1L1　An outsider's take on autism spectrum disorders

○**Martin Raff**
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Autism spectrum disorders（ASDs）are among the commonest neuropsychiatric disorders. Although, until recently, ASDs were one of the least understood of these disorders, they are now one of the best understood. Progress has come largely through recent advances in human genetics that have identified rare large-effect mutations that cause or greatly increase the risk of these disorders, together with studies of genetic mouse models based on these mutations. Remarkably, in a number of mouse models caused by single mutations, correction of the problem in the adult brain（with either drugs or genetic manipulations）largely reverses many of the behavioral and neurobiological abnormalities, providing hope for the development of therapies for individuals with ASDs. In my talk, I will review the basic features of ASDs, using home videos of the development of my 13-year-old autistic grandson as an example, and I will discuss some of the recent advances in ASD research, consider current puzzles, and speculate on possible ways forward.

2L1 A role of drebrin A in the activity-dependent trafficking of NMDA receptors to the plasma membrane

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NMDA receptors（NMDARs）are central molecular agents that enable the activity-dependent modification of synaptic strengths at excitatory synapses. However, relatively little is known about the molecular mechanisms regulating NMDAR levels at each synapse. We have previously used electron microscopic（EM）immunocytochemistry（ICC）to quantify the NMDAR levels at dendritic spines and to further differentiate the proportion of NMDARs occurring specifically at the plasma membrane, where they can bind to ligands, versus the cytoplasm, reflecting their reserve pools. In 2003, we demonstrated that this approach can successfully capture the activity-dependent trafficking of NMDARs from the dendritic shaft into the spine cytoplasm and to the plasma membrane following 30 min exposure of intact pyramidal neurons within cerebral cortex to the NMDAR antagonist, D-APV. We hypothesized that decreased activation of synaptic NMDARs by D-APV may increase NMDAR trafficking to the plasma membrane by promoting the tethering of NMDAR-containing saccules along the F-actin lattice. What might be the molecular agent that translates synaptic activity to the F-actin-mediated trafficking of NMDARs? Drebrin A is a good candidate to be this agent, because drebrin A finds to F-actin and is trafficked more into the spine head in response to D-APV blockade. This idea was tested by subjecting cortices of mice with global KO of drebrin A to D-APV blockade. Drebrin A KO cortices failed to up-regulate the NMDAR level at the plasma membrane within the hemisphere treated with D-APV, relative to the vehicle-treated hemisphere（0% ± 13% increase［mean±SEM］), while the WT cortices up-regulated NMDAR levels at spines 94% ± 28% following D-APV treatment, relative to the vehicle-treated side. Comparisons of spine head size and NMDAR levels at spines of vehicle-treated hemispheres comparisons did not reveal any difference across the KO-WT genotypes, suggesting that drebrin A is involved in the activity-dependent regulation of synaptic strength, rather than their basal levels.

3L1　Genetically encoded tools for brain analysis

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The striking progress in genome science and gene technology has led to numerous discoveries and the rapid development of new technologies in the life sciences. These new technologies include “optogenetics”—a growing suite of techniques that combine optical and molecular genetic methods. The technologies employ genetically encoded tools and are becoming popular particularly in neuroscience, where the central challenge is to understand the mechanisms by which neurons process and integrate synaptic inputs and how these mechanisms are modified by activity.

Since the isolation of the green fluorescent protein from the bioluminescent jellyfish in 1992 and the subsequent development of related molecules from non-bioluminescent marine animals, genetically encoded sensors that enable fluorescence imaging of excitable cell activity have been constructed by fusing fluorescent proteins to functional proteins that are involved in physiological signaling. Because these sensors can be introduced by gene transfer techniques, they may extract neuronal signals from an intact brain more efficiently than conventional organic dyes. Also, their expression is driven in a certain population of neurons by the use of a specific promoter；this has made visualization of the connectivity between two or more different（sub）populations of neurons all the more exciting.

On the one hand, many genetically encoded sensors have been developed to investigate the function of specific signaling mechanisms in synaptic transmission, integration, and plasticity. The sensors that monitor signals resulting from electrical activity, such as free-Ca^{2+} concentration and pH, instead of transmembrane voltage, function as low-pass filters. On the other hand, optogenetic control of neuronal activity allows us to selectively activate or inactivate genetically defined populations of neurons in order to examine how the activity of these neurons contributes to the function of neural circuits in the brain. Due to recent remarkable progress in gene transfer techniques, including electroporation, virus-mediated gene transfer, and germline transmission of transgenes, the experimental animals to be studied are not limited to mice but extended to primates. Newly emerging genetically encoded tools will surely stimulate the imagination of many neuroscientists, and this is expected to spark an upsurge in the demand for them.

1L2 Therapeutic strategy for brain disorders as systemic diseases

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In this talk, changes of conceptual framework of neurodegenerative disorders are reviewed and new therapeutic strategies are discussed for the disorders. Historically, neural cell death was the hallmark for diagnosing neurodegenerative disorders. Besides the cell death, in recent years, neuronal cell dysfunction has received more attention in pathophysiological aspects of the disorders. Studies of glial cells raised importance of their contribution in neural information processes, and a tripartite synapse hypothesis became a hot topic in neuroscience. More recently, prionoid-spreading hypothesis of disease-causing molecules attracted a great deal of attention, which has led, for example, to a wave of exsosome analyses of fluid samples from patients. On the other hand, involvements of the immune system and metabolism in the onset of neurodegenerative disorders were studied even before the modern molecular biology was introduced in science. Clinically, in addition to core symptoms, associated symptoms（for example, behavioral and psychological symptoms in dementia） are cues for clinical treatment of neurodegenerative disorders. Biomarkers for Parkinson's disease could be detected outside the brain, for example, using the cardiac scintigraphy method. Based on these trends, it is quite reasonable the idea of systemic disorders occurring in neurodegenerative disorders. Accordingly, brain-other organ-networks become important when considering the targets for clinical intervention of the disorders. Thus, therapeutic strategies must be reconsidered in the aspect that neurodegenerative disorders are the systemic diseases. Genetic factors as well as environmental factors must be studied in the new concept of neurodegenerative disorders.

1L3 Oxytocin and more：what we have learned from the brain development and its disorders

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Autism spectrum disorders（ASD）is not a sole disorder but a group of disorders, which is characterized by persistent deficits in social communication and social interaction as well as restricted, repetitive patterns of behavior and/or interests. So far, there exist no verified treatments for ASD. Oxytocin has attracts much attention for its potential as a new pharmacological treatment for ASD. Several studies demonstrated that an application of a single-dose of oxytocin nasal spray results in an improvement of the social communication of ASD patient for a while, but whether a continuous oxytocin application has pro-longed therapeutic effects or not remains open. Our Research Center for Child Mental Development at University of Fukui found that such continuous application is effective for a limited group of patients. This observation reinforced the concept that ASD is a mixture of disorders of which etiologies are varied. To elucidate such etiologies, we examined the role of specific neurons, of which function is likely to be involved in ASD characteristics as well as neural networks. In my talk, I will also introduce our recent attempts of such studies.

2L2 Is delivery a critical period in the pathogenesis of autism?

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The developing brain is not a small adult brain. The immature brain undergoes progressive alterations in molecular composition and in synchronized currents that enable neurons to fire and wire together and construct functional neuronal circuits. In the immature brain, large, synchronized patterns of neuronal activity engage many or possibly most neurons of developing brain networks, and are in contrast with the sparse firing, time-locked behaviourally relevant oscillations that occur in the adult nervous system. As well as the gradual and progressive molecular and brain activity changes that occur during development, there is also a large step-change during delivery (i.e. birth) that involves the maturation of various systems including microbiotic, endocrine, vascular and immunological. In contrast to the extensive amount of clinical, epidemiological and experimental information available on the links between genetic mutations and the cellular-molecular pathology of brain disorders, little is known on the impact they have on the sequential transition of brain activity characteristics during development and particularly birth. Collectively, these observations raise the possibility that the deleterious effects of intrauterine genetic mutations and environmental insults are mediated by the deviation of these developmental sequences of changing brain activity. It also raises the possibility that if these immature signatures persist into maturity, they are likely to continue to disrupt brain function over a lifetime. I have suggested the neuro-archeology concept according to which the persistence of these immature currents is instrumental in the subsequent clinical manifestations and a way to develop novel strategies based on specific antagonists that block immature currents in an adult brain. I shall illustrate this with our recent discoveries showing that in animal models of autism, the delivery GABA excitatory to inhibitory shift is abolished and its restoration by maternal administration of a diuretic attenuates the electrical and behavioural manifestation of autism in young and adult off springs. I suggest that delivery confirms, attenuates or aggravates embryonic pathogenic mechanisms. I shall also show the results of our clinical trials with a diuretic that confirm the usefulness of drugs that block immature properties in an adult pathological brain.

