not show clear correlations with changes in the immobility. These data suggest that the anterior cingulate cortex is involved in the neural mechanism of the developmental change in Transport Response after the maternal separation. (COI:No)

2P-128

Natural respiratory types on deductive reasoning during human discrimination task

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Harmonization of breathing together with posture and mind is taught as crucial in Zen and Buddhism. Such a coordinated respiratory state is thought to play important roles in achieving or succeeding task performance in a variety of fields in modern society. Indeed, human prefrontal cortex controls not only judgment and decision-making but also autonomic function including respiratory function, suggesting the relationship between decision-making and respiration during cognitive tasks. In sports, for example, tennis, soccer, martial arts and so on, a player often succeeds to make an action as opposed to the opponent decision-making, and importantly, there are many cases in which such an action is synchronized with the opponent respiratory timing. First of all, to understand respiratory patterning while people does decision-making, we investigated changes in respiration during discrimination task with switching premises and rules as higher deduction demands of logical reasoning in right-handed healthy subjects. We found that the deductive reasoning task produced 4 natural breathing types composed of 3 elements, periodicity, extension of expiration, and randomness: i) periodic type (55%), ii) periodic and expiration-extending type (25%), iii) long-term expiration-extending type (10%), and iv) random type, in which respiratory swings quickly between inspiration and expiration (10%). From these results, we will discuss about a possible correspondence of respiratory patterning to large-scale brain networks regarding cognitive performance. (COI:No)

2P-129

Dopamine D2L receptor is required for cognitive learning in visual discrimination task

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Deficits of cognitive functions are commonly observed in various psychiatric disorders. Cortico-striato-thalamic circuit regulates the processing of cognition via dopamine neurotransmitter in human, primates and rodents. Dopamine D2 receptors have two specific isoforms, D2S and D2L, and participate in presynaptic and postsynaptic dopaminergic transmission in the indirect pathway of basal ganglia network, respectively. Previously, we demonstrated that D2L receptors in the indirect pathway of nucleus accumbens play an important role in aversive learning (Hikida et al, 2013). Here we performed reward-based cognitive task to test whether D2L receptors could be involved in cognitive learning using D2L receptors knockout (D2L-KO) mice. D2L knockout mice showed normal operant conditioning but delayed both visual discrimination and reversal learning. These results demonstrated that D2L receptors play important roles in cognitive learning and flexibility. (COI:No)

2P-130

Asymmetrical activity in rat anterior cingulate cortex in fear expression and extinction

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Bilateral inactivation of the anterior cingulate cortex (ACC) blocks recall of conditioned fear memory. However, the role of inhibitory neural transmission in the left-ACC (L-ACC) and right-ACC (R-ACC) in expression and extinction of fear remain unknown. Here, we examined inhibitory postsynaptic transmission and c-Fos expression in the L-ACC and R-ACC of rats subjected to fear conditioning and extinction learning. After memory retention test, pyramidal neurons in layer 2/3 of bilateral ACC showed the enhanced tonic currents through GABAA-Rs by fear extinction but not conditioning. Cumulative distribution of mIPSC amplitudes was significantly shifted toward greater events in R-ACC pyramidal neurons of rats subjected to extinction learning as compared to conditioned rats. Increases in c-Fos positive cells in the R-ACC of conditioned rats were observed in comparison with those in the L-ACC. Animals undergo extinction learning showed increases in c-Fos expression in the L-ACC compared with the R-ACC. We discuss the roles of these asymmetrical inhibitory and neuronal activities between the L-ACC and R-ACC in expression and extinction of fear. (COI:No)

2P-131

No relationship between decreased aggressive behavior by monosodium L-glutamate and osmotic pressure regulatory pathways in spontaneously hypertensive rats

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We previously reported that oral intake of monosodium L-glutamate (MSG), a taste substance for umami, for 5 weeks from postnatal day 25 (P25) to P60 decreased aggressive behavior in attention deficit/hyperactivity disorder (ADHD) model rat, which is mediated by vagus nerve from gut (gut-brain communication). To consider the mechanism of MSG in the brain, we tried to separate MSG effect from sodium effect as osmotic pressure regulatory pathways projects to paraventricular nucleus that contains oxytocin. In this study, we investigated the effect of NaCl on social behavior in ADHD model. Spontaneously hypertensive rats (SHR) at P25 were housed in an isolated condition (one rat per cage) and treated with H2O (n=6) and 0.6% NaCl (n=6) until P60. Open-field test, cylinder test and social-interaction test were used to assess locomotor activity and anxiety-like behavior. We also measured serum osmotic pressure. No significant difference was shown in locomotor activity and anxiety-like behavior between controls and NaCl-treated rats. Serum osmotic pressure in NaCl treatment is comparable to that of control. Date suggest that sodium action is not involved in MSG effect, indicating no relationship between decreased aggressive behavior and osmotic pressure regulatory pathways. (COI:No)

2P-132

Cocaine and amphetamine regulated transcript in central nucleus of amygdala regulates anxiety-like behaviors in attention deficit/hyperactivity disorder model rat

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Spontaneously hypertensive rat (SHR) is an animal model for attention deficit hyperactivity disorder. We previously reported that enriched environment (EE) for 5 weeks from postnatal day 25 (P25) to P60 decreased hyperactivity and anxiety-like behaviors in SHR and EE increased cocaine- and amphetamine-regulated transcript (CART) mRNA expression in the amygdala (Amy). As CART peptide is a factor that effects on locomotion activity, anxiety and stress, we first carried out CART staining to confirm its localization in the Amy and we then investigated the mechanism of CART action in the Amy. Many CART-positive cells were especially observed in the central nucleus of Amy (CeA), although CART expression in the CeA was decreased in EE-grown rat as compared to rat grown in standard environment (SE). To confirm that reduced CART expression is related to less anxiety-like behaviors in EE-grown rat, CART neutralizing antibody was injected into both CeA to check whether suppression of CART action causes in less anxiety-like behaviors in SE-grown rat. By the injection, the anxiety behavior (a tendency to walk close to center area) was apparently changed although total distance and the velocity in open-field test are similar to controls. Data suggested that less CART expression in CeA links to less anxiety-like behaviors in EE-grown SHR. (COI:No)

2P-133

Heart-specific disruption of Bmal1 leads to age-dependent hyperglycemia in mice

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Disruption of the circadian clock system not only causes abnormal physiological rhythms but also evokes disorders in energy metabolism such as obesity and diabetes. The circadian system is composed of molecular clocks, which reside in most cells throughout the body, and their function in major metabolic tissues such as liver and pancreas is thought to have a great influence on the regulation of glucose metabolism. However, it remains unknown whether the function of molecular clocks in non-metabolic tissues also plays an important role in the maintenance of systemic glucose metabolism. Here we show that heart-specific disruption of the *Bmal1* gene, a core component of the circadian clock, not only results in a significant reduction in cardiac function but induces impaired glucose metabolism in mice. Heart-specific *Bmal1* knockout mice show a significant increase in blood glucose levels with age. In addition, the insulin tolerance test reveals a decrease in insulin sensitivity in knockout mice, indicating that hyperglycemia observed in these animals is due to systemic insulin resistance. Importantly, the levels of expression of gluconeogenic genes in the liver is not suppressed by systemic administration of insulin in knockout animals, which further indicates the existence of insulin resistance at the molecular level. Together, our results suggest that, in addition to molecular clocks in major metabolic tissues, those in the heart also have an impact on systemic glucose metabolism in mammals. (COI:No)

2P-134

Characteristics of Postural Control Disturbance in Rats with Brain Lesions: Sensorimotor Cortex versus Cerebellar Vermis Lesions

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We aimed to reveal characteristics of postural control disturbances in rats with sensorimotor cortex (SMC) or cerebellar vermis (CV) lesions. Animals were divided in 2 groups: the left SMC lesion and the CV lesion groups. Using a posturography technique, we measured changes of center of pressure (COP) of the animals during floor inclination in antero-posterior and left-right directions, at angles from 0 to 30 degrees with different angle velocities. In 2 animals of each groups, we made chronic EMG recordings of extensor muscles of fore- and hind-limbs during postural changes and treadmill walking. All measurements were made in 2 days before lesion induction and 2, 7, 14 days after lesion. SMC and CV group showed remarkable increases of COP changes in left-right and antero-posterior directions, respectively. Both groups exhibited uncoordinated EMG activities during postural changes and treadmill walking. In SMC group, postural control disturbances almost recovered to normal postural reaction before lesion induction on 7 days later after lesion. However, the postural control disturbances of CV group were sustained, and did not recover with the time lapsed after lesion. These results show that the SMC and CV have differences in their functional roles in postural control and functional recoveries after their lesions. (COI:No)

2P-135

Comparison of neuronal activity in supplementary motor area between bipedal and quadrupedal locomotion of unrestrained monkeys

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To further understand cortical mechanisms for controlling bipedal gait in humans, we recorded single-unit activity from trunk/hind-limb regions of supplementary motor area (SMA) in unrestrained Japanese monkeys exerting quadrupedal and bipedal locomotion (Q and BL) on a treadmill. We then compared modulation pattern of activity during BL with that during QL. We found that majority of cells modulated their discharge during at least one of the two locomotor modes and were divided into two categories: stepping-related and stepping-unrelated. The stepping-related cells displayed phasic and/or tonic component(s) of activity along the step. Compared with QL, activity of these cells for BL was characterized by 1) various combinations of activity component(s), which were different from those for QL at a single-neuron level (locomotor-mode dependent), 2) the peak of phasic components locating around mid-stance phase, similar to that of antigravity muscle, and 3) gently-sloping, bi-phasic modulation spanning the step cycle. On the other, the stepping-unrelated cells were characterized by brief burst just preceding the timing of transition from QL to BL. Considering the facts that locomotor movements are bilaterally symmetrical and out-of-phase about mid-sagittal plane, and that SMA is bilaterally organized, our results suggest that the monkey SMA significantly contributes to the locomotor control, so as to possibly coordinate not only stepping movements and truncal posture, but movements on the left and right sides of the body. (COI:No)

2P-136

Distributed yet functionally divided nervous system of ophiuroids: evidence from local nerve block experiments

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Ophiuroids locomote on the seafloor by the coordinated rhythmic movements of their multijoint arms. How such coordinated movements are achieved is a focus of interest from the viewpoint of neurobiology as well as robotics, because ophiuroids lack the central nervous system that exerts integrative control over the five arms. To explore the underlying mechanism of the arm coordination, we examined the effect of local anesthesia to various parts of their body on locomotion. We found the followings. 1) Anesthesia of the circumoral nerve ring completely blocked the initiation of locomotion. On the other hand, initiation of single arm movement such as food-retrieval was unaffected. 2) When midsections of all the arms were anesthetized, the arm movements were rendered completely uncoordinated. In contrast, the inter-arm coordination was preserved when even only one arm was left intact. 4) Locomotion was unaffected by the anesthesia of the distal arms. 5) Radial nerve block to its proximal region abolished the coordination among the joints of the affected arm, rendering that arm motionless. These findings indicate that the ophiuroids' circumoral nerve ring and the radial nerves play differential roles in the intra- and inter-arm coordination in locomotion. The internal control scheme of their arm usage for locomotion was discussed. (COI:No)

2P-137

Comparisons of kinematics and EMG activity between bipedal and quadrupedal locomotion of unrestrained monkeys

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Through long-term training, Japanese monkeys can acquire the ability to exert bipedal locomotion (BL) on a treadmill. To distinctly probe what strategies animals used to deal with biomechanical problems unique to BL, we compared kinematic features and EMG activity between BL and quadrupedal locomotion (QL) in single subjects. To do this, we filmed side and back views of walking monkeys using high-speed video cameras, and simultaneously recorded trunk and limb EMGs. We found that, first, compared to QL, the monkey during BL stepped with shorter stride length and wider step width. Proportion of stance-phase duration within a step was larger for BL than QL. Second, patterns of extension-flexion sequences in the major hindlimb joints of QL were roughly preserved in those of BL. However, angular excursion during BL of the hip joint narrowed and substantially shifted toward extension, while that of knee and ankle joints remained relatively unchanged. Third, body axis during BL inclined forward slightly from the vertical and clearly oscillated along lower-limb stepping. Finally, trunk and hindlimb EMGs bursted once or twice along the step during both QL and BL. However, such EMG burst for BL was drastically, particularly in the trunk muscles. Additionally, some of the antagonistic muscle pairs were co-activated around the incidence of touchdown and mid-stance phase. These results reflect neural mechanisms the monkey CNS recruited to overcome biomechanical problems inherent in BL, such as instability of upright posture and kinetic burden on the lower limbs. (COI:No)

2P-138

Effects of teeth clenching on soleus H reflex during lower limb muscle fatigue

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We studied whether the soleus H reflex was depressed or facilitated in association with voluntary teeth clenching during muscle fatigue. Experiments were performed on 13 young healthy adults. Electromyograms (EMGs) were recorded from the bilateral masseter as well as the right-side soleus muscles, with bipolar surface cup electrodes. The subjects performed right-side tiptoe standing for five minutes (TS) to induce the soleus muscle fatigue. The soleus H reflex was evoked before and after TS. In addition, the isometric muscle strength during plantar flexion was measured before and after TS. The statistical significance of the results was assessed using one-way analysis of variance and Bonferroni test. The mean amplitude of soleus H reflex with teeth clenching before TS was significantly larger than that without teeth clenching before TS ($P < 0.01$). The mean amplitude of soleus H reflex with teeth clenching after TS was significantly larger than that without teeth clenching after TS ($P < 0.01$). The mean value of peak torque during isometric plantar flexion with teeth clenching before TS, without and with maximum teeth clenching after TS were 108.0, 83.7, 88.9 % of that without teeth clenching before TS, respectively. The present study demonstrated that teeth clenching could make an effect on lower limb muscle at the fatigue which lead to reduction of 16.3% in muscle strength and 21.3% in the median power frequency of soleus muscle EMG. (COI:No)

2P-139

Relationship between sitting posture control during optokinetic stimulation and physical function in stroke patients

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The present study investigated dependence on visual information in postural control among post-stroke patients. Nine patients were recruited. Physical function was assessed using Timed Up and Go test (TUG) and 10-meter walk test. The patients were asked to sit quietly on a stabilometric platform fixed on a stool in a dim room. For optokinetic stimulation (OKS), a random dot pattern was projected onto a screen placed in front of the subjects, and was moved continuously in either horizontal or torsional direction (20 °/s). The linear regression slope applied to the change in the center of pressure position (CoP) along the right-left axis was used for the evaluation of the OKS effect (slope of CoP: SCoP). Gait speed and TUG time of the stroke group were lower than those of the control group. In the stroke group, SCoP tended to shift to the paralytic side during horizontal OKS with paralytic direction, whereas torsional OKS evoked a small change in SCoP regardless of stimulus direction. Clear positive correlations were observed between SCoP and TUG, although no clear relationship was observed between SCoP and gait speed, which may be because TUG involves turning movement that requires a greater load change than that during simple straight-line gait. The results suggest that the SCoP change induced by OKS can be used as a model for the recovery of deviations in postural control among post-stroke patients. (COI:No)

2P-140

Muscular activity and energy metabolism during passive pedaling exercise with arms

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Passive exercise has been reported to contribute to rehabilitation and health care. This study aimed to investigate the level of muscular activity during passive pedaling exercise (PPE) with arms. Four healthy volunteers participated in this study. The subjects were seated on a chair with their hands placed on the pedals of an electrical ergometer, which was placed on a desk. In the PPE test, the subjects relaxed their arms and performed PPE for 3 min after a 2-min rest, followed by a 2-min rest. In the active pedaling exercise (APE) test, the subjects performed APE on an ergometer with an unloaded power output for 3 min. Throughout the tests, the ventilation level, pulmonary gas-exchange rates, heart rates, and hemoglobin concentrations of the brachialis and flexor digitorum superficialis muscles were continuously measured for all the subjects. Moreover, electromyograms (EMGs) of the brachialis and flexor digitorum superficialis muscles were obtained. The oxygen uptake increased exponentially by approximately 100 and 200 ml/min during PPE and APE, respectively. The concentration of deoxyhemoglobin slightly increased during APE, whereas it remained nearly steady throughout the PPE test. The EMG amplitude increased in the case of all the subjects during APE, but only in some of the subjects during PPE. The mean power frequency of EMG was lower during PPE than during APE. These results showed that the level of muscular activity was lower in PPE than in APE, and the proportion of the mobilized fast-twitch muscles was higher during PPE than during APE. (COI:No).