2L3 Neuronal calcium sensor proteins：contribution to the diversity of neuronal calcium signaling

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Calcium signaling has crucial roles in the control of neural function, and its abnormalities have been implicated in many aspects of neuropathology, neurodegeneration and psychiatric disorders. Ca^{2+}-regulated events occur within microseconds and persist over longer time scales, ranging from minutes to days. The specificity of the effects of Ca^{2+} on neuronal function is determined by the magnitude, kinetics and spatial localization of the Ca^{2+} signal. The transduction of changes in Ca^{2+} signaling requires Ca^{2+}-binding proteins. Neurons express a large number of Ca^{2+} sensor proteins ranging from synaptotagmin and annexins to over 250 different EF-hand containing proteins. The ubiquitous protein calmodulin is a well-characterized EF-hand containing protein with neuronal functions. A number of other EF-hand containing proteins are enriched or expressed only in the nervous system, where they have distinct roles in the regulation of neuronal function. These include the neuronal calcium sensor (NCS) protein family, members of which have been implicated in a very wide range of Ca^{2+} signaling events in neurons and photoreceptors. These range from very specific single functions for particular NCS proteins in the retina to more broad ranging functions in neurotransmitter release, channel and receptor regulation, control of gene transcription, neuronal growth and survival.A key issue regarding the NCS proteins is how they can differentially affect specific aspects of neuronal function. NCS protein function is determined by several factors according to their intrinsic properties, including their ability to interact with and regulate different target proteins. Here, I will discuss recent advances in the understanding of their physiological roles and the underlying target protein interactions that determine their specific functions. I will also highlight the abnormalities that have been implicated in many aspects of neuropathology, neurodegeneration and psychiatric disorders.

3L2 Gliomagenesis and GRIA2–An Integrated vertical study from Gene to Disease

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Glioblastoma multiforme is the most notorious bran cancer, and affects preferentialy adult with more than 60 years of age. Neural stem cells and/or glial progenitors of the subventrilar zone（SVZ）are suspicious of origin of gliomas. Recent studies unravelled that human and other mammalian brains contain a pool of neural stem cells throughout the life. Glutamate–mediated signaling plays a pivotal role for developmental and adult neurogenesis through ionotoropic glutamate receptor such as NMDA receptors（NMDAR）, and AMPA receptors（AMPAR）. Although NMDAR activation is essential for neuronal birth and survival at the developmetal stage, in contrast, adult neurogenesis is stimulated by blockade of NMDAR. AMPARs participate in fast neuronal transmission. GluA2 encoded by GRIA2 gene, a subunit of AMPA receptors, is the determinant of Ca2＋–permeability by downregulation of GRIA2, an impairment of RNA editing processes, and trafficking of GluA1 encoded by GRIA1. We have previously shown that activation of Ca2＋–permeable AMPA–type glutamate receptors facilitates the migration and proliferation of human glioblastoma cells（Nat med. 2012, J Neurosci 2007, J Neurosurg 2014）. Furthermore, there is accumulating evidence that Ca2＋–permeable AMPA receptors more widely play important roles in various human cancers than originally thought. We have found that blockade of NMDAR in the dentate gyrus of hippocampus restored disturbance of neurogenesis which was induced by radiotherapy. Adult hippocampal cell derived from GRIA2KO mice induced gliomagenesis indicating important role of GRIA2 in gliomagenesis. Regulation of NMDAR and AMPAR using specific inhibitors will be a novel attractive therapy for this devasting disease.

3L3 Searching a novel neurotransmitter/hormone through G–protein coupled receptor：Where now and where next?

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The G protein–coupled receptor（GPCR）superfamily includes approximately 1000 seven–transmembrane receptors that are involved in diverse physiological functions and many diseases. GPCRs are the most successful targets of all modern medicine, and –40% of marketed pharmaceuticals target human GPCRs. However, the endogenous ligands of some 80 GPCRs remain unidentified, leaving the natural functions of those GPCRs in doubt. These are the so–called orphan GPCRs, a great source of drug targets. Because of tremendous efforts toward deorphanizing GPCRs, striking successes such as ghrelin have been achieved, and greater understanding of many physiological responses has resulted from this success. Many GPCRs still remain to be deorphanized, but the rate of novel neurotransmitter/hormone discovery has dramatically slowed down in recent years. In this respect, I'll show you the reasons why the progress is very difficult, then, discuss the current approaches to overcome the hurdles in the coming years.

1L4 Promotion of glial cell research by virtue of transgenic approach

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Glial cells are present in every brain region and show complex interactions with neurons. Therefore, when observing or manipulating glial cell functions, glial cell-specific expression of functional molecules is vital. These functional molecules should be fully expressed in transfected glial cells in a cell type-specific manner to exert their activities. In general, however, it is not easy to simultaneously achieve both a high level and a cell type-specific expression. To accomplish cell type-specific expression of functional probes in the brain, many researchers have employed local viral injections as a means to introduce those genes. However, viral injection via a needle causes cerebral parenchyma injury and will induce substantial alterations in the nature of glial cells. Injury-induced augmentation of glial cell activity and subsequent cross-interactions between glia and neurons are inevitable to some degree. Yes, glial cells respond to injuries. If one compares this response to a "scream," and responses during normal interactions between glia and neurons to a "whisper," then the most intriguing scientific phenomenon for glia researchers is the "whisper" being washed out by the "scream". To date, there is no definitive evidence indicating responses to injury are extensive and normal responses are imperceptible. However, glia researchers need to implant functional molecules into glial cells like a "spy" to extract information and to alert them of any tiny response in glial cells. Using genetically modified mice, functional molecules can be expressed specifically in glial cells without injury. Furthermore, it is desirable that outcomes of the manipulation is extracted without injury. I believe that only through these types of efforts we can understand the glial cell function. I would like to emphasize that expression of functional probes in sufficient amounts in cell-type specific manner is the first step toward promoting glial cell research, and knockin-mediated enhanced gene expression-tet system (KENGE-tet) provides a strategy for achieving this. The second step is that we challenge glia to unveil overlooked their function by taking advantage of sufficient probe expression.

1S1-1 GABA as an inhibitory neurotransmitter in the mammalian central nervous system

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In the early 1960's any neurotransmitter substances had not been identified in the mammalian central nervous system except for acetylcholine in the motoneuron axon collaterals. γ–Aminobutyric acid（GABA）was demonstrated commonly in the nervous tissue but its inhibitory action was not considered identical to the synaptic transmission. Synaptic inhibition is mediated by membrane hyperpolarization but GABA did not induce it in the spinal cord. Then, we started identifying an inhibitory neurotransmitter of the cerebellar Purkinje cells which form inhibitory synapses upon neurons of the lateral vestibular nucleus（Deiters）and deep cerebellar nuclei in the cat. Following criteria were investigated by electrophysiological and neurochemical experiments：1. mimicry of synaptic transmission and pharmacological properties, 2. selective distribution in the synapses and 3. release during synaptic activation. Among several candidates, only GABA satisfied all items of the criteria. In those days no histological demonstration was available and high GABA content was shown in Purkinje cells and their terminals by the sensitive enzymic assay. These studies on Purkinje cells led a concept of GABA as a principal inhibitory neurotransmitter in the mammalian brain.In the 1990's gene targeting was introduced in neuroscience. We produced knockout mice for two isoforms of GABA–synthesizing enzyme（GAD）65 and 67 and disclosed several roles of GABA in development and behavior.

1S1-2 Identification and characterization of the muscarinic acetylcholine receptor and the high–affinity choline transporter

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Acetylcholine（ACh）is the first substance which was identified as a neurotransmitter. ACh is synthesized from acetyl–CoA and choline by choline acetyltransferase. The rate of ACh synthesis is limited by the rate of choline uptake from the extracellular space. In 1973, we identified the high–affinity choline uptake activity in rat brain synaptosomes, which was shown to be dependent on the presence of Na^+ ion and to be sensitive to inhibition by hemicholinium–3（Haga & Noda）. In 2000, we identified the molecular entity of the high–affinity choline uptake activity as a transmembrane protein, which was named as CHT1（Okuda et al. ）. A single nucleotide polymorphism（SNP）of CHT1 with the activity of 50–60% of that of wild–type CHT1 was identified. The frequency of the SNP was high among Asians including Japanese（13.0%）than among Caucasians（4.1%）or Africans（1.2%）. Supply of choline in the diet might be important for those with the SNP, particularly when they suffered from Alzheimer or other diseases with the lower cholinergic activity. In 1985, we purified muscarinic acetylcholine receptors（mAChRs）by using the affinity chromatography system（Haga & Haga）. In 1986, we cloned subtypes 1 and 2 of mAChRs by using partial amino acid sequences of purified mAChRs and identified the function of mAChR as G protein activator by using reconstitution system of purified mAChRs and G proteins in collaboration with groups of Numa, Matsuo and Ui. Thus studies on mAChRs, together with those on rhodopsin and beta adrenergic receptors, served to establish the concept of G protein–coupled receptors（GPCRs）. In 1992–1994, we demonstrated mAChRs to be phosphorylated by G protein–coupled receptor kinases in an agonist–dependent manner and to be internalized in a phosphorylation–dependent manner, which provides explanation in terms of molecular interactions, at least partly, for stimulus–dependent desensitization in cholinergic systems. In 2012, we determined a tertiary structure of mAChR subtype 2 by X–ray analysis in collaboration with groups of Kobilka and Kobayashi, which is expected to contribute to the theoretical design of drugs acting on mAChRs. Molecular properties of mAChRs and CHT1 were recently reviewed in Proc. Japan Acad. 9, 226（2013）and J. Biochem. 156, 181（2014）, respectively.

1S1-3 How to create exciting trends in Neurochemistry!