2P-141

Evaluation of postural control ability during targeted CoP movement in the anteroposterior direction in young and elderly subjects

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Owing to the aging population in society, there is a need for monitoring devices, methods, or algorithm that identifies people at a higher risk of falling. In the present study, center of foot pressure (CoP) dynamics were evaluated in young and elderly subjects while performing targeted CoP movement to determine whether the phase coupling between the CoP and target is predictive of age related changes in postural control ability.
A total of 21 elderly and 32 young subjects performed CoP movement to the target that moves cyclically at a period of 6 s in the anteroposterior direction with the amplitude of 7 cm. The degree of the phase coupling (λ) and the amplitude of CoP (A_{CoP}) were quantified from the analytic signals of CoP and target movements. Then, principal component (PC) analysis was applied to quantify the contribution of measures, followed by discriminant analysis.
The λ and A_{CoP} decreased significantly in the elderly compared with young subjects. Elderly had greater coefficients of variations (CV) in λ and A_{CoP}. PC analysis showed that the 1st and 2nd PC vectors account for ~88 % of the total variance. The discriminant function identified 14 elderly were different from young group.We interpret the decreases in λ and A_{CoP} and increased their CVs in the elderly as reflecting their reduced ability to adjust CoP to the target and a narrow safe margin of stability. Results might illustrate the possibility of obtaining classifiers of postural control ability through the phase coupling analysis during targeted postural sway. (COI:No)

2P-142

Bidirectional cortico-muscular coupling between the tongue and the cortex during isometric tongue protrusion in humans: a MEG study

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The functional connection between the cortex and the tongue was investigated using cortico-muscular coherence (CMC) between whole-head magnetoencephalographic (MEG) signals and electromyographic (EMG) signals from both tongue sides during human tongue protrusion. Somatosensory evoked fields (SEFs) were also recorded following electrical tongue stimulation. The CMC was observed over both hemispheres for each tongue side at two frequency bands: the beta (β) band (15-35 Hz) and the low-frequency band (2-10 Hz). The cortical sources of CMC at the low-frequency band (low-CMC) were located significantly posterior to the sources of CMC at the β band (β-CMC) in the primary motor cortex (M1), but were in the same area as tongue SEFs in the primary somatosensory cortex (S1). Time-domain analysis showed that the MEG signal followed the EMG signal of low-CMC, which was contrary to the finding that the MEG signal preceded the EMG signal of β-CMC. These results suggest that during tongue protrusion, the β-CMC may reflect motor commands from M1 to the tongue muscles, and the low-CMC may be driven by proprioceptive afferents from the tongue muscles to S1. Bidirectional neuro-muscular coupling between the tongue and the cortex may contribute to the coordination of sophisticated tongue movements in humans. (COI:No)

2P-143

Cerebral blood flow during adjusting voluntary lip-closing force using the visual feedback

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This study aims to examine the change of blood flow during adjusting voluntary lip-closing (LC) force in the front-lateral area of the cerebral cortex using functional near-infrared spectroscopy.Nine healthy young adults were recruited as the subjects. The system for LC task using the visual feedback system consisted of the apparatus that could measure the directional LC force and a display showing the exerted LC force for each direction in real time, along with a target. Subjects were instructed to control the LC force to maintain the target value shown in the display using visual-feedback. Same system was used for the finger task in that the subjects were instructed to control the force between the thumb and index finger. The 18 probes of spectroscope was fastened to the front-lateral region of the cortex to cover the prefrontal and right and left sensorimotor cortices during the lip-closing task and left finger task.Cerebral blood flow during LC task was increased in large cortical area including primary motor cortex on both sides. Increase of blood flow during left finger task was observed in right cortical area. Changes of blood flow during left finger task were milder than that during LC task. These results suggest that adjusting LC force needs larger cortical area on both sides whereas finger task mainly need contralateral cortical area. (COI:No).

2P-144

Motor Training Promotes Both Synaptic and Intrinsic Plasticity of Layer II/III Neurons in the Primary Motor Cortex

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To analyze both synaptic and intrinsic plasticity in the layer II/III area of the primary motor cortex (M1), we subjected rats to a rotor rod test and then prepared acute brain slices. Motor skill consistently improved within two days of training. Voltage clamp analysis showed significantly higher AMPA/NMDA ratios and miniature EPSC amplitudes in 1-day trained rats compared to untrained rats, suggesting increased postsynaptic AMPA receptors in the early phase of motor learning. Compared to untrained rats, 2-days trained rats showed significantly higher miniature EPSC amplitude and frequency. Paired-pulse analysis further demonstrated lower rates in 2-days trained rats, suggesting increased presynaptic glutamate release during the late phase of learning. In contrast, 1-day trained rats exhibited decreased miniature IPSC frequency, and increased paired-pulse analysis of evoked IPSC, suggesting a transient decrease in presynaptic GABA release. Moreover, current clamp analysis revealed lower resting membrane potential, higher spike threshold, and deeper afterhyperpolarization in 1-day trained rats compared to untrained rats, while 2-days trained rats showed higher membrane potential, suggesting dynamic changes in intrinsic properties. Our present results indicate dynamic changes in both glutamatergic and GABAergic plasticity as well as intrinsic plasticity in M1 layer II/III neurons after motor training. (COI:No)

2P-145

Study of forepaw function and posture using the Reaching Task of Parkinson's disease model rats

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Applying originally developed image analysis method, we quantitatively analyzed forepaw motor function and posture during reaching task(RT) in rat model of hemi-Parkinson's disease(PD rats). We used two groups of rats in the experiment:one damaged the motor cortex contralateral to dominant forepaw (contra group). The other animal was damaged the cortex ipsilateral to dominant forepaw in RT (ipsi group). PD rats was injected with 20μg of 6-hydroxydopamine (6-OHDA:4μg/μl) in the striatum. Followings were analyzed by image analysis of RT: i)the success rate of the RT, ii) forepaw pronation, iii)the maximum distance between the second fingers and the fifth finger (fingertip-distance), iv)the distance between each fingertip and pellet at the time of grab (distance between the pellet and each fingertip), v)an inclination angle of the head as an index of the posture. The results were compared before and seven-days after the 6-OHDA injection. We found that the inclination angle of the head was significantly increased, while the success ratios were slightly reduced by the 6-OHDA injection. Both the fingertip-distance and the those between pellet and each fingertip were found to be significantly increased by the injection. These results indicate that changes in posture are significantly accompanied with abnormal forepaw function in PD rats during RT performance. (COI:No)

2P-146

Kir7.1 expression after neonatal hypoxic-ischemia relates to the inhibition of oligodendrocyte differentiation: in vitro analysis

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Developmental white matter injury (DWMI) caused by hypoxia-ischemia (H-I) is associated with permanent neurodevelopmental disabilities in preterm infants. DWMI model rat that received H-I (RCAO and 6% hypoxia for 1 hour) at P3 shows hindlimb motor dysfunction without neuronal loss. Oligodendrocyte (OL) maturation seems to be inhibited in the DWMI model as mature OL loss and myelination disturbance were detected at cortex. To clarify the factors that associate with inhibition of OL maturation, we first performed cDNA microarray analysis. Among several factors, we focused on inward rectifier K channel Kir7.1, as its protein was specifically increased in the ipsilateral white matter after H-I. To know the mechanism of Kir7.1 on delayed OL differentiation in DWMI model, OL progenitor cells (OPCs) isolated from P1 rat brain were exposed to 1% O_2 in vitro. Cell proliferation of OPC was increased compare to controls (20% O_2) along with Kir7.1 protein increase. After OPC differentiation with T3 and CNTF for 10 days, MBP expression was obviously decreased in 1% O_2 condition accompanied with Kir7.1 accumulation in MBP-negative immature OLs. Data suggest that high expression of Kir7.1 in OPC after H-I might be involved in the inhibition of OL differentiation/maturation resulting in myelination disturbance. (COI:No)

2P-147

The quality of component in the binary taste mixture affects the recognition of the partner component in this mixture.

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We investigated how the rats recognize the component in the binary taste mixture, when the concentration of partner component was changed. When a sweetner was mixed in a bitter taste solution, rats could not recognize the bitter stimulus in this mixture, even if this bitter stimulus was recognized when it was presented as a pure taste stimulus. These results suggest that rats can recognize the component in the taste mixture generally but they fail to recognize it depend on the quality of the partner component in the mixture. (COI:No)

2P-148

Expression of store-operated Ca^{2+} entry (SOCE) mediated by Ca^{2+} release-activated Ca^{2+} (CRAC) channels in rat odontoblasts

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Ca^{2+} signaling in dentin formation and/or sensory transduction in odontoblasts is mediated by Ca^{2+} release from the intracellular Ca^{2+} stores and/or Ca^{2+} influx from the extracellular medium. In a previous study, we reported that depletion of Ca^{2+} stores activated store-operated Ca^{2+} entry (SOCE) in odontoblasts. However, the detailed process and molecular mechanism for activation as well as pharmacological properties of SOCE remain unclear. In this study, we examined the pharmacological properties of SOCE in acutely isolated odontoblasts. From these cells we measured intracellular free calcium concentration ($[Ca^{2+}]_i$) by fura-2 fluorescence. In the absence of extracellular Ca^{2+}, thapsigargin, a sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitor, elicited transient $[Ca^{2+}]_i$ increases. After $[Ca^{2+}]_i$ returned to the near-resting levels, subsequent application of 2.5 mM extracellular Ca^{2+} increased $[Ca^{2+}]_i$ as SOCE. The increases in $[Ca^{2+}]_i$ were inhibited by pretreatment of synta66 and BTP2, Ca^{2+} release-activated Ca^{2+} (CRAC) channel inhibitors. In addition, application of lanthanium suppressed SOCE, which were induced by thapsigargin-pretreatment. These results suggested expression of CRAC channels and SOCE activated by depletion of Ca^{2+} stores in odontoblasts. (COI:No)

2P-149

Involvement of the vestibular nuclear complex in the facilitation induced by stimulation of the red nucleus on the jaw-opening reflex

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Our previous studies found that stimulation of the red nucleus (RN) facilitated the low-threshold afferent-evoked jaw-opening reflex (JOR), and that stimulation of the vestibular nuclear complex (VN) modulated the JOR. It has been reported that the RN projects to the superior (SVN), lateral (LVN) and inferior vestibular (IVN) nuclei. We investigated whether electrically induced lesions of the VN or microinjection of muscimol into the VN affects RN-induced facilitation of the JOR. The experiments were performed on anesthetized rats. The test stimulation was applied to the inferior alveolar nerve to evoke the JOR. The EMG was recorded from the digastric muscles. The control JOR responses were recorded as well as the modulation induced by stimulation of the RN. The VN lesion was made. The effect of the RN stimulation on the JOR was tested at the termination of lesion. Additionally, microinjections were made into the VN. The control JOR and the effects of RN stimulation on the JOR were tested, beginning from the end of the injection. Electrically induced lesions of the LVN, medial vestibular nucleus (MVN), SVN and microinjection of muscimol into the LVN, MVN, and SVN both reduced the RN-induced facilitation of the JOR. Electrically induced lesions of the IVN and microinjection of muscimol into the IVN both increased the RN-induced facilitation of the JOR. These results suggest that RN-induced facilitation of the low-threshold afferent-evoked JOR is mediated by a relay in the VN. (COI:No)

2P-150

Gene expression changes during primary culture of salivary acinar cells

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Objectives: Tissue injuries of salivary glands result in atrophy of acinar cells and consequent hyposecretion of saliva. As a model of salivary gland dysfunction, we investigated the change of gene expression pattern in primary culture of parotid acinar cells. Methods: Parotid acinar cells were dispersed from glands by digestion with collagenase and hyaluronidase, which mimics tissue injuries of glands. Isolated acinar cells were cultured in the absence or presence of PP1, a Src kinase inhibitor, or SB203580, p38 MAP kinase inhibitor. Cells were harvested just after cell isolation and after culture for 1-3 days. Total RNA were purified and mRNA levels were examined using Affymetrix GeneChip Rat Genome 230 2.0 Array. Results: Principal component analysis indicates that the pattern of gene expression greatly changed in the first 24 h and that addition of PP1 or SB203580 modestly suppressed the changes. Correlation analysis showed that the effects of PP1 and SB203580 on the gene expression pattern were similar and showed a positive correlation between changes of mRNA expression in their presence. The time-dependent change in the absence of inhibitors and the difference between in the absence and presence of PP1 showed a negative correlation. Conclusion: PP1 and SB203580 have similar effects on the changes of gene expression in the primary culture of parotid acinar cells. These results support that Src and p38 MAP kinase mediate the common signal pathway to induce dedifferentiation and dysfunction of salivary glands. (COI:No)

2P-151

V-ATPase subunit a3 KO mouse shows reduced salivation

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Vacuolar H^+-ATPases (V-ATPases) function not only to acidify a variety of intracellular compartments, but also to pump protons across the plasma membranes of various cell types. We previously reported that 1) major salivary glands commonly expressed a2, a3, d1, B2, C1, E2 subunit isoforms of V-ATPases; 2) B2 subunit isoform was localized in ductal cells, but not in acinar cells, of the major salivary glands; and 3) B2 subunit isoform was localized in apical, basolateral membranes and basal infoldings as well as in cytosol of striated ductal epithelial cells. In order to clarify functional roles of the V-ATPase in the salivary gland, we have examined the phenotype of knockout mouse of a3 subunit isoform (a3-KO mouse). In a3-KO mice, there is little saliva secretion and the size of major salivary glands was smaller than that in wild type mice. Reduction of saliva secretion was prominent by pilocarpine stimulation, whereas no difference was detected by isoproterenol in a3-KO mice. The osmolarity of secreted saliva was increased, concentrations of Na^+, Cl^-, Mg^{2+} in secreted saliva were changed, and pH of intraoral salivary tended to be slightly acidified in a3-KO mice. These results suggest that V-ATPases play a critical role in salivary ductal function, such as ion transports involved in epithelial electrolyte absorption, anion secretion and modifying pH. (COI:No)

2P-152

A new method for the study of velopharyngeal function using digital barometer

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Evidence supports the idea that changes of pressure in pharynx reflect swallowing function. Manometer has been used to assess the intrapharyngeal pressure, but direct influence of boluses to the sensor confounds data interpretation. We introduce our newly developed device to better understand the changes of baropressure during swallowing by transducer in a balloon and clarify its validity to assess the swallowing. Baropressure was measured in pharynx from 12 healthy subjects. Four series of task (dry, 15 and 45-ml water, and 15-ml thickened water swallowing) were performed. All tasks displayed positive pressure. Interestingly, bolus swallowing had biphasic responses that consisted of early and late phase. Response in late phase was much larger than early phase in all tasks. Late phase showed similar response between tasks, but response in early phase by 45-ml water was greater than 15-ml water swallowing, suggesting that early phase could be sensitive to the volume of boluses. Dry swallowing had no response of early phase, suggesting that mechanisms for dry swallowing could be different from bolus swallowing. These findings indicated that our sensor has abilities to measure the baropressure in pharynx and has advantages to assess the swallowing more precisely (i.e. 0.15 kp of resolution) sufficient to detect small changes of pressure in early phase with less influence by a direct contact of boluses to the sensor. (COI:No)

2P-153

Shogaol and gingerol inhibit oral ulcer-induced pain via sodium channel blockage

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The traditional Japanese medicine hangeshashinto, composed of seven herbal extracts, alleviates oral ulcer-induced pain. However, it is unclear which and how ingredients are effective to the analgesic effect. In this study, we explored main analgesic ingredients in hangeshashinto in two screening tests and investigated pharmacological mechanism underlying the anesthesia of the identified ingredients using a rat oral ulcer model. In the first screening using automated patch-clamp recordings, [6]-shogaol and [6]-gingerol among tested 19 ingredients showed antagonistic effects on sodium channel $Na_v1.8$, as same as or more than lidocaine. In the second screening in rat cultured sensory neurons, both [6]-shogaol and [6]-gingerol that are contained in Processed Ginger extract inhibited stimulant-induced substance P releases dose-dependently. In the model rat, Processed Ginger extract and a mixture of [6]-shogaol and [6]-gingerol induced analgesic effects on oral ulcerative mucositis-induced pain following co-application with abundantly-saponin containing Ginseng extract, which accelerated substance permeability into oral ulcer. These results suggest that the anesthesia of hangeshashinto on oral ulcer is caused by cooperative action of both Na_v channel blockage effects of shogaols/gingerols in Processed Ginger extract and drug delivery acceleration due to other saponin-containing herbal extracts. (COI:Properly Declared)

2P-154

Parasympathetic vasodilation in major salivary glands in type 2 diabetic rats.

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Previously, we demonstrated that parasympathetic vasodilation in rat salivary glands was evoked by stimulation of orofacial sensory nerves, and noted the importance of hemodynamic regulation because of the rapid increases in blood flow. Recent investigation has shown that parasympathetic vasodilation is impaired in the submandibular glands of streptozotocin-diabetic rats, a model of type 1 diabetes mellitus (DM), indicating that short-term hyperglycemia is responsible for hemodynamic disturbance. However, the effects of long-term hyperglycemia in type 2 DM on parasympathetic vasodilation of salivary glands are poorly understood. In the present study, we analyzed the hemodynamics in the major salivary glands during electrical stimulation of the central cut end of the lingual nerve in urethane-anesthetized spontaneously-developed type 2 diabetic rats and nondiabetic control rats. Lingual nerve stimulation induced intensity- and frequency-dependent blood flow increases in three glands in both diabetic and nondiabetic rats, and the glandular blood flow increases were significantly inhibited by intravenous administration of the autonomic ganglion blockade hexamethonium. The magnitude of the changes in vascular conductance in the parotid gland of diabetic rats was significantly lower than that of nondiabetic rats, but this was not the case in the submandibular and sublingual glands. Our results indicate that long-term hyperglycemia selectively inhibits parasympathetic vasodilation in the parotid gland and suggest that long-term hyperglycemia disturbs the regulation of glandular hemodynamics. (COI:No)

2P-155

Study of mouse temporomandibular joint induced by unilateral anterior malocclusion using metal crown

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In order to investigate the etiology of temporomandibular joint osteoarthritis (TMJ-OA), an unilateral anterior malocclusion model was made by using metal crown. Three male C57BL6 mice (7-9 weeks old) were used. Under anesthesia, the metal crown, made of 18G injection needle, was fixed on the left mandibular incisor by zinc phosphate cement. Then, the crown was kept for 6 weeks. MR images were obtained by a 7 T MR system. After MRI experiments, mice were euthanized with an overdose of pentobarbital, then, histological slices were prepared. Mice presented no significant decrease of body-weight throughout the experimental period. Six weeks after wearing metal crown, position of the mandibular condyle were moved to posterior direction in the sagittal MR image. In the transverse MR image, the angle of right ascending ramus with respect to the sagittal plane was larger than that of right ascending ramus. Under optical microscope, arrangement of chondrocytes in the posterior condylar cartilage became irregular, and we found cell-free area and cluster-formation in the posterior condylar cartilage. These findings are early stage pathological changes of TMJ-OA in condylar cartilage. In order to establish the reproducibility of TMJ-OA caused by unilateral anterior malocclusion, we continue experiments to increase the number of samples, and also try to detect time-dependent changes in position and morphology of the mandibular condyle. (COI:No)

2P-156

Vascular permeability of the synovial membrane in the temporomandibular joint of the collagen-antibody-induced-arthritis mouse measured by MR imaging

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We analyzed the anatomical structure of the temporomandibular joint (TMJ) and the vascular permeability of the synovial membrane of the collagen-antibody-induced-arthritis (CAIA) mouse using 7 T magnetic resonance (MR) imaging. Arthritis was induced by intraperitoneal injection of a 5-clone cocktail of monoclonal antibodies (mAb) against collagen type II, followed by lipopolysaccharide. Male C57BL6 mice (5weeks old, n=25) were observed by MR imaging on days 0, 4, 7 after the mAb injection. For a dynamic study, T_1-weigted MR images were measured every 45 s before and after intravenous injection of a contrast reagent (Gd-albumin; 74 kDa). After MRI examination, all mice were euthanized with an overdose of pentobarbital for the histological analysis. From T_1-weight MR images and histological results, the temporal bone and mandibular condyle showed no morphological change, and the T_2-weight MR images presented no change in the synovial fluid both 4 days and 7 days. However, in the dynamic study, we observed an increase of signal intensity in the synovial fluid at 4 days, but did not observed significant increase at 7 days. These results suggest that the vascular permeability of the TMJ synovial membrane was increased in the early phase of arthritis. (COI:No)

2P-157

Gross and fine temporal signaling patterns of postcentral somatosensory neurons in area 2 during sequential tongue movements

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The lateral-most part of the postcentral somatosensory cortex is an important brain region for dexterous motor control of the tongue and lips. We examined the temporal signaling patterns of neurons in area 2 during a sequential tongue motor task. One female macaque monkey (*Macaca fuscata*) was trained to open two small sliding windows successively and get rewards through the windows by the tongue, with its eyes blinded with an eye mask. Neuronal activities were recorded extracellularly and single units were identified off-line using Spike 2 softwares (CED). For further numerical analyses, the data were exported to Scilab (Scilab Consortium / Digiteo). To quantify the temporal structure (regular, random, bursty), we used a metric of local variation (LvR) that evaluates the cross-correlation between consecutive interspike intervals (Shinomoto et al., 2009). The metric can evaluate the regularity (or irregularity) of interspike intervals independently of the firing rate fluctuations. The confidence intervals of the metric were constructed with 1000 bootstrap samples. Among neurons which were activated during the task (n=56), LvRs were ranged widely from 0.3 to 2.4. Among neurons which had low LvRs (regular spiking patterns), we found neurons that showed both depression and excitation during the task. Such neurons might subserve monitoring a certain phase of the sequential tongue movements. The animal used in this research was provided by NBRP, the National BioResource Project of the MEXT Japan. (COI:No)

2P-158

A regulatory mechanism for apical surface expression of ENaC through the ubiquitin-proteasome pathway in renal epithelial A6 cells

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Epithelial Na+ channel (ENaC)-mediated Na+ reabsorption in the cortical collecting duct plays an important role in regulation of extracellular fluid volume and blood pressure. As the Na+ entry step via ENaC located at the apical membrane is the rate-limiting step for transepithelial Na+ reabsorption, the activity and surface expression of ENaC are finely regulated. So far, it is considered that ENaC is poly-ubiquitinated by Nedd4-2 and subsequently degraded in the endosome-lysosome system, although the inhibition of proteasome actually increases apical surface expression of ENaC in renal epithelial A6 cells. However, to degrade apical membrane proteins in the proteasome, the proteins should be pulled out of apical membrane and delivered to the proteasome. In this study, we report that a possible role of p97 (an AAA+ ATPase)-dependent retrotranslocation-like mechanism for pulling ENaC out of apical membrane and the ubiquitin-proteasome pathway might be a main mechanism for degradation of ENaC in endogenously ENaC expressed renal epithelial cells. Supported by JSPS (15K08183). (COI:No)

2P-159

Ligands for Toll-like receptors 2, 3, and 4 differentially affects the functions of microglial cells in culture

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Microglial cells in the brain rapidly become activated in response to even minute pathologic changes. Among the factors causing pathologic activation of microglial cells, endogenous Toll-like receptor (TLR) ligands may play critical roles. TLR ligands act through MyD88-dependent and independent pathways. A typical TLR4 ligand LPS caused translocation of NFkB into nuclei and subsequent microglial activation mainly through MyD88-dependent pathway. In this study, we compared the actions of lipoteichoic acid (LTA) a ligand for TLR2 and pIC for TLR3 with that of LPS. TLR2 links to only MyD88-dependent pathway and TLR3 to the independent pathway. Total RNA was collected from microglial cells that were incubated with one of the TLR ligands for 4 h and subjected to quantitative real-time RT-PCR. LPS most strongly, LTA moderately and pIC weakly enhanced expression of mRNA encoding iNOS and IL-6. STAT1 and IRF1 mRNA expression was most strongly accelerated by pIC. NO was most abundantly released by LPS-treated microglial cells, and pIC did not induce significant NO release. As revealed by immunoblotting, pIC caused rapid phosphorylation of STAT1 and 3 and IRF1 expression. LPS and LTA caused loss of IkB, phosphorylation of MSK1, MK2, p38 and ERK. The loss of IkB may lead to the translocation of NFkB. Because a MSK1 inhibitor strongly suppressed LPS-induced NO release, MtD88-dependent phosphorylation of MSK1 may be critical to activation of microglia in addition to translocation of NFkB. (COI:No)

2P-160

The possible involvement of microglial cells in sleep-wake cycle

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We found that microglial cells display circadian changes of their morphology and functions. To investigate the circadian changes in mRNA expression in the cerebral cortex, total RNA was prepared from frontal cerebral cortices of male Wistar rats (8 weeks old) every 6 h from 7 AM. As revealed by quantitative real-time RT-PCR (qPCR), mRNA encoding CX3CR1 and F4/80, microglia-specific markers in the brain, displayed circadian changes; higher expression during the sleeping time (AM7 - PM3) than the awaking time (PM7 - AM3). Similarly, mRNA encoding MFG-E8 recognizing "eat-me" signals, and matrix metalloproteases (MMPs) 2, 9, 13, and 14. These qPCR results were coincided with immunoblotting results. Synaptic proteins synapsin I and PSD95 decreased at AM7 compared to PM7, in accordance with reported results. Immunohistochemical study has revealed that microglial cells at AM7 engulfed a synaptic protein synaptophysin, suggesting the synaptic pruning by microglial cells during the sleeping time. By in vitro study using rat primary microglial cells, it was revealed that glutamate enhanced their phagocytic activity and nor adrenaline abolished it. On the other hand, cultured microglial cells did not show circadian changes in their activity or expressions of factors responsible for phagocytosis. Collectively, microglial cells change their morphology and activities in response to the changes in neuronal activities in the cerebral cortex, while they are engaged in formation of circadian rhythm or sleep-wake cycle. (COI:No)

2P-161

Possible involvement of microglia in compensation for dopaminergic neuron loss in Parkinson's disease

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Parkinson's disease (PD) is a common degenerative neurological disorder in the elderly. The main pathology of PD is the progressive dopaminergic (DArgic) neuron loss in the substantia nigra, pars compacta (SNpc). The PD symptoms are said to become apparent only after substantial degeneration of the DArgic neuron system; the threshold has been estimated 70 or 80 % depletion of the striatal dopamine and 50 or 60% loss of DArgic neurons. The observation suggests that some compensatory mechanisms cover the decrease in dopamine content in the striatum and masks the appearance of the PD symptoms. In this study, we propose a compensatory mechanism, in which microglial cells are involved by phagocytosis of glutamatergic synapses arising from subthalamic nuclei. This notion was on the following observations. In the 6-OHDA-treated rat PS model rat brains, microglial cells were activated in the internal segment of globus pallidus (GPi) and the SN pars reticulata (SNpr), the basal ganglia outputs, which are devoid of neuronal cell death. Microglial cells in GPi and SNpr phagocytosed synaptic elements. Glutamate enhanced microglial phagocyosis. These results suggest that disinhibited glutamatergic neurons in the subthalamic nuclei caused microglial activation in the SNpr and GP. Then, activated microglia may be engaged in elimination of glutamatergic synapses in the SNpr and GPi, leading to the suppressed activity of GABArgic neurons. (COI:No)

2P-162

Circadian changes in IL-6 expression in normal mature rat brain and its action on non-activated (or resting) microglial cells

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Although interleukin-6 (IL-6) has been recognized mainly as a pro-inflammatory cytokine, it can also act as an anti-inflammatory cytokine. Circadian changes in IL-6 expression in the brain or in the cerebrospinal fluid have been reported. Our recent data by quantitative real-time RT-PCR (qPCR) assay have shown that IL-6 mRNA expression is the highest at PM 23, awakening time for rats. In the same investigation, expression of mRNA encoding a microglial marker F4/80 and matrix metalloproteases (MMPs) 2 and 9 was suppressed during the awakening time. To elucidate the relationship between high expression of IL-6 and low expression of F4/80 and the MMPs during the awakening time, rat primary microglial cells were incubated with IL-6 for 3 h and subjected to qPCR analysis. Then, it was found that IL-6 suppressed the expression of these factors. The data suggest that IL-6 may act as a suppressive cytokine on microglial cells on the contrary to the major notion that IL-6 is pro-inflammatory. In fact, when added to microglial culture with LPS, IL-6 suppressed the expression of proinflammatory cytokines TNFα and IL-1β. The suppressive effects may be exerted in a concentration dependent-manner. Our preliminary experiments showed that IL-6 suppressed proinflammatory cytokine expression even in the absence of LPS. Collectively, these results suggest that IL-6 is involved in circadian rhythm or sleep-wake cycle formation through the regulation of microglial functions. (COI:No)

2P-163

Effects of IL-6 on LPS-treated rat primary cultured microglial cells

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Interleukin 6 (IL-6) is a multifunctional cytokine that can function as in both pro-inflammatory and anti-inflammatory manners. IL-6 is released by many kinds of cells including macrophages. Microglial cells are the resident macrophages in the brain and known to express IL-6 at a very high level when they are activated. In spite of its long history, IL-6 actions on microglial cells have not been fully elucidated. We investigated IL-6 actions on cultured rat primary microglial cells that were activated by the treatment with lipopolysaccharide (LPS). First, we incubated microglial cells with LPS and IL-6, and total RNA was collected from the microglial cells 3 h later. When incubated with IL-6, LPS-induced elevated expression of proinflammatory cytokines TNFα and IL-1β was suppressed. Because LPS-treated microglial cells release very large amounts of IL-6, it may be necessary to remove the actions of endogenous IL-6 to investigate the effects of exogenous IL-6. Therefore, expression of gp130, a indispensable component of IL-6 receptor, was knocked down. Many other cytokines such as IL-11 utilize gp130 as their receptor component, whereas IL-6 may be the far more abundant than other cytokines. LPS-treated gp130-KD microglial cells reduced expression of TNFα and IL-1β, suggesting that IL-6 is a proinflammatory cytokine. Thus, we did not yet obtained a collective conclusion whether IL-6 is pro- or anti-inflammatory. We suspect that some other cytokines or the context of experimental protocols affect the overall effects of IL-6. (COI:No)

2P-164

A truncated form of CD200 (CD200S) expressed on glioma cells prolonged survival in a rat glioma model by induction of a dendritic cell-like phenotype in tumor-associated macrophages.