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Japanese Society for Neurochemistry（JSN）is the oldest and biggest society in the International Society for Neurochemistry.“Neurochemistry” is a science to understand the mechanism of the brain function at the molecular level in order to correlate behavior, morphology and molecules. After deepeningour understanding to correlate molecules and behavior, it is possible to manipulate the molecules to modify behavior and morphology. We have been actively working in JSN in these decades. I here describe some of the examples of the activity of neurochemistry taking the examples of the research going on in my laboratory. To understand the complex structure of the brain which exerts variety of functions including learning & memory and behavior, it is necessary to introduce variety of strategies such as biochemistry, molecular biology, biophysics, structural biology etc. It is sometimes necessary to introduce the developmental aspects and comparative analysis of the abnormal diseased brain with the control one. Combination and fusion of differentresearch areas gives us unexpected ideas to solve the unknown mechanism of mysterious brain function and structure. I will describe here 1）the mechanism of myelination taking the example of introducing *shiverer* and *mld* mutations **Nature** 299 357–359（1982）, **Annual Rev. Neurosci.** 14 201–17（1991）. 2）the mechanism of neuronal positioning in the cortical layers in the brain taking an example to revealing the molecular mechanism by introducing *reeler* and *yotari* mutations **Neuron** 14899–912（1995）**Nature** 385 70–74（1997）**Nature** 389 730–733（1997）. 3）the mechanism of IP_3 receptor/calcium signaling whichwediscovered from the analysis of the P400–protein deficient mice by introducing *pcd*, *nervous* mutant deficient of Purkinje neurons and also *staggerer* mutant in the cerebellum **Nature** 342 32–38（1989）**Science** 257 251–255（1992）**Cell** 73 555–570（1993）**Science** 292 920–923（2001）**Nature** 379 168–171（1996）. All these unexpectedway of doing research has given us glorious results which are so important for revealing the mechanism of the function of the brain. These may make newtrends in neurochemistry.

1S2-1 Thyroid hormone and the nervous system

○Douglas Forrest[1], Hong Liu[1], Jeff Huang[1], Xuefeng Wu[1], Anand Swaroop[2], Lily Ng[1], Yulong Fu[1]
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Thyroid hormone (T3) promotes diverse functions in the development and function of the nervous system. This is evident from the mental retardation that can result from defects caused for example, by congenital hypothyroidism or endemic iodine deficiency. Although the requirement for T3 is well-recognized, less is known of the underlying cellular functions that T3 regulates and how T3 acts at appropriate stages of development. We have addressed these questions by investigation of thyroid hormone receptors (TR), which act as ligand-regulated transcription factors. Two genes, Thra and Thrb, encode three TR isoforms, TRa1, TRb1 and TRb2 which serve a variety of functions in the nervous system. Mutagenesis in mice and observations in human patients indicate that the Thrb gene is important in sensory systems. In the mouse retina, TRb2 is unexpectedly critical for generating diversity in cone photoreceptors, which mediate colour vision. TRb2-deficiency results in a form of blue monochromacy with the presence of blue (or S) cones for response to short wave light but lack of green (or M) cones for response to medium/long wave light. Evidence indicates further developmental plasticity in photoreceptor precursor cells that extends to rods, the photoreceptors for vision in dim light. Differential expression of two factors, TRb2 and Nrl, a leucine zipper transcription factor, can direct common precursors to three photoreceptor outcomes : M cone, S cone or rod. Cones are also sensitive to the level of T3 at immature stages, such that excessive T3 acting on TRb2 eliminates cones by apoptotsis. Cone survival is safeguarded by type 3 deiodinase, a thyroid hormone-degrading enzyme. The findings suggest that in neurodevelopment, cell-specific responses to T3 are determined by specific TR isoforms acting in cooperation with ligand-metabolizing deiodinases in the neural tissue environment.

1S2-2 Neuroendocrine Regulation of Iodothyronine Deiodinases

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Thyroid hormones play important roles in normal brain development and function. Thyroxine (T_4), which is a major secretory product of the thyroid gland, needs to be converted to 3, 5, 3'-triiodothyronine (T_3) by iodothyronine deiodinase to exert its biological activity.

Two different isozymes, type 1 (D1) and type2 (D2) iodothyronine deiodinase, catalyze T_4 activation. D1 is mainly present in thyroid gland, liver, and kidney, whereas D2 is present in brain, anterior pituitary, brown fat, and pineal gland, in the rat. D2 activity increases in the hypothyroid state and plays a critical role in providing local intracellular T_3. Type 3 iodothyronine deiodinase (D3) inactivates T_4 and T_3 to 3,3',5'-triiodothyronine (rT_3) and 3,3'-diiodothyronine (T_2), respectively.

Iodothyronine deiodinases play important roles in the regulation of cell-specific thyroid hormone action. In the rat pineal gland, D2 shows nocturnal increase by a beta-adrenergic mechanism. Indeed, cAMP response elements have been demonstrated in D2 gene promoter, TSH receptor-cAMP-mediated expression of D2 in human thyroid gland and osteoblast, and rat brown adipose tissue has been reported.

In the central nervous system, D2 is present in astrocytes, whereas D3 is predominantly expressed in neurons. In humans, D2 and D3 have been demonstrated in normal brain tissues and brain tumors. We identified that D2 is highly expressed in anaplastic oligodendroglioma tissue. Among the glial cells, it is well known that thyroid hormones play important roles in the development of oligodendrocytes. Increased T_3 production by D2 in oligodendroglioma may play roles related to the functions of thyroid hormones in oligodendrocytes.

To elucidate the deiodinase-regulated thyroid hormone action in ologodendroglioma cells, we studied the expression and regulation of deiodinases in human oligodendroglioma (HOG) cells. D2 was expressed in HOG cells, and its expression was increased by beta-adrenergic stimulation and inhibited by glucocorticoid. Hypothyroid state increased D2 expression in HOG cells, and thyroid hormones decreased D2 activity through the ubiquitin-proteasome pathway. Increased T_3 production by D2 in HOG cells may play pathophysiological roles in oligodendroglioma and oligodendrocytes-specific thyroid hormone action.

1S2-3 **Attenuation of local thyroid hormone signaling due to reduction of DIO2：SAMP8 mouse as an unique animal model of developmental anomalies and later-onset cognitive deficits**

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Thyroid hormone (TH) regulates many aspects of neural network formation including neural cell migration, synaptogenesis, and myelination. Studies using rodent models show that perinatal hypothyroidism strongly affects the development of the GABAergic system, resulting in attenuation of inhibitory neurotransmission. While the number of total GABAergic neurons was not altered, subpopulations expressing parvalbumin and neuropeptide Y (NPY) were preferentially reduced in the hippocampus of hypothyroid rats which could be rescued by TH replacement after birth. A key molecule in post-synaptic switching of GABA action from excitatory to inhibitory, the neuron-specific K^+/Cl^- co-transporter (KCC 2), was also TH-responsive. The senescence-accelerated mouse prone 8 (SAMP8) is a spontaneous model of neurodegeneration exhibiting age-related cognitive deficits with little physical impairment. Before the onset of cognitive impairment, young SAMP8 mice show signs of developmental anomalies such as marked hyperactivity and reduced anxiety. While exploring the possible involvement of the TH system, we found a significant reduction of the TH-activating enzyme, type 2 deiodinase (DIO2), in the hippocampus and the cerebral cortex of SAMP8 compared with the normally-aging SAM resistant 1 (SAMR1) starting from the onset of its expression in the early postnatal weeks. Attenuation of TH signaling was confirmed by down-regulation of TH-responsive genes in SAMP8 including KCC2. Although distribution of total GABAergic neurons was similar in both strains, NPY-positive neurons in the SAMP8 hippocampus were reduced by 22-30%. Electrophysiological comparison of hippocampal slices at 4 weeks revealed that epileptiform activity induced by high frequency stimulation lasted 4-times longer in SAMP8 compared with SAMR1, indicating dysregulation of excitability. The results suggest that local attenuation of thyroid hormone signaling without changes in plasma TH levels may lead to behavioral and cognitive disorders.

1S3-1 Design of circadian clock by two ATPases in cyanobacterial clock protein KaiC

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We reconstituted the self-sustainable circadian oscillation of the KaiC phosphorylation state by incubating purified cyanobacterial clock proteins, KaiC with KaiA, KaiB, and ATP. This in vitro oscillation is the primary pacemaker of the cyanobacterial circadian clock, and revealed a novel function of proteins as timing devices that govern cellular metabolism. We further found that the ATPase activity of KaiC defines the period length and its temperature compensation. KaiC possesses extremely weak but stable ATPase activity (15 molecules of ATP per day). As the ATPase activity of KaiC is inherently temperature-invariant, suggesting that temperature compensation of the circadian period could be attained by simple ATPase reaction. Interestingly, the activities of five period-mutant proteins are directly proportional to their in vivo circadian frequencies, indicating that the ATPase activity defines the circadian period. We propose that KaiC ATPase activity constitutes the most fundamental reaction underlying circadian periodicity in cyanobacteria. Based on these observations, we propose a model of the protein circadian clock, in which the clock is composed from two units, pacemaker and driver that respectively take charge of C1 and CII domain of KaiC ATPase. Functionally, this design is important to mix two basically different processes function together to achieve precision of period length and robustness of the oscillation. The first unit can be achived by intramolecular feedback that generate mechanical tension inside the protein and the second unit could be energy-dependent phosphorylation cycle that is basis for robustness of the oscillation. These two processes could be combined by a unique mechanism similar to escapement mechanism of the pendulum clock.