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CD200 exerts immunosuppressive effects on myeloid cells expressing its receptor CD200R. Human carcinoma tissues have been found to express not only full-length CD200 (CD200L) but also its truncated form, CD200S. The role(s) of CD200S in tumor immunity has never been investigated. In this study, we established rat C6 glioma cell lines that expressed either CD200L or CD200S; the original C6 cell line did not express CD200 molecules. Upon transplantation into the neonatal rat fstriatum, rats transplanted with C6-CD200S cells survived for a significantly longer period than those transplanted with the original C6 and C6-CD200L cells. The C6-CD200S tumors were smaller than the other tumors, and many apoptotic cells were found in the C6-CD200S tumor. Tumor-associated macrophages (TAMs) in C6-CD200S tumors displayed dendritic cell (DC)-like morphology with multiple processes and CD86 expression. Furthermore, CD8+ cells were more frequently found in C6-CD200S tumors, and the expression of DC markers, granzyme, and perforin was increased in C6-CD200S tumors. Isolated TAMs from original C6 tumors were co-cultured with C6-CD200S cells and showed increased expression of DC markers. These results suggest that CD200S activates TAMs to become DC-like antigen presenting cells, leading to the activation of CD8+ cytotoxic T lymphocytes, which induce apoptotic elimination of tumor cells. (COI:No)

2P-165

TRPC3 amplifies B cell receptor-induced ERK signaling via protein kinase D-dependent Rap1 activation.

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Sustained activation of extracellular-signal-regulated kinase (ERK) has an important role in cell fate decision of B lymphocytes. Recently, we demonstrated that the diacylglycerol-activated nonselective cation channel, canonical transient receptor potential 3 (TRPC3) is required for the B cell receptor (BCR)-induced sustained ERK activation. However, the signaling mechanism underlying TRPC3-mediated ERK activation remains elusive. Here, we showed that TRPC3 mediates Ca^{2+} influx to sustain activation of protein kinase D in a protein kinase C dependent manner in DT40 B lymphocytes. The later phase of ERK activation depends on the small G protein Rap1, known as a downstream target of protein kinase D, while the earlier phase of ERK activation depends on the Ras protein. Interestingly, the sustained ERK phosphorylation is required for full induction of the immediate early gene *Egr-1*. These results suggest that TRPC3 reorganizes the BCR signaling complex by switching the subtype of small G proteins to sustain ERK activation in B lymphocytes. (COI:No)

2P-166

A spontaneous distant metastasis model of cancer using normal Wistar rats.

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Malignant tumors metastasize from the primary site to other parts of the body. Particularly, it is called distant metastasis of cancer when the primary tumor migrates and deposits in the distant organs or tissues. Because distant metastasis usually carries the worst prognosis, it is eagerly waited for novel therapeutic interventions to suppress distant metastasis. For this purpose, the appropriate and convenient animal models with distant metastasis must be prepared. The most frequently employed model of distant metastasis is the direct injection of tumor cells into the systemic circulation that leads to the multiple metastases in many organs. This model, however, detachment of cells from the primary tumor site, the critical step for the metastasis, cannot be observable in this model. In this study, we have established a distant metastasis model using normal immunocompetent Wistar rats. C6 glioma cells (200, 000 cells /rat) were transplanted into the subcutaneous tissue in the back within 24 h after birth. Because of immatureness of the immune system, neonatal rats did not reject the cells. Three weeks later small hemispheric tumor was formed. By five weeks after birth, the back tumors became larger than 2 cm in diameter and the rats appeared unhealthy. Such unhealthy rats were found to have metastatic tumors in the lung. The metastasis was also observed by magnetic resonance imaging. Currently. Immunohistochemical analyses are currently in progress. (COI:No)

2P-167

Hunt for novel anti-tumor drugs mimicking the anti-tumor actions of CD200S molecule using a novel rat cancer model with distant metastasis.

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We recently found that a truncated form of an immunosuppressive molecule CD200 termed CD200S has a marked anti-tumor effect in a rat glioblastoma model. When C6 glioma cells expressing CD200S were transplanted into the striatum of neonatal Wistar rats, large number of glioma cells in the tumor underwent apoptotic cell death. This was supposedly caused by the action of cytotoxic T cells that had been activated by tumor-associated macrophages (TAMs). TAMs in the C6-CD200S tumor displayed dendritic cell phenotype and induced activation of cell-based anti-tumor immunity, resulting in the suppression of tumor growth. The anti-tumor effect of CD200 has inspired and urged us to find the agents that mimic the CD200S actions. For hunting the agents, we established a rat cancer model, in which we transplanted normal C6 glioma cells into the subcutaneous tissue on the back or the head of neonatal Wistar rats. Hemispheric tumor (diameter ~10mm) grew on the back or the head three weeks later after the transplantation. Further two or three weeks later, the tumor on the back metastasized mainly to the lungs. With the use of these cancer models, we have found several candidate drugs that have been applied through injection or oral administration. Among the agents, some might ones appeared to suppress the distant metastasis. We are now investigating the mechanisms of the actions as well as the significance of the anti-cancer effects. (COI:No)

2P-168

An old hypnotic bromvalerylurea ameliorate E.coli-induced accute respiratory distress syndrome of rats

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Bromvalerylurea (BU) is an old hypnotic/sedative, which we have found of its marked immunosuppressive effects on LPS-treated macrophages. BU promotes the survival of septic rats prepared through cecum ligation and puncture. In this study, we infused E. coli into the lungs through bronchi, and caused acute lung injury (ALI). Shortly after infusion of E. coli, BU and/or antibiotic (AB) solutions were once subcutaneously injected to rats. As controls, dexamethasone (Dex) + AB and vehicle were injected. The survival of ARDS rats were examined every 12 h. As a result, all rats (n=10) injected with BU+AB survived for 48 h. On the other hand, 40 % of rats injected with Dex+AB, 60 % of those with AB alone, and 70 % of those with vehicle alone of rats died. We have tried to find the differences in the mechanisms underlying the ameliorative effects of BU. Although in vitro study has shown that BU suppressed a variety of LPS-induced proinflammatory reactions by alveolar macrophages such as proinflammatory cytokine expression and nitric oxide release, the BU effects were less significant than Dex. Both BU and Dex suppressed LPS-induced phosphorylation of mitogen and stress-activated kinase 1 (MSK1) presumably leading to inhibition of NFkB binding to DNA. Taken a possibility that Dex suppressed the immune reaction to E. coli allowing expansion of the bacteria, the weaker immunosuppressive action of BU might be attributable to the better outcome caused by BU. (COI:No)

2P-169

Expansion of Alveoli Induced ATP Release in *ex-vivo* Rat Lung

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ATP is now recognized as a ubiquitous extracellular messenger of local intercellular signaling in the body. However, ATP releasing mechanisms are still obscured and no real-time observation of ATP release in the tissues is presented. In the lung, ATP regulates diverse processes of its normal functions and also contributes to the pathogenesis of a wide range of respiratory diseases. Expansion of alveoli is an essential physical stimulus in the lung but its effect on ATP release is not known. Here, we designed perfusing *ex-vivo* lung sample to investigate the expansion induced ATP release using the real-time ATP luminescence imaging system. After perfusion with medium via pulmonary artery, the lung with trachea and heart was excised. Luciferin-luciferase medium was introduced through trachea by instilling air space with the medium or through perfusing pulmonary artery. The *ex-vivo* sample was set in an upright microscope with 4x lens (NA0.28) and applied brief pressure from airway through 1 ml syringe. Expansion of lung (1s, intra-alveolar pressure of about 20 cm H_2O) induced transient ATP release in some alveoli. Prolonged expansion evoked strong and long lasting ATP release, and cyclic expansion induced cyclic ATP release. These results demonstrating direct association of ATP release with alveoli expansion. This study shows the first evidence for expansion induced ATP release in the lung. (COI:No)

2P-170

KCNJ15/Kir4.2 and intracellular polyamines couples to sense weak extracellular electric field in electrotaxis

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Weak electric fields are found at wound, regeneration site and tumor, and provide strong guidance cue for directional cell migration. Many motile cells can sense extracellular electric fields and migrate toward the cathode or the anode side: this phenomenon is known as electrotaxis. The sensor for detection of extracellular electric fields and its mediator transducing to downstream signaling pathways, however, remain largely unknown. We performed a large-scale loss-of-function screening using a siRNA library targeting ion channels/pumps/transporters in human corneal epithelial cells to find those molecules. We identified 17 genes showing defective or enhanced electrotaxis respectively after knockdown. Among them, knocking down of KCNJ15, a gene encoding inwardly rectifying K^+ channel Kir4.2, abolished electrotaxis. Knockdown of other K^+ channels tested in the present study did not affect electrotaxis. Depletion of cytoplasmic polyamines, highly positively charged small molecules participating in inward rectification of Kir4.2 function, completely inhibited electrotaxis. Expression of polyamine-binding defective mutant of KCNJ15/Kir4.2 also significantly decreased electrotaxis. These data suggest that KCNJ15/Kir4.2 and intracellular polyamines couples to play an important role to sense extracellular electric field in electrotaxis. (COI:No)

2P-171

Cytotoxicity effect towards human cancer cells of kappa and lambda carrageenan

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Carrageenan is a sulphated polysaccharide existing in red seaweeds. Prior studies reported that carrageenan is cytotoxic towards human tumor cells, however there remains little information regarding comparison of cytotoxicity effect from different types of carrageenans. In this study we determine the effects from two types of carrageenan, kappa carrageenan (κ-carrageenan) and lambda carrageenan (λ-carrageenan) on HeLa cells. Both carrageenans were applied to the culture media with the concentration from 250 to 2500 μg/mL. We found inhibited cell growth, increased cell death, and altered cell cycle progression compared to unexposed cells. After 72h carrageenan treatment, κ-carrageenan was seen to suppress growth with 75% suppression, which is higher than λ-carrageenan showing 65% suppression. Staining with calcein-AM/PI also showed κ-carrageenan treatment resulted in higher ratio of dead cells compared to λ-carrageenan. We used the fluorescence ubiquitination-based cell cycle indicator (FUCCI) to monitor cell cycle progression. We found that G1 phase population was increased in both type carrageenans. Considering these results, it is suggested that κ-carrageenan could potentially induce stronger cytotoxic effect towards human cancer cells compared to λ-carrageenan. Additionally, further study is needed to confirm this conclusion for better understanding to target best results towards carrageenan based research as a potential antitumor agent. (COI:No)

2P-172

Analysis of the regulatory mechanism of D-allose-inducible tumor suppressive factor TXNIP (thioredoxin interacting protein) for development of a new cancer therapy

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D-allose, the C3-epimer of D-glucose, has an anti-proliferative effect on various cancer cell lines. We have reported that D-allose caused up-regulation of thioredoxin interacting protein (TXNIP), an anti-tumor protein down-regulated in cancer cells. The anti-proliferative effect of D-allose is due to the up-regulation of TXNIP which causes cell cycle arrest.
We analyzed the signaling mechanisms of TXNIP up-regulation caused by D-allose in hepatocarcinoma cell line HuH-7. The D-allose-induced TXNIP up-regulation was repressed upon the inhibition of p44/p42 MAPK phosphorylation by PD98059. It was also repressed by the p38MAPK inhibitor SB203580. Several transcription factors have shown to participate in the TXNIP up-regulation through these two MAPK pathways. Meanwhile a glycolysis inhibitor 3-bromopyruvate did not show any effect on the TXNIP up-regulation caused by D-allose. Overall, TXNIP is up-regulated through both p44/p42 MAPK pathway and p38MAPK pathway, but not through the glycolytic pathway.
We also examined the mechanism of TXNIP decrease in HuH-7. The results showed that TXNIP decreased through both p44/p42 MAPK pathway and ubiquitin-proteasome pathway upon the serum stimulation.
Present works elucidated the regulatory mechanisms of TXNIP, a tumor suppressor, in cancer cells, which would make a contribution to establish a new strategy of cancer therapy utilizing D-allose and TXNIP. (COI:No)

2P-173

Human cell lines of leukocytic, erythrocytic and megakaryocytic lineages affect redox state of human serum albumin

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Human serum albumin (HSA) is composed of human mercaptoalbumin (HMA) with cysteine residue at position 34 (Cys-34) having reducing power, reversibly oxidized human non-mercaptoalbumin (HNA-1), and strongly oxidized human non-mercaptoalbumin (HNA-2). It is already known that the percentage of oxidized albumin increases in chronic renal failure, hepatic disease, and diabetes mellitus. In addition, the percentage of oxidized albumin increases with age. We already revealed that human aortic endothelial cells showed conversion of HNA to HMA, but not human dermal fibroblast cells. Here, we investigated whether human cell lines of leukocytic, erythrocytic and megakaryocytic lineages affect redox state of HSA or not. We revealed that human leukocytic, erythrocytic and megakaryocytic lineages cell lines showed conversion of HNA to HMA. Especially, human monocytic cell line and myeloma cell line had strong reducing efficiency. On the other hands, human T cell line had weak reducing efficiency. These results showed that reductive activity is probably common function among hematopoietic and endothelial cell lineages, however, the strength of reducing efficiency is different depending on whether differentiated into any type of blood cell. These cells are considered to participate in redox regulation in blood serum or bone marrow. (COI:No)

2P-174

Oxidative Stress Inhibits the Activation of Protein Phosphatase 5 by S100 Proteins

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Protein phosphatase 5 (PP5) is a serine/threonine phosphatase that is involved in the oxidative stress responses. The basal activity of PP5 is kept low and limited numbers of physiological activators have been identified. Among them, S100 proteins activate PP5 in a calcium dependent manner. We investigated the effect of oxidative stress on the interaction of S100 proteins and PP5 and the enzyme activity. S100A2 was readily oxidized in air or with Cu treatment and formed cross-linked dimers and higher molecular weight complex structures. S100A2 binding to PP5 was reduced by oxidation, resulting in decreased PP5 activation. PP5 activation was also inhibited by oxidation of other S100 proteins including S100A1, S100A6, S100B, and S100P. Hydrogen peroxide (H_2O_2) induced endogenous S100A2 oxidation in HaCaT and Huh-7 cells. Furthermore, the binding of S100A2 to PP5 was reduced and resulted in the decrease of PP5 activation by H_2O_2 treatment of Huh-7 cells. These results suggest that S100 proteins are involved in the modulation of PP5 activity upon oxidative stress. (COI:No)

2P-175

Role of Steroidogenic acute regulatory protein-related lipid transfer domain containing 10 in lipid droplet formation

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Steroidogenic acute regulatory protein related lipid transfer domain containing 10 (STARD10) is a member of the START domain containing lipid transfer protein family. We have previously shown that STARD10 is highly expressed in the liver and involved in bile acids homeostasis. When fed a high fat diet, the liver of *Stard10* knockout (*Stard10*$^{-/-}$) mice accumulated significantly less cholesterol and triglycerides than wild type (WT) mice. The aim of this study was to clarify the role of STARD10 in lipid accumulation in the liver. The area of lipid droplet (LD) of *Stard10*$^{-/-}$ mice fed with normal diet was significantly smaller than those of WT mice. We examined the effect of Choline-deficient diet (CDAA), which is known to induce liver specific fat accumulation and nonalcoholic steatohepatitis (NASH), in *Stard10*$^{-/-}$ mice. The liver of *Stard10*$^{-/-}$ mice was smaller and the area of LD was significantly smaller than those of WT mice. Gene expression levels of IL-1β and TNF-α were lower in *Stard10*$^{-/-}$ mice. Recently it was reported that STARD10 interacts with lysophosphatidylcholine acyltransferase 1 (LPCAT1) to facilitate trafficking of phospholipid from the ER to the lamellar bodies in pulmonary epithelial cells. We confirmed the interaction of STARD10 with LPCAT1 and their localization at the surface of LD in tsA201 cells. These results indicate that STARD10 is involved in regulating LD formation through the transport of phosphatidylcholine. (COI:No)

2P-176

PPARα-stimulated NOS1 phosphorylation mediated via PI3K/Akt in antral mucous cells: enhancement of Ca^{2+}-regulated exocytosis

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In antral mucous cells, the PPARα enhances ACh-stimulated exocytosis via NO produced by NOS1. In this study, we examined how PPARα activates NOS1 in antral mucous cells.The ACh-stimulated exocytosis was enhanced by a PPARα agonist (GW7647). The enhancement was abolished by a PPARα antagonist (GW6471). However, GW6471 induced a delayed increase in the ACh-stimulated exocytosis via cAMP accumulation by PDE2 inhibition. The GW6471 actions were mimicked by a PI3K inhibitor (wortmannin) and an Akt inhibitor (AKT 1/2 Kinase Inhibitor). In the western blotting of antral mucosae, GW7647 evoked phosphorylations of PI3K, Akt, and NOS1, which were inhibited by GW6471. The NOS1 phosphorylation induced by GW7647 was also inhibited by wortmannin and AKT 1/2 Kinase Inhibitor. GW6471 inhibited NO production in antral mucosae stimulated by GW7647, and wortmannin and AKT 1/2 Kinase Inhibitor also inhibited it. PPARα, PI3K and Akt exist in the cytoplasm of antral mucous cells. Thus, PPARα phosphorylates NOS1 via PI3K/Akt signal, which enhances Ca^{2+}-regulated exocytosis in antral mucous cells. (COI:No)

2P-177

Receptor activator of NF-κB ligand induces cell adhesion and integrin α2 expression via NF-κB

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Cell to the extracellular matrix interaction play critical roles in a variety of cell physiological functions. We have reported that receptor activator of NF-κB ligand (RANKL) specifically facilitates head and neck squamous cell carcinoma (HNSCC) progression in vivo; the molecular mechanism underlying this phenomena has yet to be determined, however. Here, we report a novel role for RANKL in the regulation of cell adhesion. Adhesion to type-I collagen, but not to fibronectin laminin, and Matrigel, was upregulated in RANKL-expressing cells. Among the major type I collagen receptors, integrin α2 was significantly upregulated in RANKL-expressing cells, and its knockdown inhibited cell adhesion to type-I collagen. We also found that RANKL induced integrin α2 expression via RANK-NF-κB pathway in an autocrine/paracrine manner. Interestingly, the amount of active integrin β1 on the cell surface was increased in RANKL-expressing cells through the upregulation of integrin α2 and endocytosis. Moreover, the RANK-integrin α2 pathway contributed to RANKL-dependent enhanced survival in a collagen-rich environment and inhibited apoptosis therein, demonstrating an important role for RANKL-mediated cell adhesion in three-dimensional environments. (COI:No)

2P-178

Mechanisms of PTHrP-induced smooth muscle relaxation in the guinea pig stomach

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PTHrP is known to relax vascular smooth muscle by increasing intracellular cAMP levels. Endogenous PTHrP released upon stomach wall distension may contribute to the receptive relaxation of stomach. However, the intracellular mechanisms underlying PTHrP-induced relaxation of gastric smooth muscle remain to be elucidated. In strips of gastric smooth muscle taken from the guinea pig stomach, PTHrP (1-100nM) suppressed spontaneous contractions in a dose dependent-manner. L-nitro-arginine (100μM), a nitric oxide synthase inhibitor, or ODQ (10nM), an inhibitor of guanylate cyclase reduced the inhibitory effects of PTHrP (10μM) by approximately 80 %, suggesting that PTHrP induces NO release to inhibit gastric smooth muscle. Surprisingly, SQ22536 (300nM), an inhibitor of adenylate cyclase, had only a marginal effect on the PTHrP-induced relaxations. Thus, PTHrP appears to exert its inhibitory action predominantly via a NO-cGMP pathway rather than by stimulating cAMP production. PTHrP may relax gastric smooth muscle by activating multiple intracellular pathways that can modulate each other by signalling cross-talk between cAMP and cGMP. (COI:No)

2P-179

Subcellular localization of the clock proteins, BMAL1 and CLOCK

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A circadian rhythm is a crucial factor in the regulation of a wide range of physiological processes such as the endocrine system and sleep. The systemic circadian rhythms can be broken down into cellular rhythms, which are maintained by periodic change in the status of a set of clock genes and proteins. For instance, BMAL1 and CLOCK play a key role in rhythm generation through transcriptional regulation of core clock genes. In addition to the expression levels, such proteins also oscillate at the levels of posttranslational modification. However, circadian changes in subcellular localization of clock proteins are poorly elucidated.We constructed the fluorescent biosensors for BMAL1 and CLOCK and observed their dynamics. Whereas BMAL1 and CLOCK were mainly localized in the nucleus as reported previously, CLOCK was also observed as granule-like structures in the cytosol. We next determined the organelle in which the CLOCK granules reside, by using fluorescent makers for a variety of the organelles. Constitutive expression of CLOCK biosensor and immunofluorescence with an anti-CLOCK antibody revealed that CLOCK granules were localized in the endoplasmic reticulum (ER). The BMAL1 biosensor was also co-localized with the ER marker when it was co-expressed with CLOCK, indicating that CLOCK recruits BMAL1 to the ER. Fluorescence imaging thereby revealed that BMAL1 and CLOCK also accumulated in the cytoplasmic organelle ER in addition to the nucleus, suggesting a novel role of CLOCK in regulating periodic changes in cellular function other than transcription in the ER. (COI:No)

2P-180

Cysteine persulfide-mediated sulfur transfer is required formethylthiolation of mammalian tRNAs in vivo

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The 2-methylthio (ms2) modification of tRNAs is essential for accurate decoding in all species. Deficiency of ms2 modification causes aberrant protein synthesis, which triggers the development of type 2 diabetes and mitochondrial diseases. In contrast to the important physiological contribution, the molecular mechanism of sulfur insertion in ms2 has been largely unknown. Here, we report that a considerable amount of sulfur in ms2 of tRNA was rapidly transferred from cysteine persulfide (CysSSH), which is a newly identified reactive cysteine species. The intracellular CysSSH firstly transferred sulfur atom to the Cys residues in a specific domain of ms2-modifying enzyme. The sulfur atom was then relay to the tRNA, and formed ms2-modification in combination of S-adenocylmethione. Furthermore, we show that lacking of CysSSH in pancreatic b-cells impaired ms2 modification, which attenuated insulin secretion. These results reveal that CysSSH is a major source of tRNA sulfidation and is essential for b-cell functions. (COI:No)

2P-181

Extracellular Cl^- ion dependency of hypotonic swelling of HeLa cells

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We previously reported that cell swelling after hypotonic challenge depended on extracellular Na^+ concentration. Furthermore, the cell swelling was inhibited by bumetanide and amiloride, those were NKCC and ENaC inhibitors. These data suggested that water influx after hypotonic challenge was partially driven by Na^+ influx via NKCC and ENaC. If the NKCC transporter is related on the hypotonic challenge cell swelling, the swelling depends on not only extracellular Na^+ concentration but also extracellular Cl^- concentration. In this study, we examined extracellular Cl^- dependency of hypotonic swelling of HeLa cells. Cl^- of an extracellular solution was substituted by gluconate, and the hypotonic cell swelling was measured by using flowcytometry. Although the maximum cell size after the hypotonic challenge increased linearly by the increment of extracellular Na^+, the Cl^- dependency of cell swelling showed biphasic; the cell swelling did not occur up to 10 mM extracellular Cl^- concentration, then cells started to swell in a Cl^- dependent manner. (COI:No)

2P-182

Coordinated effect of IL-17A and IL-27 inhibit the osteoclast differentiation of RANKL-stimulated RAW264.7 cells

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Periodontitis is a chronic inflammatory disease characterized by alveolar bone resorption. Inflammation-mediated bone loss is a major cause of various bone diseases, such as chronic periodontitis, and is due to an imbalance in bone remodeling that favors resorption. IL-17A is a proinflammatory cytokine that is mainly secreted by activated T cells. IL-27 is known to have multifaceted actions during immune responses, with both activating and regulatory roles. Previous studies reported the direct effect of RANKL-induced osteoclast differentiation from osteoclast precursors in IL-17A or IL-27 alone. However, the mechanism of the coordinated effect of IL-17A and IL-27 on osteoclastogenesis has not been reported. In this study, we confirmed the coordinated effect of IL-17A and IL-27 on RANKL-induced osteoclastogenesis. High expression of IL-17RC and IL27-R&alpha was detected on RAW264.7 cells. A coordinated effect of IL-17A and IL-27 suppressed osteoclast differentiation from RAW264.7 cells treated with RANKL without reduction of cell proliferation. Furthermore, the phosphorylation of JNK stimulated with RANKL was inhibited by the coordinated effect of IL-17A and IL-27. These data suggest the possibility that the coordinated effect of IL-17A and IL-27 inhibited the phosphorylation of JNK which is involved in the suppression of RANKL-induced osteoclast differentiation in RAW264.7 cells. (COI:No)

2P-183

Calcium imaging of dorsal root ganglionic neurons and fibroblast-like synoviocytes to mechanical stimulation in co-culture system

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In early phase of osteoarthritis, synovial inflammation may be related to pain or hydrarthrosis. Although neurogenic inflammation has been included in pathogenesis of osteoarthritis synovium, any interactions between the synovium and the sensory nerve are not sufficiently investigated. The aim of the present study was to investigate the interaction of sensory mechanisms to mechanical stimulation (MS) of fibroblast-like synoviocytes (FLS) in co-culture system with dorsal root ganglionic (DRG) neurons. Primary FLS isolated from adult mouse synoviums and primary sensory neurons isolated from DRG were co-cultured for 48 hours. Intracellular Ca $^{2+}$ concentrations ($[Ca^{2+}]_i$) of FLS and/or DRG neurons was measured with loaded Fluo-3AM. MS was performed with a glass micropipette. In single-culture of FLS, MS elicited immediate $[Ca^{2+}]_i$ increasing responses. This response of FLS remained in Ca $^{2+}$-free conditions, although the duration of the response was shorter than that in the presence of Ca^{2+}. The result suggested the possibility that the response of FLS to MS involved Ca $^{2+}$ release from the intercellular store (endoplasmic reticulum). In the co-culture system, MS elicited immediate $[Ca^{2+}]_i$ increasing responses in FLS and delayed ones in DRG neurons. MS of synovium Ca $^{2+}$-dependently elicited signal transmission from FLS to sensory neurons. (COI:No)

2P-184

Observation of exocrine organs in open aqueous solution by atmospheric scanning electron microscopy

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Mammals have 3 pairs of major salivary glands, the parotid glans, submandibular glands and sublingual glands. These glands secrete serous, mucous or mixed saliva via the proper main excretory ducts connecting the glandular bodies with the oral cavity. The hallmark characteristic of Sjögren's syndrome is diminished secretory production from the primary exocrine gland and the lacrimal or salivary glands resulting in symptoms of dry eye and mouth. The disease is believed to be mediated by an inflammatory and autoantibody response directed against salivary and lacrimal gland tissues. However, the mechanism underlying this disease remains poorly understood. We analyze the cell morphology of the salivary glands using an atmospheric scanning electron microscope (ASEM). In the ASEM, wet tissues are placed on a silicon nitride-film window in the base of an open sample dish, immersed in radical scavenger D-glucose solution, and a 2- to 3-μm specimen thickness from the film was directly observed from below by an inverted SEM . Thus, the time-consuming pretreatments generally required for biological samples to withstand the vacuum of a standard electron microscope are avoided. The secretion granules in salivary glands were fixed, stained with phosphotungstic acid and visualized in aqueous liquid using the ASEM. The results indicate that the ASEM has a potential that can be a new method of detecting an abnormality of the secretory granules of Sjögren's syndrome. (COI:No)

2P-185

Suppression of the HeLa cell proliferation by Cs without the effect on the membrane potential

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We have already reported that the application of CsCl to the culture medium suppressed the HeLa cell proliferation without the G0/G1 arrest. Cs is known as the K^+ channel blocker and many reports have shown that several types of the K^+ channel are involved in the cell proliferation The membrane potential reflects the reversal potential of K^+ in case K^+ permeability is dominant and the blocking of K^+ channels depolarizes the cell membrane. Therefore the membrane potential is considered as the important factor for the cell proliferation. In this study we measured the membrane potential of the HeLa cell and evaluate the effect of Cs on the membrane potential of the HeLa cell. To measure the membrane potential, HeLa cells are fused to form a giant HeLa cell using poly-ethylene glycol (PEG). The membrane potential was measured using the glass micro-electrode. The membrane potential of HeLa cells was around -20 mV. The perfusion of 10 mM CsCl did not affect on the resting membrane potential. We also applied 0.5mM quinine, known Ca^{2+}-activated K^+ channel blocker, which also failed to change the resting membrane potential. These data suggest that the membrane potential is not the important factor for the HeLa cell proliferation and Cs suppresses the HeLa cell proliferation by the different mechanism from the membrane potential. (COI:No)