1S3-2 Disruption of MeCP2 attenuates circadian rhythm in CRISPR/Cas9-based Rett syndrome model mouse

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Methyl-CpG-binding protein 2 (Mecp2) is an X-linked gene encoding a methylated DNA binding nuclear protein which regulates transcriptional activity. The mutation of MECP2 in humans is associated with Rett syndrome (RTT), a neurodevelopmental disorder. RTT patients frequently exhibit abnormal sleep patterns and sleep-associated problems, in addition to autistic symptoms, raising the possibility of circadian clock dysfunction in RTT. In this study, we investigated circadian clock function in Mecp2-deficient mice. We successfully generated both male and female Mecp2-deficient mice on the wild-type C57BL/6 background and PER2Luciferase (PER2Luc) knock-in background by utilizing the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system. Generated Mecp2-deficient mice recapitulated reduced activity in mouse models of RTT, and their activity rhythms were diminished in constant dark conditions. Bioluminescence rhythms were analyzed using photomultiplier tubes (PMT) and high-sensitivity EMCCD camera-based microscopy in order to evaluate the molecular clockwork in the master pacemaker suprachiasmatic nucleus (SCN) with or without lacking Mecp2, PER2Luc. Real-time bioluminescence imaging revealed that the amplitude of PER 2Luc driven circadian oscillation was significantly attenuated in Mecp2 deficient SCN neurons. On the other hands, in vitro circadian rhythm development assay using Mecp2 deficient mouse embryonic stem cells (ESCs) showed slight period-length changes of PER2Luc bioluminescence rhythms without apparent dampening. Together, these results demonstrate that Mecp2 deficiency abrogates the circadian pacemaking ability of the SCN, which may be a therapeutic target to treat the sleep problems of RTT patients.

1S3-3 Understanding the mechanism of seasonal time measurement

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Animals living outside the tropics use changes in day length to adapt to seasonal changes in environment, but mechanisms underlying seasonal time measurement are not fully understood. Japanese quail is an excellent model for the study of these mechanisms because of its rapid and dramatic response. We have demonstrated that local thyroid hormone catabolism within the mediobasal hypothalamus (MBH) by thyroid hormone-activating enzyme (type 2 deiodinase : DIO2) regulates photoperiodism. Functional genomics analysis demonstrated that long day stimulus induces thyrotropin (thyroid stimulating hormone : TSH) production in the pars tuberalis (PT) of the pituitary gland, which triggers *DIO2* expression in the ependymal cells of the MBH. In mammals, nocturnal melatonin secretion provides an endocrine signal of the photoperiod to the PT that contains melatonin receptors in high density. We have also demonstrated the involvement of TSH signaling pathway in mammals by using the TSH receptor null mice. Well known function of TSH derived from pars distalis (PD) of the pituitary gland is stimulation of thyroid gland. However, it was unclear how these two TSHs avoid functional crosstalk. We demonstrated that tissue-specific glycosylation is central to this mechanism. Although fish also exhibit clear seasonal responses, they do not possess an anatomically distinct PT. We found expression of TSH, DIO2, and rhodopsin family genes in the coronet cell of the saccus vasculosus (SV), suggesting the existence of a photoperiodic signaling pathway from light input to neuroendocrine output. Functional analysis suggested that the SV acts as a seasonal sensor in fish. We are currently trying to develop transformative bio-molecules that improve animal production and human health.

1S3-4 Regulation of memory retrieval by forebrain circadian clock

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Cognitive performance in people varies according to time-of-day, with memory retrieval declining in the late afternoon-early evening. Here we show that mice exhibit a similar time-of-day retrieval profile following weak hippocampus-dependent learning, with reduced retrieval efficiency correlating with low forebrain activity of circadian transcription factor, BMAL1. To test whether BMAL1 activity regulates retrieval efficiency, we inducibly expressed a dominant negative BMAL1 (dnBMAL1) in mouse forebrain. dnBMAL 1 expression had no effect on memory encoding but disrupted retrieval at Zeitgeber Time 8-12, and not at other time. Importantly, these effects were observed across multiple hippocampal memories and were independent of retention delay, time of encoding and Zeitgeber entrainment cue. Additionally, cAMP signals were suggested to mediate regulation of retrieval efficiency by BMAL1. Furthermore, forebrain dnBMAL1 expression did not affect locomotor rhythm or BMAL1-mediated transcription in the SCN. Thus forebrain clock regulates the efficiency of hippocampus-dependent memory retrieval independent of core time-keeping cells.

1S4-1 Interaction between glia and blood vessels during cortical development

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During cerebral cortical development, neurons and glia are produced directly or indirectly from neural stem cells in the ventricular zone and migrate to their final destinations. Although the behaviors of migrating neurons are well described, those of glial progenitors are not largely uncovered. The most accepted model of the migration of glial progenitor is the translocation of radial glia, but this model does not explain the even distribution of astrocytes throughout the all layers of cortical gray matter. During our observations of the cells migrating from the ventricular zone, we have noticed that some cells moved in a very unique manner that had not been previously described：these cells moved very rapidly and almost randomly within both the intermediate zone and the cortical plate and frequently underwent cell division. We named this migration erratic migration. The lineage analyses of them both in vitro and in vivo revealed that they were astrocyte progenitors destined for cortical gray matter. These cells frequently migrate along blood vessels and spread widely throughout the cortical plate.

1S4-2 Macrophage-independent programmed regression of fetal ocular vasculature triggered by neurons

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Vascular development involves not only vascular growth but also regression of transient or unnecessary vasculature such as ductus arteriosus and umbilical arteries. Hyaloid vasculature is the temporary circulatory system in fetal eyes, which normally degenerates soon after birth. Failure to regress these vessels leads to an ocular pathology called the persistent hyperplastic primary vitreous, which causes severe intraocular hemorrhage and impairs visual function. This programmed regression of hyaloid vessels is generally thought to be triggered by ocular macrophages inducing endothelial cell death. Here, we found a novel switch of this regression controlled by neurons independently of macrophages. Striking upregulation of VEGFR2 occurs in retinal neurons just after birth via activation of the Distal-Multipotent-Mesodermal-Enhancer（DMME）, known as a hemangioblast-specific enhancer of VEGFR2. Lack of neuronal VEGFR2 interrupts this program resulting in massive hyaloid vessels that persist even during late postnatal days. This abnormality is caused by excessive VEGF proteins in the vitreous cavity due to the impairment in neuronal endocytosis of VEGF, recently described to account for neuronal avascularity in neonatal retinas. Taken together our data indicate neurons trigger transition from the fetal to the postnatal circulatory systems in retina independently of macrophages.

1S4-3 Role of blood vessels in neuronal regeneration

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Neuronal migration is an important process in brain development and homeostasis. It occurs in the adult brain, following adult neurogenesis, not only in the embryonic brain. In fact, throughout life, numerous new neurons generated by stem cells in the adult ventricular–subventricular zone (V–SVZ) take the long journey to the olfactory bulb (OB) through the rostral migratory stream (RMS). The neural stem cells in the adult V–SVZ also have the capacity to partially regenerate new neurons after various insults. After ischemic injury in rodents, the V–SVZ–derived new neurons migrate from the V–SVZ towards the injured site along blood vessels. In this talk, I will present recent studies on the molecular mechanisms of the blood vessel–guided neuronal migration. Our in vivo and in vitro data suggest that cell–to–cell interactions mediated by the laminin–integrin signaling is important for the efficient chain migration of new neurons along the blood vessel scaffold. Transplantation of laminin–rich porous sponge promoted the migration of new neurons towards the injured cortex, suggesting that artificial blood vessel–like scaffold may enhance regenerative property of endogenous new neurons in the brain.

1S4-4 Control of oligodendrocyte precursor cell survival and proliferation by vascular endothelial cells

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We showed previously that transplantation of brain microvascular endothelial cells (MVECs) greatly stimulated remyelination in the white matter lesion induced by endothelin–1 (ET–1) injection and improved the behavioral outcome (Puentes *et al.*, 2012). In this study, we examined the effects of MVECs on the behavior of oligodendrocyte precursor cells (OPCs) *in vivo* and *in vitro*. MVECs prepared from rat cerebral cortices were transplanted into ET–1–induced demyelinating lesion in the internal capsule (IC) of rat brains. Cell density, apoptotic death, and proliferative state of OPCs in and around the ET–1–induced lesions in IC of MVEC–transplanted animals were examined. The effects of exosomes prepared from MVEC cultures on survival and proliferation of OPCs isolated from cerebra of young rats were also examined. MVECs promoted survival of OPCs both *in vivo* and *in vitro* and stimulated their proliferation *in vitro*. Elucidation of the molecular mechanisms by which MVECs control survival and proliferation of OPCs may lead to the establishment of a therapeutic strategy against demyelinating diseases.