2P-186

Mg^{2+} homeostasis of adult rat ventricular myocytes in primary culture

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To study physiological roles of Mg^{2+} channel/transporter proteins, we investigated, in a preliminary manner, the effects of transfection with non-targeting shRNA on transmembrane Mg^{2+} transport functions in ventricular myocytes of adult rats. Acutely isolated cells were plated onto laminin-coated glass-bottom dishes for culture, and were transfected with non-targeting shRNA and green fluorescence protein (GFP) by recombinant adenoviruses. After 72 hours, we used the GFP-positive and rod-shape cells for experiments of Mg^{2+} transport activities. The rates of Mg^{2+} influx and efflux were measured with a fluorescent indicator furaptra, as previously reported for acutely isolated cells (PLoS One 8:e73171, 2013). In the transfected cells, resting $[Mg^{2+}]_i$ was 0.99±0.05 mM, which was similar to that of acutely isolated cells, 0.88±0.03 mM. The rates of Mg^{2+} influx and efflux in the transfected cells were 0.24±0.09 μM/s (with initial $[Mg^{2+}]_i$ at 0.39±0.02 mM), and -1.44±0.15 μM/s (with initial $[Mg^{2+}]_i$ at 1.59±0.12 mM), respectively. These values were not significantly different from those obtained from acutely isolated cells: the influx rate, 0.27±0.043 μM/s at 0.35±0.016 mM and the efflux rate, -1.12±0.16 μM/s at 1.45±0.15 mM. With apparently unaltered function of cellular Mg^{2+} regulation, the primary cultured ventricular myocytes of adult rats may be used to study physiological pathways of Mg^{2+} transport by gene silencing of Mg^{2+} channel/transporter proteins. (COI:No)

2P-187

Comparative study on the oxidative modification of recombinant and plasma-derived human serum albumin: thiol oxidation and carbonylation

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Commercially available human serum albumin (HSA) products have been widely used in both laboratory and clinical fields. As various kinds of recombinant HSA (rHSA) can be available now, we examined the oxidative chemical modification (thiol oxidation and carbonylation) of rHSA, compared with those of plasma-derived HSA (pHSA) (all were obtained from Sigma-Aldrich Co. USA). For rHSA, product nos. A9731, A6608 and A7223 are expressed in Oryza sativa, Saccharomyces cerevisiae and Pichia pastoris, respectively. For pHSA, the A1653 is an initial product from large-scale pooled human sera and the A3782 is a final product. The thiol-redox state and carbonyl content were analyzed by using both HPLC and Ellman methods, and Carbonyl Assay Kit (Cayman Chem., USA), respectively. The HMA values of A6608 and A7223 were significantly higher than that of A1653 ($P < 0.01$), and those HNA-1 values were significantly lower than that of A1653 ($P < 0.01$). Dimer fraction was observed in all products except for A6608. Positive correlations were observed between HMA (%) and thiol content ($r = 0.857$), and between HNA (%) and carbonyl content (nmol/mg albumin) ($r = 0.480$). These results suggest that various degree of oxidative modification of rHSA may occur during their expression and purification processes. Therefore, it is necessary to consider the heterogeneity of rHSA products, when researchers use these recombinant products in their own research field. (COI:No)

2P-188

The role of cyclin D3 in enucleation of mouse erythroblasts

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During the late stage of mammalian erythropoiesis, an erythroblast is divided from four to five times. Erythroblasts gradually decrease the size as divided. Finally, a nucleus moves in one cytoplasmic direction and an erythroblast divides into a reticulocyte and a condensed nucleus covered by a cell membrane.Cyclin D3 is one of the members of the cyclin D family that play critical roles as core members of the mammalian cell cycle machinery. Once induced, cyclin D binds to and activates the CDK4 or 6 and promotes progression from the G1 to S phase of the cell cycle. Afterward, cyclin D3 was polyubiquitinated and degraded by proteasome. As the results of the analysis of Cyclin D3 knockout mice, it is thought that cyclin D3 regulates the number of cell divisions that erythroblasts undergo during terminal differentiation, thereby controlling erythrocyte size and number.Here, we performed the analysis about the role of cyclin D3 in the enucleation of the erythroblast. We use in vitro enucleation system of mouse erythroblasts. And extruded nuclei are analyzed by using the cell-permeable DNA staining dye SYTO16 and flow cytometer. As the results, we show that Cyclin D3 protein is degraded during enucleation and erythroblasts are not enucleated in the presence of proteasome inhibitors. These results suggest that the degradation of cyclin D3 correlates with progression of enucleation of mouse erythroblasts. (COI:No)

2P-189

Effect of Dehydroepiandrosterone for CPD stored blood on rheological function of erythrocytes

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Dehydroepiandrosterone (DHEA) has a preventive effect over nerves system, under which oxidative stress is pathologically important. On the other hand, blood viscosity was increasing during storage at 4 °C, and one of the causes of that was oxidative damages of erythrocyte membrane. We study to evaluate the rheological properties on stored blood with DHEA at 4 °C. A Viscosity of one week stored blood contain with citrate-phosphate-dextrose (CPD) that was measured by cone-plate viscometer, increased. However, Viscosity of stored CPD blood with 1uM DHEA increased lesser than without them. Erythrocyte deformability was measured with high-shear rheoscopy, the deformability of red blood cell during 1 week storage decreased. However, the decrement of erythrocyte deformabilites during blood storage with 1uM DHEA decreased. We report the preventing mechanism against oxidative stress during blood preservation on rheological properties by DHEA. (COI:No)

2P-190

Evaluation of activities of tissue-type plasminogen activators expressed and retained on vascular endothelial cells

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We reported that tissue-type plasminogen activator (tPA) secreted from vascular endothelial cells (VECs) is retained on the cell surface and effectively evokes both plasminogen activation and fibrin clot dissolution (fibrinolysis) on VECs. To elucidate the findings, here we quantitatively analyzed these distinct events evoked by secreted green fluorescent protein (GFP)-conjugated tPA from cultured human VECs (EA.hy926 cells). Strong correlations were obtained between the amount of secreted tPA-GFP and the plasminogen activation activity (r=0.88) as well as the fibrinolytic activity (r=-0.96). Two variant tPAs, TNK-tPA (T103N, N117Q, K296A, H297A, R298A, R299A) and K-tPA (K296A, H297A, R298A, R299A)-GFP, showed less surface retention and less VEC-associated plasminogen activation activity, which coincided with less accumulation of plasminogen on the VEC surface, whereas they showed stronger fibrinolytic activity on VECs compared to wild-type tPA. Our novel quantitative assay demonstrated that cell-generated tPA evoked plasminogen activation and fibrinolysis on VECs. However, TNK-tPA, which dissociates easily from the membrane and is known to be more resistant to PA inhibitor-1, evoked less activation of plasminogen and much more effective fibrinolysis, which may explain its superior clinical efficacy in thrombolytic therapy with a longer half-life. (COI:No)

2P-191

Effect of high-fat diet on α_2-antiplasmin knock out mice .

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α_2-Antiplasmin (α_2-AP) is an important factor in the fibrinolysis system, which works as a endogenous inhibitor of plasmin, a key enzyme of fibrin degradation. In this study, we examined the influence of high-fat diet (HFD) on mice with α_2-AP gene deficient (α_2-APKO). Both α_2-APKO mice and wild type control (α_2-AP WT) mice were fed HFD over 17weeks, and blood sample was taken from Juglar vein every 2 weeks. Then, plasma biochemical parameters and histological analysis were performed. In KO mice weight gain was more remarkable than WT mice. Especially, liver weight, but not fat tissue, was significantly higher in KO mice. In addition, plasma alanine transaminase (ALT) was significantly increased in KO mice but not in WT mice after HFD feeding. These results suggested that α_2-APKO mice caused non-alcoholic steatohepatitis (NASH) by HFD feeding. (COI:No).

2P-192

Effect of oral administration of Saponins from Panax Ginseng on redox status of mice erythrocyte membrane proteins

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Erythrocyte membrane proteins' thiol is good index of the oxidative damage. And an age-dependent decrease in membrane proteins' thiol was reported. We studied to evaluate protective effects against oxidative stress on redox status of mice erythrocyte membrane by oral administration of saponins extracted from Panax ginseng. Adult C57BL/6 mice were used as experimental animals. Crude saponins were given orally (0.2μg/g weight mice). After the administration, mice blood was collected under anesthetizing. Thiol contents of mice erythrocyte membrane proteins were measured by Ellmans' reagents. Thiol-group of membrane proteins increase by administration of saponins. And we have screened the components of saponins extracted from Panax ginseng and identified ginsenoside Rh1 as the active ingredient. Rh1 did not have antioxidant activity in an aqueous phase. However, it inhibited the oxidation-induced decrease of thiol in membrane proteins. (COI:No)

2P-193

Early life exposure of perfluorooctane sulfonate causes cerebellar dysfunction possibly through suppression of iodothyronine deiodinase 2 activity.

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Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been used in a wide variety of industrial and commercial products. The adverse effects of PFOS in the developing brain are becoming of a great concern. To investigate the effect of PFOS or PFOA, we examined the change in TH-induced dendrite arborization of Purkinje cells in primary rat cerebellar culture. As low as 10^{-7} M PFOS but not PFOA suppressed thyroxine (T4)-induced dendrite arborization. Interestingly, triiodothyronine (T3)-induced dendrite arborization was not. We examined the effect of early life exposure of PFOS using 10-12 w mice by rotarod test and showed that pre- and postnatal exposure of PFOS resulted in markedly shorter time on rotarod compared to control. Reporter gene assay showed that either PFOS or PFOA did not affect TH receptor-mediated transcription in CV-1 cells. Semi-quantitative RT-PCR showed that PFOS suppressed iodothyronine deiodinase 2 (D2) mRNA expression in primary cerebellar cells. D2 activity was suppressed by PFOS in C6 cells. In summary, early life exposure of PFOS disrupts TH-mediated cerebellar development possibly through disruption of D2 activity and/or mRNA expression, which may cause cerebellar dysfunction in adult mice. (COI:No)

2P-194

Involvement of vitamin D receptor in the generation of rhythmic Ca transient in murine atrial myocyte-derived cell line HL-1

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The biologically-active form of vitamin D_3, 1,25-dihydroxyvitamin D_3, has diverse effects on various tissues, including the cardiovascular system, through vitamin D receptors (VDR). In this study, to clarify the role of VDR in the cardiovascular system, we examined the effects of VDR knockdown on Ca transient, action potentials and gene expressions in murine atrial myocyte-derived cell line HL-1. VDR knockdown using siRNA resulted in approximately a 50% reduction of its mRNA. HL-1 cells treated with control siRNA (Cont cells) showed 80%, 0% and, 20% of rhythmic, arrhythmic and sporadic/none Ca transients, respectively, while the cells treated with siRNA for VDR (VDR^- cells) showed 20%, 13.3% and 66.7%, respectively, which indicates the reduction of rhythmicity of HL-1 cells. This reduction was recovered by the concurrent transfection of human VDR plasmid, resulting in the rhythmicity classification of 81.3%, 12.5% and 6.3%, respectively. In addition, membrane potentials measured with the whole cell patch clamping were synchronized with the changes of Ca transient both in Cont and VDR^- cells. DNA array analysis between Cont and VDR^- cells showed the significant reductions in mRNA levels of cardiac myosin-binding protein C gene, PDZ and LIM domain protein 3, actinin 2, Zac1. These results suggest that VDR is involved in the generation of rhythmic Ca transient in HL-1 cells, possibly regulating several gene expressions. (COI:No)

2P-195

Perinatal mild hypothyroidism induced learning and memory disorders in adult mice

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Thyroid hormone (TH) is one of the critical factors for brain development. Untreated severe hypothyroidism in infancy causes irreversible cretinism. Milder hypothyroidism may also affect cognitive development. However the effects of mild and/or moderate hypothyroidism on brain development is not fully understood. In this study, we examined the behavior of adult mice which were rendered mild hypothyroid during perinatal period using low dose propylthiouracil (PTU). PTU was given through in drinking water (5 or 50 ppm) from gestational day 14 to postnatal day 21. Cognitive performances studied by novel object location test (OLT) were impaired in PTU-treated mice on postnatal week 8. This result suggests that even hypothyroidism is mild; it may partially prevent cognitive function. Thus we next measured the concentrations of neurotransmitters (glutamate, gamma amino butyric acid (GABA), and glycine) in the hippocampus using in vivo microdialysis under OLT. We found that the concentrations of neurotransmitter, particularly glutamate and glycine, were decreased in PTU-treated mice. These data indicate that a mild perinatal hypothyroidism cause learning and memory disorder in adult mice induced by decrease in concentrations of neurotransmitters. (COI:No)

2P-196

Reduction of aggressive behavior by pre-pubertal, but not adult site-specific knockdown of estrogen receptor β in the medial preoptic area of male mice

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Testosterone (T) is known to play an essential role in the regulation of male social behaviors. T regulates these behaviors via estrogen receptors (ER), ERα and ERβ, after being aromatized. We previously reported that ERα in the medial preoptic area (MPOA) is necessary for the facilitation of sexual but not aggressive behavior (Sano et al, EJN, 2013). However, site-specific role of ERβ in the MPOA for these behaviors is still unclear. Thus, we site-specifically knocked down ERβ (βERKD) in the MPOA during pre-pubertal period and examined the effect on male social behaviors. At 3 weeks of age, gonadally intact male mice were injected bilaterally either with viral vectors silencing ERβ or a control vector in the MPOA. They were tested for sexual and aggressive behavior in adult. Knockdown of ERβ reduced the levels of aggressive behavior, without affecting sexual behavior. Thereafter, we conducted βERKD in the MPOA post-pubertally, at 12.2±1.00 weeks of age. Two weeks after injection, they underwent behavioral tests. Surprisingly, βERKD only in adulthood affected neither sexual nor aggressive behavior. These results suggest that ERβ in the MPOA during pubertal period may necessary for facilitatory action of T on male aggressive behavior in adult. (Supported by KAKEN #23240057 & #15H01844 to SO.) (COI:No)

2P-197

Characteristics of fatty acid metabolisms and contractile responses of uterus isolated from ovariectomized rats.