1S4-5 Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors

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Recent work from our lab and others has come to the surprisingconclusion that some aspects of aging are reversible and are under thecontrol of circulating factors. Parabiotic coupling of young and oldmice has demonstrated that systemic factors present in young blood canpromote improvements in the aged nervous system including enhancedvasculature and blood flow in the CNS, augmented neurogenesis in theadult brain, and increased performance in behavioral tests. Inparticular, we find that systemic administration of growthdifferentiation factor 11 (GDF11), a TGFβ family member that decreaseswith aging, can recapitulate some of the beneficial aspects of youngblood. Additionally, we find that GDF11 and its receptors areexpressed within the central nervous system, and that neurons and gliarespond to direct stimulation with the ligand. These findings haveimportant clinical implications and may form the basis for novelavenues of treating age related neurological decline.

1S5-1 An overview of non-coding microsatellite repeat expansion disorders

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Members of hereditary neuromuscular disorders caused by the expansions of various microsatellite repeat motifs are still growing after the first identification of respective mutations in spinal and bulbar muscular atrophy and fragile X syndrome in 1991. They are classified into two categories based on the location of repeat expansions in the genes, one is the expansions in the coding region, and the other is that in the non-coding region. The most frequent mutation in the coding region is the CAG trinucleotide expansion that is translated into the polyglutamine stretch in spinocerebellar ataxias such as SCA1, SCA2, Machado-Joseph disease/SCA3, Huntington disease, and SBMA, etc. The pathogenesis of this type of mutation falls into the toxic gain-of-function mechanism due to polyglutamine-mediated toxicity in neurons. In contrast, the microsatellite repeat motifs of non-coding expansions are more variable and complicated such as（CTG）n in DM1 and SCA8,（CGG）n in FXTAS and FXS,（GAA）n in Friedreich ataxia,（CCTG）n in DM2,（ATTCT）n in SCA10,（TGGAA）n in SCA31,（GGCCTG）n in SCA36/Asidan, and（GGGGCC）n in C9-linked ALS/FTD. The common pathological hallmark of a subset of these disorders is found in the affected cells as RNA foci that are derived from an accumulation of the expanded repeat transcripts. Several lines of evidence suggested that the molecular mechanism associated with RNA foci underlies RNA gain-of-function. However, loss-of-function mechanism is also suggested in some non-coding expansion mutations. Surprisingly, in some disorders it is revealed that the non-coding expanded repeat transcripts can express homopolymeric proteins by the mechanism called as repeat-associated non-ATG（RAN）translation. In this presentation, the hereditary neuromuscular disorders caused by the coding and the non-coding microsatellite repeat expansions are overviewed.

1S5-2 Myotonic dystrophy-toxic RNA and spliceopathy

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Myotonic dystrophy（DM）is the most common type of muscular dystrophy in adults, caused by unstable genomic expansions of simple tandem repeats. Myotonic dystrophy type 1（DM1）results from expansion of a CTG repeat in the 3′untranslated region of DMPK. In myotonic dystrophy type 2（DM2), the expanded repeat is a CCTG tetramer in intron 1 of CNBP/ZNF9. The transcripts containing the expanded repeat form ribonuclear inclusions, thereby retained in the nucleus. The mutant RNA gives rise to a toxic gain-of-function by perturbing splicing factors, leading to misregulation of alternative pre-mRNA splicing. The misregulated splicing is thought to be responsible for multisystemic symptoms in DM, such as myotonia, cardiac conduction defects, and glucose intolerance.

1S5-3 A Novel ALS/SCA Crossroad Mutation Asidan

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Recently we found intronic hexanucleotide GGCCTG gene expansion in NOP56 gene as the causative mutation (＝SCA 36；nicknamed "Asidan") in nine unrelated Japanese familial SCA. In the nine families, 14 patients were clinically examined and genetically confirmed to Asidan. The age at onset of ataxia was 53.1 ± 3.4 years, with the most frequent symptoms being truncal ataxia (100% of patients), ataxic dysarthria (100%), limb ataxia (93%), and hyperreflexia (79%). Tongue fasciculation and subsequent atrophy were found in 71% of cases, particularly in those of long duration. Skeletal muscle fasciculation and atrophy of the limbs and trunk were found in 57% of cases. Lower motor involvement was confirmed by EMG and muscle biopsy. The neuropathologic study revealed significant cerebellar Purkinje cell degeneration with obvious loss of lower motor neurons. Immunohistochemical analysis showed that NOP56 was localized to the nuclei of various neurons. Cytoplasmic or intranuclear inclusion staining of NOP56, TDP-43, and ataxin-2 was not observed in the remaining neurons.Taken together, these patients showed unique clinical features of cerebellar ataxia and motor neuron disease (MND), locating on the crossroad of these two diseases. In this symposium, we would like to introduce the clinical features of Asidan, and discuss the possible mechanism of hexanucleotide GGCCTG expansion leading to both Purkinje cell degeneration and motor neuron loss.

1S5-4 Cytotoxic properties of dipeptide repeat proteins generated by repeat-associated, non-ATG (RAN) translation on c9ALS/FTD

○**Daisuke Ito(伊東　大介)**
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Advances in genetics and pathological studies have revealed that amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) constitute a disease spectrum that shares a common molecular basis. The expansion of the GGGGCC hexanucleotide repeat in the non-coding region of the chromosome 9 open reading frame 72 (C9orf72) gene is the most common cause of ALS and FTD (c9ALS/FTD). Recently, it was reported that an unconventional mechanism of repeat-associated non-ATG (RAN) translation arises from C9orf72 expansion. Sense and anti-sense transcripts of the expanded C9orf72 repeat, i.e., the dipeptide repeat protein (DRP) of glycine-alanine (poly-GA), glycine-proline (poly-GP), glycine-arginine (poly-GR), proline-arginine (poly-PR), and proline-alanine (poly-PA) are deposited in the brains of patients with c9ALS/FTD. However, the pathological significance of RAN-translated peptides remains unknown. To elucidate the impact of individual DRP, we generated synthetic cDNAs encoding 100 repeats of poly-GA, -GP, -GR, -PR, and -PA with start codon, avoiding GGGGCC repeats and evaluated the effects of these proteins on cultured cells and cortical neurons in vivo. Our results revealed that the poly-GA protein formed highly aggregated ubiquitin/p62-positive inclusion bodies in neuronal cells. In contrast, the highly basic proteins poly-GR and PR also formed unique ubiquitin/p62-negative cytoplasmic inclusions, which colocalized with the components of RNA granules. The evaluation of cytotoxicity revealed that overexpressed poly-GA, -GP, and -GR increased the substrates of the ubiquitin-proteasome system (UPS), including TDP-43, and enhanced the sensitivity to a proteasome inhibitor, indicating that these DRPs are cytotoxic, possibly via UPS dysfunction. These findings demonstrate that DRP affect the protein quality control system, resulting in cytotoxicity and are potential therapeutic targets for c9FTD/ALS.

1S5-5 Mechanism and Function of RAN translation in FXTAS and related microsatellite expansion disorders

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Fragile X-associated Tremor Ataxia Syndrome (FXTAS) is a neurodegenerative disorder caused by a CGG trinucleotide repeat expansion in the 5'UTR of the Fragile X gene, FMR1. FXTAS is thought to arise primarily from an RNA gain-of-function toxicity mechanism. However, recent studies demonstrate that the repeat also elicits production of a toxic polyglycine protein, FMRpolyG, via Repeat-Associated Non-AUG (RAN) translation- an atypical form of translational initiation that occurs in association with a variety of pathologic repeats in the absence of an AUG start codon. Here we describe how different RAN translation products from CGG repeats contribute to toxicity in model systems and provide insights into the mechanisms by which RAN translation occurs in different reading frames. Our findings demonstrate that the FMR1 5'UTR supports RAN translation across multiple repeat reading frames, follows some but not all canonical eukaryotic translational initiation steps, and contributes to both the normal translational control of Fragile X mRNA and FXTAS disease pathogenesis. These findings provide a model for Repeat associated Non-AUG (RAN) translation and shed light on the roles of unconventional translational initiation in both normal and disease states.

1S6-1 Neurodegeneration promotes angiogenesis in the adult CNS

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Angiogenesis is a prominent feature of central nervous system (CNS) pathology and is crucial for regulating disease progression. Although neuronal damage is a primary process of CNS disease progression, the role of neuronal damage in pathological angiogenesis remains poorly understood. Here we show that lactate dehydrogenase A (LDHA) release from degenerating axons drives vascular endothelial cell proliferation in the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. Silencing neuronal LDHA expression suppressed angiogenesis around EAE lesions and in response to controlled cortical impact (CCI) brain injury. LDHA-mediated angiogenesis was dependent on surface vimentin expression and p44/42 mitogen-activated protein kinase (MAPK) activation in vascular endothelial cells. Silencing of vimentin expression in vascular endothelial cells prevented angiogenesis around EAE. These results elucidate a novel aspect of pathological neurovascular interactions and provide a potential target for treating CNS diseases that involve angiogenesis.

1S6-2 Cortical astrocytes rewire somatosensory circuits for neuropathic pain

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Neuropathic pain following peripheral nerve injury is characterized by mechanical allodynia, a painful response to innocuous tactile stimulation. Although this chronic pain is known to be induced by glial activation and altered nociceptive transmission within the spinal cord, an effective treatment is still insufficient, indicating that novel therapeutic targets are critically needed. One such target may be the synaptic rewiring in the primary somatosensory (S1) cortex that is correlated with the severity of neuropathic mechanical allodynia. However, its causal relationship to mechanical allodynia and its cellular/molecular mechanisms remain unknown. In addition, glial contribution to the S1 synaptic plasticity is unclear. Here we show that partial sciatic nerve ligation (PSL) injury induces an early re-emergence of immature metabotropic glutamate receptor 5 (mGluR5) signaling in S1 astrocytes, which elicits spontaneous somatic Ca2+ transients, thrombospondin-1 release and synapse formation. Such activation of S1 astrocytes was apparent only during a critical period (～1w post-injury), correlating with the temporal changes in S1 extracellular glutamate levels and dendritic spine turnover following PSL injury. Blocking this astrocytic signaling pathway suppressed mechanical allodynia, while activating this pathway in the absence of injury induced long-lasting (>1 month) allodynia. Thus, these synaptogenic astrocytes are a key trigger for S1 synaptic circuit rewiring that causes neuropathic pain mechanical hypersensitivity.

1S6-3 Calcium signals in astrocyte processes：its visualization and manipulation

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Astrocytes are not electrically excitable, but are excitable in term of calcium signals. Such calcium excitability is observed throughout of the brain in both animals and humans. Calcium excitability is spatiotemporally dynamic phenomena. Thus, calcium signals in astrocytes are thought to be relevant to brain functions and brain disorders. It has been proposed that astrocytes regulate synapses using calcium via releasing gliotransmitters. However, the role of calcium excitability is still enigmatic since the mechanisms underlying the calcium signals are largely unknown due to the lack of the methods to analyze those calcium signals in astrocytes especially at peripheral processes where astrocytes intimately contact with synapses and may regulate synaptic transmission. To achieve better understanding of calcium signals in astrocyte processes, we used two novel methods. *First*, to visualize astrocytes processes, we expressed genetically encoded calcium indicator（GECI）, Lck-GCaMP3 or GCaMP3[1], into astrocytes. To introduce GECI into astrocytes, we injected adeno-associated viruses with GFAP minimal promoter into the brain or used transgenic mice generated by Cre-loxP mediated recombination. GECI successfully reports numerous calcium signals at processes in acute brain slices. *Second*, to manipulate calcium signals in astrocyte processes, we generated transgenic mice to overexpress P2Y1 receptors（P2Y1）, which is known to elevate calcium signals in astrocyte processes, using tetracycline inducible system. Astrocytes overexpressing P2Y1 showed　～3 fold increase in calcium signals in astrocytes from the dentate gyrus of acute brain slices. Astrocyte with P2Y1 overexpression displayed calcium signals highly correlated between neighboring astrocytes. Two approaches described above will give us an unique opportunity to analyze the role of astrocyte processes in neuronal circuits in（patho）physiology.

Reference
1 Shigetomi et al.（2013）Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. J. Gen. Physiol. 141（5）：633-647.

1S6-4 The role of microglia in the adult CNS of systemic inflammation.

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Microglia are haematopoietic-cell derived glial cells in the central nervous system（CNS）that function as the only resident immune cells of the CNS. Traditionally, effects of microglia as immune cell in CNS have been thought to be mainly in pathological conditions where they exert neuro-protective or neuro-toxic effects to modify disease progression. However, recent studies have reported that microglial cells play a role in brain homeostasis in the normal physiological state, promoting programmed cell death in both neural development and in adult neurogenesis, and monitoring and phagocytosing synapses. On the other hands, substantial evidence has demonstrated that immune condition can have effects on to the neuronal circuits. However little has been known whether those immune condition could affect on to the function of neuronal circuits. Here we use systemic inflammation model to study the interaction of systemic immune cells and microglia. And we also show the functional regulation of synapses by microglia contacts and the alteration of the synapse response in systemic inflammation model and their affect on the behavior responses. Those data indicate that microglia changes induced by the interaction with systemic immune cells can modulate function of neuronal circuits.

1S7-1 Neural Restrictive Silencing Factor (NRSF/REST) 1995-2015

○**Nozomu Mori**(**森　望**)
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Brain is a tissue with higher complexity, and its complexity rely, at least in part, on a complexity in the gene regulation of a vast set of neuron-specific genes. It has been well known that many neuron-specific genes are under the control of a transcriptional repressor called Neural-restrictive silencing factor (NRSF) or RE-1 silencing transcription factor (REST). NRSF/REST was initially identified as a transcription factor that regulates neuron-specific genes, such as SCG10 and Na^+-channel type II, but its target gene repertoire is now estimated to be more than 1,000 neuron-specific genes. Since its discovery in 1995, accumulative evidence indicates that roles of NRSF/REST are not restricted to neuronal gene regulation during neural differentiation, but also are involved in stem cell regulation, brain aging, and neurodegeneration. NRSF/REST was initially thought to be expressed exclusively in non-neuronal cells and/or neuronal progenitor cells ; however, recent evidence indicate that it is also expressed and play some role（s）in mature adult neurons. Recently, we came to know that several laboratories in our country are working on various aspects of NRSF/REST, and published some interesting results. Regrettably, however, those works stay independent, and researchers do not aware of other works. I think it would be beneficial if these researchers come together and have an opportunity to discuss those findings. Thus, this symposium is indicated to overview recent findings on the NRSF/REST biology, and discuss perspectives of these studies, thereby stimulating mutual interaction for potential collaboration and/or exchange ideas and materials.

1S7-2 Remarkable differences in NRSF/REST target genes between human ESC and ESC-derived neurons

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The neuron-restrictive silencer factor (NRSF) is a zinc finger transcription factor that represses neuronal gene transcription in nonneuronal cells by binding to the consensus repressor element-1 (RE1) located in regulatory regions of target genes. NRSF silences the expression of a wide range of target genes involved in neuron-specific functions. Previous studies showed that aberrant regulation of NRSF plays a key role in the pathological process of human neurodegenerative diseases. However, a comprehensive set of NRSF target genes relevant to human neuronal functions have not yet been characterized. We attempted to perform genome-wide data mining from chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) datasets of NRSF binding sites in human embryonic stem cells (ESC) and the corresponding ESC-derived neurons, retrieved from the database of the ENCODE/HAIB project. By using bioinformatics tools such as Avadis NGS and MACS, we identified 2,172 NRSF target genes in ESC and 308 genes in ESC-derived neurons based on stringent criteria. Only 40 NRSF target genes overlapped between both. By motif analysis, binding regions showed an enrichment of the consensus RE1 sites in ESC, whereas they were mainly located in poorly defined non-RE 1 sites in ESC-derived neurons. Molecular pathways of NRSF target genes were linked with various neuronal functions in ESC, such as neuroactive ligand-receptor interaction, CREB signaling, and axonal guidance signaling, while they were not directed to neuron-specific functions in ESC-derived neurons. Remarkable differences in ChIP-Seq-based NRSF target genes and pathways between ESC and ESC-derived neurons suggested that NRSF-mediated silencing of target genes is highly effective in human ESC but not in ESC-derived neurons (Satoh et al. Bioinform Biol Insights 7 : 357-368, 2013).

1S7-3 Post-translational modification of Charlatan, a Drosophila NREF/REST, is required for neuron specific genes expression

○**Leo Tsuda（津田　玲生）, Yasutoyo Yamasaki（山崎　泰豊）, Young-Mi Lim（林　永美）**
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Neuronal networks in the brain are consisted with many types of neuronal cells. On the way to construct matured neuronal circuits, there are number of irreversible regulatory steps, and each steps must be tightly regulated by the expression level of neuron specific genes. Neuron-restrictive silencing factor or RE1 silencing transcription factor（NRSF/REST）has pivotal role for many kinds of target genes expression during neuronal development in mammalian cells. Recent study revealed that post-translational modification, especially the balance between ubiquitylation and de-ubiquitylation, provides rapid regulatory networks of NRSF/REST during neuronal development. In this symposium, we will present a data that the similar ubiquitylation-deubiquitylation system also plays an important role for Charlatan, a Drosophila NRSF/REST. We further reveal that the ubiquitylation leads to truncation of the C-terminal repressor domain and stabilized the N-terminal DNA-binding region of Chn. This post-translational modification of Chn drastically changed the transcriptional activities from repressor to activator. We found that the post-translational modification of Chn seems to be required for maintaining expression level of dopamine receptor gene in the mashroombody. Therefore, the dynamic changes of Chn modifications might be a determinant of the irreversible regulation of neuron specific genes expression during neuronal development.

1S7-4 Neuroepigenetics in negative signs of chronic pain

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Mechanisms underlying chronic pain are closely related to a kind of long-term memory process in the somatosensory nervous system. Emerging evidence has been accumulated that epigenetic mechanisms, such as DNA methylation and histone modifications, are engaged in the memory process of chronic pain. We have previously clarified the epigenetic regulation of pain-related genes in the primary afferent neurons underlying abnormal pain sensations and resistance to morphine in a model of peripheral nerve injury-induced chronic neuropathic pain in mice（J Neurosci, 2010）. Specifically, we have demonstrated that neuron-restrictive silencer factor（NRSF, also known as RE-1-silencing transcription factor；REST）orchestrates histone deacetylation-mediated transcriptional repression of pain-related genes to cause pathological and pharmacological dysfunction of C-fibers after nerve injury. In this symposium, I will discuss the molecular mechanisms of epigenetic gene regulation underlying chronic pain, and the therapeutic potential of epigenetically modifying compounds in the treatment of chronic pain.