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The ovarian hormones (OH) indicate various effects on smooth muscles. To investigate the effects of OH on uterus, we determined fatty acid composition and the contractile responses of uterus isolated from ovariectomized rat. Eight-week-old female rats were divided into two groups: sham-operated group (Control) and ovariectomized group (OVX). Four weeks later, fatty acid composition and fatty acyl coenzyme As (CoA) were determined in uterus, and isometric contraction of uterus was recorded with a strain gauge transducer. Concentration of stearic acid and arachidonic acid in OVX were about 3 times less than those in Control. Oleic acid, linoleic acid and linolenic acid significantly increased in OVX. The rate of n6/n3 fatty acids significantly increased, but CoA significantly decreased in OVX. KCl (80 mM)- and $PGF_{2\alpha}$(1μM)-induced contractions were significantly greater than those in Control.Ca^{2+} concentration dependent contraction in Ca^{2+}-free Krebs solution was significantly greater in OVX than that in Control. Tonic component of $PGF_{2\alpha}$-induced contraction was significantly suppressed in uterus from rat treated with eicosapentaenoic acid (300 mg/Kg/day) by oral administration for 2 weeks in OVX. These findings suggested that OH affected characteristics of fatty acid metabolism, Ca^{2+} regulation system and $PGF_{2\alpha}$ receptor in uterus. (COI:No)

2P-198

Elevation of blood pressure in F2 offspring of dams delivered from carbohydrate-restriction during pregnancy

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[Background & Aims] It has been reported that more than 25% of 20th and 14% of 30th women are lean in Japan. Moreover, average birth weight is decreasing and incidence of low birth weight delivery is increasing from 80th to the present. According to DOHaD hypothesis, low birth weight is reportedly related to an increased prevalence of common cardiovascular and metabolic disorders later in life. However, the mechanisms underlying low birth weight-induced hypertension has not been fully understood. We therefore tried to clarify the effect on blood pressure of offspring and grandoffspring of female rats fed low carbohydrate diets during pregnancy.[Methods] Female Wister rats (F0) were mated with control males and restricted their carbohydrate and calorie to 60% of control during pregnancy. F1 offspring were then weaned to adequate diets into adulthood. F1 dams were maintained on an adequate diet consumed ad libitum post weaning, and then their offspring (F2) were obtained. Their blood pressure and plasma hormone levels were measured.[Results] Mean body weight at birth of F1 offspring delivered from carbohydrate-restricted dams and their grandoffspring (F2) was significantly lower than controls. F1 and F2 offspring showed elevated systolic and diastolic blood pressure compared to controls. Plasma corticosterone but not aldosterone concentrations of both F1 and F2 offspring were significantly higher than controls.[Conclusion] It is possible that the intergenerational transmission of blood pressure regulation is programmed in part by the mothers nutritional status during pregnancy. (COI:No)

2P-199

A novel purification method for mouse ES cell-derived hypothalamic progenitors

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Mouse embryonic stem cells (mESCs) can differentiate into hypothalamic progenitors via embryoid body (EB) formation in a growth factor-free medium. In this method, about 70% of cells acquire hypothalamic identity, however, the remaining portion contains undifferentiated cells that may interfere the survival of induced neurons. To overcome this problem, we have previously purified hypothalamic progenitors by GFP labeling and fluorescence-activated cell sorting (FACS), which was often cytotoxic for the progenitors. In this study, we newly developed an antibody-based purification method without genetic manipulation and significant cytotoxicity. We have identified several antigens specific to non-hypothalamic lineage cells by analyzing surface antigen profiles of EB cells. Using the antibody cocktail against these antigens, we labeled non-hypothalamic cells with fluorophore or magnetic beads and then depleted the labeled cells by FACS or magnetic-activated cell sorting (MACS), which retrieved hypothalamic progenitors with purity >90%. MACS surpassed FACS in the recovery rate and viability of the purified cells. Subsequent differentiation steps generated a variety of hypothalamic neurons without emergence of undifferentiated cells. The regenerative hypothalamus with method would be useful on basic sciences, drug-development, and regenerative medicine. (COI:No)

2P-200

Oxytocin protects primary hippocampal neurons from corticosterone-induced apoptosis

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Oxytocin (OT), a neuropeptide produced in the paraventricular nucleus of the hypothalamus, plays an active role in adaptive stress-related behavioral responses. The hippocampus, a critical structure for spatial learning and memory, is particularly vulnerable to stress-induced glucocorticoid damage, evidenced by stress-induced atrophy and loss of neurons in the adult hippocampus. In contrast, chronic antidepressant treatment reverses the atrophy and damage in hippocampus caused by stress. OT is released in response to stressful stimuli, and has been shown to have an antidepressant- and antianxiety-like effects in animal studies. OT may exert anti-stress effects by protecting hippocampal neurons from the damaging effects of glucocorticoids. In this study, OT significantly reduced the number of TUNEL-positive cells in primary cultures of hippocampal neurons treated with corticosterone. The caspase-3 activity in OT-treated hippocampal slices was significantly lower than that of controls. OT also increased phosphorylation of Akt and CREB in primary hippocampal cultures. Both Akt and CREB are known to be involved in cell survival and anti-apoptosis. These results suggest that OT protects primary hippocampal neurons from corticosterone-induced apoptosis via phosphorylation of Akt and CREB. OT has a therapeutic potential for the treatment of stress related disorders. (COI:No)

2P-201

The Gper1 agonist G1 inhibits IGF-1-induced lactotroph proliferation

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In addition to their well-known stimulatory action, estrogens have an anti-proliferative effect. Estrogen binds to the nuclear estrogen receptor (ER) to modulate gene expression and thereby regulates essential functions of estrogen-responsive cells. However, recent studies have shown that estrogen also binds to the membrane ER to modulate cytoplasmic protein kinase signaling cascades leading to non-genomic actions. We have previously shown that suppression of gene expression of Bcl3 is involved in the anti-proliferative action of estrogen in pituitary lactotrophs. However, these results does not necessarily exclude the possibility that estrogen binds to the membrane ER to induce the anti-proliferative action. Therefore, we investigated whether either the nuclear or membrane ER including G protein-coupled estrogen receptor 1 (Gper1) mediates the inhibiting action of estrogen on insulin-like growth factor-1 (IGF-1)-induced proliferation of pituitary lactotrophs in primary culture. 17β-estradiol (E2) inhibited IGF-1-induced proliferation while cytoplasmic membrane-impermeable bovine serum albumin-conjugated E2, which activates cytoplasmic membrane ERs but not nuclear ERs, had no effect on IGF-1-indued proliferation. Similar to E2, G-1, an agonist for the membrane ER Gper1, inhibited IGF-1-induced lactotroph proliferation in a dose-dependent manner while it scarcely modulated mRNA expression of estrogen-responsive genes. Our results suggest that G-1 inhibits IGF-1-induced lactotroph proliferation. (COI:No)

2P-202

Personalized metabolic profile estimations using oral glucose tolerance tests

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Oral glucose tolerance tests are used commonly to diagnose diabetes mellitus. However, blood glucose data and the changes in insulin induced by OGTTs contain information regarding intestinal absorption, hepatic control of glucose and insulin, pancreatic insulin secretion and peripheral tissue glucose and insulin control. Therefore, an appropriate dynamic model could reveal the above information from OGTT data.We developed an OGTT model containing five compartments for insulin dynamics and two compartments for glucose dynamics based on previous reports. Anthropometric data of individuals were used to assume the cardiac output. Simplex and Levenberg-Marquardt algorithms were then used to fit the data obtained from 42 normal subjects and eight subjects with DM. We found clear gender differences in the intestinal glucose absorption kinetics, glucose sensitivity in the pancreas, maximal insulin production capacity and endogenous glucose production. There were also differences between normal and DM subjects. For example, pancreatic and liver dysfunctions were evident in DM cases. The differences between normal and DM subjects in glucose and insulin dynamics in the pancreas, liver and peripheral tissues, such as insulin resistance, insulin secretion and the relative roles of glucose disposal in each organ, were demonstrated clearly and quantitatively in a time-dependent manner. 2015M3A9B6028310 (COI:No)

2P-203

How Salivary Oxytocin Levels Correlated to Maternal Emotionality during Late Pregnancy, Labour, and the Early Postpartum

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The purpose of this study was first to examine human oxytocin levels during late pregnancy, labour and the early postpartum and then to explore the associations between oxytocin levels and maternal emotionality. The subject was a healthy pregnant woman, whose saliva was examined on a daily basis. The collection of her saliva was conducted at a fixed time (10:00a.m) to minimize the influence of diurnal variations and extended from 37 weeks of gestation to four days postpartum. The subject was requested to describe in detail what she did and how she felt in her journal. The oxytocin level was measured by an enzyme-linked immunosorbent assay (ELISA) using the ELISA kit (Abcam, UK). The level of cortisol, the stress hormone, was also measured as a comparative indicator and assessed using ELISA. This study was approved by the Juntendo University Research Ethics Committee. The fluctuations of two hormones and the records the subject kept in her journal suggested a correlation: when she experienced stressful event, the oxytocin levels were reduced while those of cortisol augmented, and vice versa when she was relaxed, not agitated. These data indicated that oxytocin and cortisol levels were negatively correlated in a significant manner (r=-.460, p<.05). The findings of this study suggest that the measurement of salivary oxytocin levels during pregnancy, labour and the postpartum period has the potential to serve as a useful index of maternal emotionality. (COI:No)

2P-204

Ghrelin ameliorates ionizing irradiation-induced acute hematopoietic injury in mice

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Hematopoietic system is one of the most radio-sensitive tissues in the body. Ghrelin, a brain-gut peptide hormone, has been reported to attenuate gastro-intestinal injury in rats after 10 Gy-whole body irradiation (WBI). However, there have been few reports concerning the effect of ghrelin against the hematopoietic-injury induced by the "myelo-supressive doses" (3-5 Gy) of ionizing irradiation. In this study, we evaluated the effects of ghrelin against hematopoietic-injury induced by 3 Gy-irradiation using a mouse model of WBI. We also examined the *in vitro* cell-protective effects of ghrelin on the 3 Gy-irradiated peripheral blood lymphocytes (PBL). Dynamics of ghrelin levels in plasma and the stomach (a main source of ghrelin) of mice were also examined after 3 Gy-irradiation. In 3 Gy-WBI mice, administration of ghrelin significantly ameliorated the decline-ratio of peripheral white blood cell (lymphocytes and granulocytes) counts and red blood cell counts. Ghrelin also increased the survival-ratio of cultured PBL after the 3 Gy-irradiation. Plasma levels of active ghrelin in mice increased significantly 7 days after the WBI, and also did that of total ghrelin 7-30 days after 3 Gy-WBI. The current data indicate that ghrelin could be a potent protector or mitigator ameliorating the radio-injury of hematopoietic system at least in mice. (COI:No)

2P-205

A new animal model for hyperuricemia: in vivo evaluation of uric acid-lowering effects by calcium channel blockers

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<Background>The oxonic acid-treated rats which generally serve as an animal model for the study of hyperuricemia, were not uric acid (UA) "underexcretion type", but "overproduction type". We created UA underexcretion type hyperuricemia mice using a pyrazinamide (PZA), which promotes UA reabsorption by UA transporter 1 (URAT1). Moreover, we used this model to clarify the mechanism of urate-lowering effects by calcium channel blockers (CCBs).<Method>ICR mice were given PZA 400 mg/kg body weight (BW) (n =9) and control group was given DMSO (n =9). Benzbromarone (BENZ) group was given 3 mg/kg BW (n =7) or 10 mg/kg BW (n =6), CCBs group was given nilvadipine (NV) (n =9), nifedipine (NF) (n =9), and nitrendipine (NT) (n =8) after PZA administration. The blood and urine was obtained to measure its UA and creatinine, then UA excretion rate (UAER) was evaluated after 6.5 hours.<Result&gtUrine UA (15.2 in control and 1.2 in PZA, p&lt0.001) and UAER (13.3 in control and 1.2 in PZA, p =0.006) were significantly reduced in PZA group. UAER was significantly elevated in BENZ group (6.2 in 3 mg/kg BENZ, p =0.012 and 21.3 in 10 mg/kg BENZ, p =0.016) and CCBs group (7.3 in NV, p&lt0.001, 7.01 in NF, p&lt0.003, 7.3 in NT, p&lt0.001) compared with PZA group.<Conclusion>In this study, we clarified that urine UA and UAER was reduced in PZA group. The data could provide a useful model for hyperuricemia research. In addition, we elucidated the mechanism of urate-lowering effects by CCBs by the inhibition of renal UA reabsorption by interacting with URAT1. (COI:No).

2P-206

Metabolic Acidosis with Alkalinuria in Phosphate-Deprived Mice: a Role of Pendrin in Kidney Type B Intercalated Cells

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Ca-sensing receptor (CaSR), originally cloned in rat kidney by Riccardi et al. (1995), was very recently identified in the basolateral membrane of cortical collecting duct type B intercalated cells (IC-B) as well as of thick ascending limb of Henle?s loop in mice kidneys (Yasuoka et al, 2015). Physiological roles are certainly unknown. Methods: C57Bl/6J mice (10 weeks, male) were fed either normal diet (1% Pi + 1%Ca) or low-Pi (LP) diet (0.02% Pi + 1% Ca). On day 7, a 24-hr urine, blood, and kidney samples were collected. Results: Serum and urine Ca were significantly ($^{**}P < 0.005$) increased in mice with LP diet [serum, 9.9** mg/dl; urine, 2,279** μg/day], compared with control [serum, 7.4 mg/dl; urine, 88 μg/day]. Plasma pH was decreased significantly from 7.37 (control) to 7.26** in LP diet mice. Surprisingly, urine pH was unexpectedly increased from 6.3 (control) to 7.4** in mice with LP diet. Interestingly, cell-heights of IC-B and IC-A were significantly increased and decreased, respectively. Finally, immunostaining of luminal Pendrin and basolateral AE4 in IC-B was significantly ($^{*}P < 0.05$) and cooperatively increased. Conclusion: Cooperative stimulation of luminal Pendrin and putative H^+ back flux across the basolateral membrane of IC-B may cause "metabolic acidosis with alkalinuria". Basolateral CaSR in IC-B may be involved in hypercalcemia-induced, inappropiriate stimulation of Pendrin. (COI:No)

2P-207

The Physiological Roles of Moesin, an Actin-Binding Protein, in Renal Salt Reabsorption

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Tubular reabsorption of electrolytes in the kidney is an essential function in regulating fluid balance in the body. In the thick ascending limb of Henle (TAL), 20-40% Na^+ filtered by the glomeruli are reabsorbed by Na^+-K^+-$2Cl^-$ cotransporter type 2 (NKCC2). In humans, mutations in the gene coding for NKCC2 were identified in patients of Bartter syndrome type I, which is characterized by severe salt losing tubulopathy. Despite of the physiological importance of NKCC2 in NaCl homeostasis, the molecular mechanisms for its membrane trafficking have not been fully elucidated. An actin-binding protein, moesin was reported to play an important role in the cell surface expression of NKCC2 by *in vitro* experiments using LLC-PK1 cells. Here, we examined the physiological roles of moesin in the regulation of renal function *in vivo* by using male moesin-null ($Msn^{-/y}$) mice. Fractional excretions of electrolytes were significantly increased in $Msn^{-/y}$ mice compared to $Msn^{+/y}$ mice. GFR and blood pressure were decreased in $Msn^{-/y}$ mice. We revealed that cell surface expression level of NKCC2 was not significantly different between $Msn^{+/y}$ and $Msn^{-/y}$ mice by protein biotinylation and western blot, whereas the distribution of NKCC2 in the lipid raft was decreased in $Msn^{-/y}$ mice. Our results suggest that moesin might play an important role in the regulation of lipid raft localization of NKCC2 and in appropriate reabsorption of electrolytes in TAL. (COI:No)

2P-208

An extracellular calcium induces tension force development in pig urethra smooth muscle

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Back ground Recently we found the specific novel mechanism which is sensitive to extracellular calcium corelatd with the force development of contraction in pig urethral smooth muscle but not in detrusor. Thus, the aim of this study is designed to clarify the Ca2+-sensing system diversed on urethral smooth muscle.Materials and methods Pig lower urinary tracts were obtained from the abattoir. We peformed Tension Force Measurement, Ca^{2+}-imaging study, and Molecular Study.Results Using intact tissues, only urethral but not detrusor smooth muscle enhanced tension force by the gradual increase of extracellular calcium ($[Ca^{2+}]$out) in a concentration-dependent manner (up to 10 mM) . Various types of Ca^{2+} channel inhibitors failed to complete inhibition of the force development induced by 5mM $[Ca^{2+}]$out. On the other hand, Ca^{2+}-activated Cl^- channel inhibitor, niflumic acid well-inhibited the 5mM $[Ca^{2+}]$out-induced tension force by 22.0 ± 4.7 % . Further, the combination of 10 μM CPA and 10 mM caffeine completely diminished this 5mM $[Ca^{2+}]$out-induced tension force without any channel inhibitors. Neither Ca^{2+} sensing receptor (CaSR) related drugs nor Mg^{2+}, La^{3+}, Gd^{3+} had no effect on this 5mM $[Ca^{2+}]$out-induced tension force. In the molecular study, not only quantitative PCR but also Western blot analysis indicated the predominat expression of CaSR in urethral than in detrusor smooth muscle.Concluding message This specific novel mechanism in urethra but not in detrusor would be the most physiological function and be the target for the treatment of lower urinary tract dysfunctions. (COI:No)

2P-209

Physiological roles of ezrin in the formation of glomerular podocyte foot process

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Ezrin is highly expressed in the glomerular podocytes, and reported to form multi-protein complex with a scaffold protein Na^+/H^+ exchanger regulatory factor 2 (NHERF2), and podocalyxin, a major sialoprotein. Podocalyxin deficient mice died within 24 hrs after birth with anuric renal failure, whereas NHERF2 knockout mice did not show apparent renal phenotype. On the other hand, physiological roles of ezrin in the glomerular podocytes still remain unclear. Thus, we investigated the physiological importance of ezrin in the glomerular podocyte by using ezrin knockdown mice ($Vil2^{kd/kd}$) in this study. $Vil2^{kd/kd}$ mice exhibit no apparent glomerular dysfunction and morphological defects in podocytes. We also investigated the influence of ezrin defect on the Rho GTPase activities, since ezrin interacts with Rho GTPase dissociation inhibitor (Rho-GDI), which plays an essential role in the regulation of podocyte actin organization. In $Vil2^{kd/kd}$ glomeruli, RhoA was significantly activated than WT glomeruli at basal condition. Furthermore, In Adriamycin-induced nephrotic condition, $Vil2^{kd/kd}$ mice showed reduced susceptibility to the adriamycin induced glomerular dysfunction compared to WT mice. RhoA activity in $Vil2^{kd/kd}$ glomeruli was significantly upregulated, suggesting that loss of ezrin substantially protect the podocytes from injury-induced dynamic morphological change by stabilizing podocyte structure. Our results suggest that ezrin plays important roles in the regulation of podocyte structure rather than scaffolding of membrane protein in glomerular podocytes. (COI:No)

2P-210

Tyrosine phosphorylation-independent regulation of hyperactivation by extracellular Na^+ in hamster sperm

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Mammalian sperm have to undergo "capacitation" to become fertilization-competent. Capacitated sperm show a specialized flagellar movement called hyperactivation. We previously reported that the appearance of hamster sperm hyperactivation was delayed as the extracellular Na^+ concentration ($[Na^+]_e$) increased, and this delay was likely to be caused by an action of Na^+/Ca^{2+} enchanger (NCX).In the present study, we examined whether the capacitation-associated tyrosine phosphorylation of fibrous sheath (FS) proteins of sperm were changed by $[Na^+]_e$ using mTALP media where NaCl concentrations were varied from 75 to 150 mM. The results showed that the tyrosine phosphorylation of FS proteins were unaffected by the $[Na^+]_e$, unlike hyperactivation. SN-6, an inhibitor of NCX, also did not affect tyrosine phosphorylation of FS proteins. Next, we investigated the detailed change of the parameters of the flagellar movement by $[Na^+]_e$. Flagellar bend angle and sliding velocity are suppressed by $[Na^+]_e$ at the onset of incubation, and the suppression was time-dependently abolished. Na^+ concentration of the oviductal fluid was higher than that of mTALP. K^+ concentration of the oviductal fluid was also higher than that of mTALP, and such K^+ concentration also delayed hyperactivation when compared to standard mTALP. These results suggest that release from suppression by oviductal ions via tyrosine phosphorylation independent pathway has important role in expression of hyperactivation. (COI:No)

2P-211

RNA interference of BCAR3 and FOSL1 genes induces the inhibition of proliferation of MDA-MB-231 breast cancer cells stably transfected with estrogen receptor

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Treatment with E2 (17β-estradiol) inhibits the proliferation of estrogen receptor (ER) negative breast cancer cells stably transfected with ERα cDNA. The elucidation of the ER-mediated anti-proliferative mechanism will provide a novel strategy for the treatment of breast cancer. The ER negative MDA-MB-231 breast cancer cells were stably transfected with ERα cDNA and 5 clones (MDA-ERs) were established. Although the treatment with 10 nM E2 for 72 h inhibited the proliferation of all MDA-ERs, 24 h treatment with E2 induced the inhibition of proliferation only in MDA-ER#3 clone. To find specific genes involved in the E2-induced inhibitory mechanism in MDA-ER#3, cDNA microarray analysis was applied to MDA-ER#1 and MDA-ER#3 treated with 10 nM E2 or vehicle for 4 h. A higher responsiveness for E2-upregulated gene expression was observed in IGFBP3, JAZF1, TOB1, and INHBB in MDA-ER#3 than in MDA-ER#1. The knock down of those genes by RNA interference, however, had no effect on the E2-induced inhibition of proliferation in MDA-ER#3. On the other hand, a higher responsiveness for E2-downregulated gene expression was observed in BCAR3, FOSL1, ENC1, FOXQ1, and TNFAIP8 in MDA-ER#3 than in MDA-ER#1. The RNA interference of only the two genes, BCAR3 and FOSL1, induced the inhibition of proliferation in MDA-ER#3. These results suggest that E2-induced inhibition of specific gene expressions such as BCAR3 and FOSL1 may be involved in the anti-proliferative mechanism of E2 in MDA-ER. (COI:No)

2P-212

Clarifying the relationship between saliva amylase upon waking and sleep quality during phases of the menstrual cycle in healthy women

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The present study aimed to clarify the relationship between saliva amylase upon waking and sleep quality during phases of the menstrual cycle in healthy women. Sleep quality during the menstrual cycle of 11 women was measured for about 1 month. Basal body temperature and saliva amylase during the ovarian follicular, corpus luteum, and menses phases were measured before rising from bed in the morning, and sleep quality was assessed using a mat-type device. No significant differences were observed in saliva amylase between sleep start time and wake time. Basal body temperature was found to be higher before the menses phase began, and lower during the menses phase (r=-0.173, p=0.033). Significant differences were observed in basal body temperature between deep sleep (r=0.157, p=0.02) and sleep onset latency (r=0.143, p=0.035). Significant differences in saliva amylase were observed between total sleep(r=0.176, p=0.009), nocturnal awakening(r=0.166, p=0.017), deep sleep(r=0.259, p=0.000), sleep onset latency(r=0.279, p=0.000), and body motion(r=0.178, p=0.008). Women's basal body temperature is high when falling asleep, but it increases further during deep sleep. Our results may indicate differences in sleep quality among menstrual cycle phases. The quality of sleep may also affect the production of saliva amylase in the morning. (COI:No)

2P-213

Regulation of hamster sperm hyperactivation by progesterone and GABA

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Mammalian spermatozoa are capacitated in the oviduct before fertilization. Capacitated spermatozoa are firstly hyperactivated to create the propulsive force necessary for penetration of the zona pellucida, and next are acrosome reacted to bind to the oocyte. It has been recently shown that mammalian spermatozoa were hyperactivated by steroids, amines and amino acids.In the present study, we investigated whether hyperactivation of hamster spermatozoa is regulated by progesterone and γ-aminobutyric acid (GABA). Although hyperactivation of hamster spermatozoa was enhanced by progesterone, GABA significantly suppressed progesterone-enhanced hyperactivation in a dose-dependent manner. Suppression of progesterone-enhanced hyperactivation by GABA was significantly inhibited by an antagonist of the $GABA_A$ receptor (bicuculline). Moreover, progesterone bound to the sperm head, and this binding was decreased by GABA.Because the concentrations of GABA and progesterone change in association with the estrous cycle, these results suggest that GABA and progesterone competitively regulate the enhancement of hyperactivation through the $GABA_A$ receptor. (COI:No).

2P-214

Minocycline did not affect the suppression by lipopolysaccharide of pulsatile luteinizing hormone secretion in ovariectomized rats.

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Inflammatory/immune challenge is known to suppress both pulsatile luteinizing hormone (LH) secretion and LH surge. We previously reported that pretreatment with minocycline, a potent inhibitor of activation of microglia/macrophage, significantly alleviate the inhibition of ovarian steroid-induced LH surge by lipopolysaccharide (LPS) in ovariectomized (OVX) rats. In this study, we examined the effect of minocycline on the suppression of pulsatile LH secretion by LPS in OVX rats. Rats were ovariectomized at least two weeks before the day of experiments. Minocycline or saline was administered intraperitoneally once a day for four consecutive days including the day of experiment. Blood samples were collected at 6-min intervals for 3 hours from free moving rats. LPS or saline was injected intravenously 1.5 hours after control sampling. Serum concentrations of LH were determined by radioimmunoassay. Minocycline treatment by itself did not affect pulsatile LH secretion. LPS treatment significantly suppressed the pulsatile LH secretion. Minocycline pretreatment did not affect the suppression of the LH secretion by the LPS treatment at all. These results indicate that minocycline attenuate the suppression of the ovarian steroid-induced LH surge but not the pulsatile LH secretion by LPS treatment, and suggest mechanisms in suppression of LH secretion by LPS might be different in LH surge and pulsatile LH secretion. (COI:No)

2P-215

Effects of postnatal nicotine exposure and maternal-like behavior on maternal behavior and spatial learning in adulthood of female rats

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Women who smoke during pregnancy and parenting are increasing risk to children's IQ. In addition, deficits in learning and memory in children have been associated with maternal smoking during pregnancy. Further, nicotine exposure to dam during pregnancy exhibited deficits in maternal behavior in rats. Though juvenile rats are known to show a subset of maternal behavior, no studies have examined the effect of experience of maternal-like behavior in the presence of nicotine on maternal behavior in adulthood of female rats. The present study investigated the effect of exposure nicotine during postnatal day (PD) 25-31 and juvenile maternal behavior at PD 25-31 on maternal behavior at PD 53-57 and Y maze test for special learning assessments at PD 71-75, and serum prolactin (PRL) concentration at PD 78 of Long-Evans female rats. Four groups of control (C), maternal-like behavior (M), only nicotine (10 μg/ ml in drinking water; N) and nicotine+ maternal-like behavior (NM) were established. In maternal behavior test, rats in each group were tested again for their latencies to induce maternal behavior. Higher Y-maze performance and PRL concentration were found in N group compared to C group. NM group but not M group showed reduced maternal behavioral latency compared to C group. Taken together, postnatal nicotine exposure increases spatial learning and PRL concentration, and potently affects maternal behavior in adulthood following experience of maternal-like behavior. (COI:Properly Declared)

2P-216

Generation of various ion channels-expressing functional airway epithelial cells from induced pluripotent stem (iPS) cells

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Background: Primary airway epithelial cells have been used for understanding of ion channel properties and airway disease such as cystic fibrosis caused by mutations of cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. However, it is not easy to acquire an adequate quantity of cells and maintain in culture for long time. Therefore, airway epithelial cells generated from iPS cells are expected to be a useful cell source instead of primary airway epithelial cells. The aim of this study is to generate functional airway epithelial cells from iPS cells. Methods: We have generated functional airway epithelial cells from iPS cells based on serum-free conditions and air-liquid interface culture. iPS cell-derived airway epithelial cells were characterized by gene expression, immunoreactivity, and functional assay.Results: The cells generated from iPS cells expressed airway epithelium markers such as *Tubb4a*, *Muc5ac*, and *Krt5*. Furthermore, the expression of various ion channels including *CFTR* and *ENaC*was successfully detected. Interestingly *CFTR* was not detected in iPS cells, but *ENaC* was also expressed in iPS cells. The formation of tight junction was also confirmed. Conclusion: Airway epithelial cells generated by our method have functional characteristics and will be useful cell source for molecular mechanisms of airway function and disease, such as cystic fibrosis. (COI:No)

2P-217

Analysis of Neuronal Morphology of Developmental White Matter Injury Model Rat without Loss of the Neuron Number

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Developmental white matter injury (DWMI) caused by hypoxia-ischemia (H-I) in preterm infants is associated with permanent neurodevelopmental disabilities (paralysis and cognitive dysfunction). DWMI model rat that received H-I (RCAO and 6% hypoxia for 1 hour) at P3 shows hindlimb motor dysfunction without neuronal loss. Cortical responsiveness by intracortical microstimultion (ICMS) revealed that motor map changed in DWMI model rat: the area of hip joint became smaller compared to control. To clarify how the motor map changes in DWMI model after development, we investigated the possibility that neuronal morphology in the sensorimtor cortex change in this model.Goldi staining was carried out in DWMI model focusing on the motor area (M1) of the hindlimb area, and the difference between the left side (control) and the right side (H-I) of their brains was evaluated. It revealed that the crosswise dendrites were increased in the middle layer (layer III-IV) on the right side, indicating that ipsilateral, contralateral, and/or thalamocortical projection could relates to the morphological change of the M1 area. Our data also suggest that this neuronal change might be involved in the motor map alteration and slight but confident motor deficit. (COI:No)

2P-218

In vivo imaging for age-dependent oxygen dynamics in brain using iridium complex probe.

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Oxygen (O_2) is critical component for brain function. It is supplied by blood flow. If it is stopped, ischemic brain becomes hypoxia resulting in functional disorders. Relationship between blood flow and ischemic neuronal cell death is well studied. However, O_2 dynamics in brain is not fully understood because of technical limitations. The functional remodeling after brain stroke occurs not only in the peri-infarcted area but also in healthy contralateral hemisphere. However the change in dynamics during functional remodeling has not yet been studied. Recently, $_2$ -sensing probe that contains iridium inside of the probe complex has been generated. This probe is small enough to spread in the extracellular space of brain and thus O_2 dynamics in brain may be studied. Thus, in the present study, we utilized one of the iridium complex probes, BTPDM1, to examine the O_2 dynamics in brain. We used multi-photon laser microscopy (FVMPE-RS, Olympus) to observe the O_2 dynamics during somatosensory stimulation in vivo. In this presentation, we would like to show the age-dependent change of O_2 dynamics in somatosensory cortex during somatosensory stimulation. We found a unique oscillation long time after stimulation in elder mice. Our results indicate that O_2 levels in brain may not be as stable as we expected. We plan to use this probe to examine O_2 dynamics during functional remodeling after stroke. (COI:No)

2P-219

Assembly of GABAergic circuitry by FoxG1, a gene associated with neurodevelopmental disorders

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The mammalian cerebral cortex is composed of a sophisticated neuronal network that processes higher order information such as perception, consciousness and memory. Thus, mutations in genes involved in the specification and migration of neurons as well as the formation of the correct synapses within the six-layered neocortex often lead to neurological diseases. Recent discoveries of both gain- and loss-of-function mutations in the transcription factor FoxG1 in patients with neurocognitive disorders strongly suggest that proper FoxG1 gene dosage is essential for mental health. By taking advantage of mouse genetic strategies, I have revealed that FoxG1 expression levels change dramatically during the course of embryonic brain development in a manner that is tightly correlated with the differentiation and maturation stage of neurons. I have demonstrated that these dynamic changes in FoxG1 expression are critical in the determination of the laminar identity of pyramidal neurons. Furthermore, I have found that FoxG1 is required at distinct developmental stages of GABAergic interneurons that play key inhibitory roles in the neocortical circuit. These findings provide clarity as to the dose-dependent requirement for FoxG1 and why even relatively minor changes in its expression during development result in severe neurological impairment. (COI:No)