1S7-5 Rest function in neuronal or neural crest cell lineage revealed by the conditional gene ablation

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Rest also known as NRSF is a regulator of neuronal development and function. *Rest* null mice have embryonic lethality which prevents further investigation. To study the Rest function *in vivo*, we focused on the development of the neuronal and neural crest（NC）cells, both expected to require REST function. Conditional knockout（CKO）of the exon 4 of *Rest* encoding the CoRest binding site during the early neural development stage was carried out by using *Sox1-Cre* promoter. Although *Rest* suppressed expression of neuronal genes *in vivo*, *Rest* conditional ablation did not affect neuronal development and thus *Rest* is dispensable for natural neurogenesis. The morphology of neural tissues, maintenance and differentiation of neuronal progenitor cells *in vivo* were all normal in *Rest* CKO mice. While the electrical stimulations of cervical vagus nerve reduce the heart rate, blood pressure, stomach contraction etc, *Rest* CKO mice were found to be resistant for the stimulus induced reduction of heart rate compared to the control mice but not blood pressure and muscular contractions of stomach. While *Sox1-Cre* induced Rest ablation leads to *in vitro*-specific derepression of neuronal genes during neurogenesis, genetic ablation of *Rest* by *Sox1-Cre* does not cause any detectable morphological abnormality in the nervous system but functional defect might be caused in vagus nerve cells. Next, we used a *Wnt1-Cre* to specifically ablate the early progenitor cells of the developing NC lineage cells. The NCC-specific *Rest* CKO mice showed neonatal lethality that were characterized by gastrointestinal tract dilation, while no histological abnormalities except the thinning of the digestive tract as a consequence of the gas accumulation. The gas collected from the swollen digestive tracts of the *Rest* CKO mice contained high concentration of CO_2. They do not have proper gastric retention and the reduction of acetylcolinesterase activity of myenteric plexus in the stomach was detected. The neonatal lethality in NCC-specific *Rest* CKO mice showed gastrointestinal distension phenotype caused by a failure of gut function in underdeveloped cholinergic transmission of enteric nerve system may provide a model for understanding the NCC defects in humans. These experiments indicate novel Rest phenotypes in various neuronal cell lineages.

1S7-6 NRSF plays an essential role in the regulation of cardiac gene expression and function

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Alterations in the cardiac gene program affect both cardiac structure and function, and play a key role in the progression of pathological cardiac remodeling and heart failure. Among the genetic alterations in cardiac diseases, reactivation of fetal cardiac genes in adults is a consistent feature of cardiac hypertrophy and heart failure. We investigated the transcriptional regulation of fetal cardiac genes, such as geens encoding atrial and brain natriuretic peptide, and revealed a transcriptional repressor, neuron-restrictive silencer factor（NRSF）, also called repressor element-1 silencing factor（REST）, to be an important regulator of multiple fetal cardiac genes. Inhibition or deletion of NRSF in the heart leads to cardiac dysfunction and sudden arrhythmic death accompanied by re-expression of various fetal genes, including those encoding fetal ion channels, such as the HCN channels and T-type Ca2+ channels. We demonstrated that inhibition of these ion channels suppressed lethal arrhythmias and sudden arrhythmic death in mice expressing dominant-negative mutant of NRSF in a cardiac-restricted manner. Our findings indicate that NRSF plays an essential role in maintaining normal cardiac structure and function.

2S1-1 Phosphorylation controls neprilysin cell surface localization and extracellular Aβ level

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Neprilysin is one of the major amyloid β peptide（Aβ）degrading enzymes. Expression of neprilysin in the brain declines during aging, leading to a metabolic imbalance of Aβ, which can induce the amyloidosis underlying Alzheimer's disease（AD）. Pharmacological activation of neprilysin during aging is a potential way to prevent AD. However, the regulatory mechanisms of neprilysin activity in the brain still remain unclear. To address this issue, we screened for pharmacological regulators of neprilysin activity and found that the neurotrophic factors brain derived neurotrophic factor（BDNF）, nerve growth factor（NGF）, neurotrophin（NT）-3 and 4 reduce cell surface neprilysin activity. The reduction of neprilysin activity was mediated by MEK/ERK signaling which enhanced phosphorylation at serine 6（S6）in the intracellular domain of neprilysin（NEP-ICD）. Increased phosphorylation of S6-NEP-ICD reduced cell surface neprilysin and subsequently led to an increase in extracellular Aβlevels in primary neurons. Further, a specific inhibitor of protein phosphatase-1a（PP1a）tautomycetin, induced extensive phosphorylation of S6-NEP-ICD and consequently reduced cell surface neprilysin activity. Accordingly, activation of PP1a elevated cell surface neprilysin activity and lowered Aβlevels. These results together indicate that the phosphorylation state of S6-NEP-ICD influences localization of neprilysin and affects extracellular Aβ levels. Therefore, maintaining S6-NEP-ICD dephosphorylated through either inhibition of protein kinases involved in the phosphorylation at S6-NEP-ICD or by activating phosphatases catalyzing dephosphorylation of S6-NEP-ICD may represent a new way to prevent reduction of cell surface neprilysin activity during aging and to maintain a physiological level of Aβ in the brain.

2S1-2 Neuroprotective function of DJ-1 in Parkinson's disease.

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Parkinson's disease（PD）occurs in approximately 1% of the population over the age of 65 years, the second most common neurodegenerative disease after Alzheimer's disease. PD is a progressive neurodegenerative disease that results from dopaminergic neuronal cell death in the substantia nigra. The cause of this selective cell death is poorly understood.
PD is comprised of sporadic and familial forms. Although familial PD cases account for about 10% of total cases of PD, investigations of the functions of familial PD gene products have provided great insights into the molecular mechanisms of the onset of PD, and familial PD gene products are thought to also play roles in the pathogenesis of sporadic PD. From these insights, major causes of neurodegeneration in PD are thought to be oxidative stress and mitochondrial dysfunction. The *DJ-1* gene has been identified by us as a novel oncogene that transforms mouse NIH3T3 cells in cooperation with activated ras in 1997. In 2003, Bonifati et al. found a large deletion and missense mutation in the *DJ-1* gene as a causative gene for familial PD *park7* with recessive inheritance. Although genetic and environmental factors suggest that DJ-1 affects the onset of PD, precise mechanisms at the molecular level have not been elucidated. The *DJ-1* gene is a causative gene for not only familial PD（*park7*）but also an oncogene. DJ-1 has various functions, including transcriptional regulation, anti-oxidative stress reaction, protease and mitochondrial regulation, and its activity is regulated by its oxidative status, mainly that of cysteine 106（C106）of DJ-1. Excess oxidation of DJ-1 has been observed in patients with sporadic PD, Alzheimer's and Huntington's disease, suggesting that DJ-1 also participates in the onset and pathogenesis of sporadic PD as well as familial PD. DJ-1 is also a stress sensor and its expression is increased upon various stresses, including oxidative stress. In this session, I introduce functions of DJ-1 against oxidative stress and possible roles of DJ-1 in the pathogenesis of PD.

2S1-3 Histone deacetylase mediates the decrease in drebrin cluster density induced by amyloid beta oligomers.

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Dendritic spine defects are found in a number of cognitive disorders, including Alzheimer's disease（AD）. Amyloid beta（Aβ）toxicity is mediated not only by the fibrillar form of the protein, but also by the soluble oligomers（Aβ −derived diffusible ligands, ADDLs）. Drebrin is an actin−binding protein that is located at mature dendritic spines. Because drebrin expression is decreased in AD brains and in cultured neurons exposed to Aβ, it is thought that drebrin is closely associated with cognitive functions. Recent studies show that histone deacetylase（HDAC）activity is elevated in the AD mouse model, and that memory impairments in these animals can be ameliorated by HDAC inhibitors. In addition, spine loss and memory impairment in HDAC2 over−expressing mice are ameliorated by chronic HDAC inhibitor treatment. Therefore, we hypothesized that the regulation of histone acetylation/deacetylation is critical to synaptic functioning. In this study, we examined the relationship between HDAC activity and synaptic defects induced by ADDLs using an HDAC inhibitor, suberoylanilide hydroxamic acid（SAHA）. We show that ADDLs reduce the cluster density of drebrin along dendrites without reducing drebrin expression. SAHA markedly increased the acetylation of histone proteins, and it simultaneously attenuated the ADDL−induced decrease in drebrin cluster density. In comparison, SAHA treatment did not affect the density of drebrin clusters or dendritic protrusions in control neurons. Therefore, SAHA likely inhibits ADDL−induced drebrin loss from dendritic spines by stabilizing drebrin in these structures, rather than by increasing drebrin clusters or dendritic protrusions. Taken together, our findings suggest that HDAC is involved in ADDL−induced synaptic defects, and that the regulation of histone acetylation plays an important role in modulating actin cytoskeletal dynamics in dendritic spines under cellular stress conditions, such as ADDL exposure.

2S1-4 Gaucher disease model in medaka displays axonal accumulation of alpha−synuclein

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Background：Recent genetic studies have revealed that mutations in *glucocerebrosidase*（*GBA*）, a causative gene of Gaucher disease（GD）, are a strong risk for Parkinson's disease（PD）. Accrding to a previous report, the odds ratio for any *GBA* mutation in patients versus controls was 5.43. However, its pathological mechanisms leading to PD remain largely unknown. Medaka（*Oryzias latipes*）are a versatile vertebrate animal model for disease research. So far, we have reported genetic PD models of medaka that develop locomotor dysfunction accompanied by the selective loss of dopaminergic and noradrenergic neurons. Medaka have the potential to be a new animal model of PD.Objective：The objective of this study was to investigate how *GBA* mutations cause PD. Methods：We generated *GBA* mutant medaka by screening a targeting induced local lesions in genomes（TILLING）library and *alpha−synuclein*（*α−syn*）deletion mutant medaka by transcription activator−like effector nucleases（TALENs）. Results：We generated GBA nonsense mutant（$GBA^{-/-}$）medaka completely deficient in glucocerebrosidase（GCase）activity. In contrast to the perinatal death of human and mice lacking GCase activity, $GBA^{-/-}$ medaka survived for months, enabling us to analyze disease progression. $GBA^{-/-}$ medaka displayed non−selective neuronal cell death accompanied by neuroinflammation, lysosomal abnormalities and α−syn accumulation in spheroids containing autophagosomes. Unexpectedly, disruption of *α−syn* did not improve the life span, spheroid formation, neuronal loss, or neuroinflammation in $GBA^{-/-}$ medaka. Conclusion：$GBA^{-/-}$ medaka display not only the phenotypes resembling human neuronopathic GD but also axonal accumulation of α−syn accompanied by impairment of the autophagy−lysosome pathway. Furthermore, the present study demonstrates this α−syn accumulation has minimal contribution to the pathogenesis of neuronopathic GD in medaka.

2S2-1 Neuronal innate immunity regulates neural development and function

○**Yi-Ping Hsueh**
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In the central nervous system, microglial cells are the well-known main immune cells in the brains in response to infection and injury. However, the recent accumulated studies have indicated that neurons also express the key pattern recognition receptors and downstream adaptors for innate immune responses. We are intrigued to explore the functions of innate immune systems in neurons. TLR3, TLR7 and TLR8 are particularly interesting, because these three pattern recognition receptors are able to recognize both pathogenic and endogenous RNAs. Using rodent cortex and hippocampus, we showed that TLR3, TLR7 and TLR8 are expressed in neurons and negatively control dendritic growth in a cell-autonomous manner. Although these TLRs share the similar effect on neuronal morphology, our studies suggested that they use different downstream signaling pathways and effectors to control neuronal growth. Induction of cytokine expression is only required for TLR7 pathway. The comparison of these TLRs will be presented. Besides, the roles of downstream adaptors SARM1 and MYD88 in neurons will also be discussed. Based on our studies, we suggest that neuronal innate immune system recognizes endogenous ligands and restricts dendritic and/or axonal growth. It is critical for neurodevelopment and brain function. Related publications by Hsueh, Y.-P.1. Chen et al. (2011) Sarm1, a negative regulator of innate immunity, interacts with syndecan-2 and regulates neuronal morphology. Journal of Cell Biology 193：769.2. Liu et al. (2013) TLR7 negatively regulates dendrite outgrowth through the Myd88-c-Fos-IL-6 pathway. Journal of Neuroscience 33：11479. 3. Lin et al. (2014) Neuronally-expressed Sarm1 regulates expression of inflammatory and anti-viral cytokines in brains. Innate Immunity 20：161. 4. Lin and Hsueh (2014) Sarm1, a neuronal inflammatory regulator, controls social interaction, associative memory and cognitive flexibility in mice. Brain, Behavior, and Immunity 37：142. 5. Lin et al. (2014) Sarm1 deficiency impairs synaptic function and leads to behavioral deficits, which can be ameliorated by an mGluR allosteric modulator. Frontiers in Cellular Neuroscience 8：87.

2S2-2 Microglial ontogeny and functions in shaping embryonic brain circuits

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Microglia are the resident macrophage population of the central nervous system（CNS）. Adequate microglial function is crucial for a healthy CNS；microglia are not only the first immune sentinels of infection and inflammation, but are also involved in the maintenance of brain homeostasis. Emerging data are showing new and fundamental roles for microglia in the control of neuronal proliferation and differentiation, as well as in the formation of synaptic connections. In parallel, recent studies on microglial origin indicate that these cells arise very early during development from progenitors in the embryonic yolk sac that produce cells able to persist in the CNS into adulthood. These unique immune cells are thus present at all stages of brain development, including the prenatal stage of neuronal circuit formation, which points to the intriguing possibility that microglia might be involved in development of the CNS. Here, we show that microglia participate to normal embryonic forebrain wiring regulating the progression of dopaminergic axons in the forebrain and the laminar positioning of subsets of interneurons in the neocortex. Our study reveals novel roles for microglia in the normal assembly of brain circuits and raises the possibility that dysregulated embryonic microglial function during pre-natal inflammation could impact forebrain connectivity and could contribute to the etiology of neuropsychiatric disorders.

2S2-3 Stress peptides and relapse to reward-seeking

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Relapse and hazardous drinking represent the most difficult clinical problems in treating patients with alcohol use disorders. Increasing our understanding of the brain circuits and chemicals that regulate alcohol intake and relapse offers the potential for more targeted therapeutic approaches to assist in relapse prevention. We have provided evidence for a role of numerous neuropeptides in cue and/or stress induced reward-seeking. This presentation will highlight recent studies on 3 neuropeptide systems, which can act independently and via circuit-level interactions to regulate relapse-like behaviour. Specifically, orexin, corticotropin releasing factor（CRF）and relaxin-3 all act, and appear to also interact, within circuits mediating cue and/or stress-induced relapse-like behaviour. For example, orexin1 receptors in the ventral tegmental area and prelimbic cortex regulate cue-induced reinstatement of alcohol-seeking in rats；relaxin-3 acts upon RXFP3 receptors in the bed nucleus of the stria terminalis to regulate stress-induced reinstatement of alcohol-seeking in rats.

2S2-4 Interleukin-1 receptor family proteins function as neuronal synapse organizers

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Interleukin-1 (IL-1) family cytokines play crucial roles in immune and inflammatory responses through the activation of a group of structurally related receptors belonging to the IL-1 receptor（IL-1R）family. IL-1R family proteins consist of extracellular three Ig-like domains and a TIR domain in the cytoplasmic portion. By morpholino-mediated gene knockdown screening of causative genes for neurodevelopmental disorders, we identified IL-1 receptor accessory protein-like 1（IL1RAPL1), a member of IL-1R family, as a key regulator of neuronal synapse formation. IL1RAPL1 expressed in fibroblasts induced excitatory presynaptic differentiation of co-cultured cerebral cortical neurons. Furthermore, we identified presynaptic protein tyrosine phosphatase（PTP）δ as a major IL1RAPL1 interacting protein. Although the presynapse-inducing activity of IL1RAPL1 was completely abolished in PTPδ knockout neurons, the postsynapse-inducing activity of PTPδ was partly suppressed in IL1RAPL1 knockout neurons, suggesting that IL1RAPL1 mediates synapse formation solely through presynaptic PTPδ, while PTPδ organizes postsynaptic differentiation through IL1RAPL1 and other proteins. We found by systematic screening of IL-1 receptor family proteins for synaptogenic activity that IL-1 receptor accessory protein（IL-1RAcP), a common subunit of receptor complexs for IL-1 cytokines, had strong activity to induce excitatory presynaptic differentiation. IL-1RAcP also required PTPδ to exert the synaptogenic activity. Accordingly, IL-1RAcP knockout mice exhibited decreased spine density in some brain regions as shown in IL1RAPL1 and PTPδ knockout mice. Moreover, X-ray crystallography of PTPδ-IL1RAPL1/IL-1RAcP complex revealed the structural basis of synapse-organizing cell adherent interaction. These results suggest that IL-1 receptor family proteins, IL1 RAPL1 and IL-1RAcP function as cell adhesion molecules in the brain to organize neuronal synapse formation.

2S3-1 Atypical brain auditory processing in young children with autism spectrum disorder.

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Magnetoencephalography（MEG）is a noninvasive neuroimaging technique that provides a measure of cortical neural activity on a millisecond time scale with high spatial resolution. In the past decade, a number of previous MEG studies have reported atypical brain responses to auditory stimuli in children with autism spectrum disorder（ASD）. Recently, using a child custom-sized MEG, we demonstrated the atypical brain auditory processing and the atypical developmental trajectory in a component of auditory evoked field（P1m）in young children with ASD. Although further studies are still necessary, we present our recent result from MEG study for young children with ASD and typically developing children.

2S3-2 Modeling Autism

○**Toru Takumi**（**内匠　透**）
RIKEN BSI（理研・BSI）

Autism is a complex psychiatric illness that has received considerable attention as a developmental brain disorder. Substantial evidence suggests that chromosomal abnormalities including copy number variations contribute to autism risk. The duplication of human chromosome 15q11–q13 is known to be the most frequent cytogenetic abnormality in autism. We have modeled this genetic change in mice using chromosome engineering to generate a 6.3-Mb duplication of the conserved linkage group on mouse chromosome 7. Mice with a paternal duplication display autistic-like behavioral features such as poor social interaction and stereotypical behavior, and exhibit abnormal ultrasonic vocalizations. This chromosome-engineered mouse model for autism seems to replicate various aspects of human autistic phenotypes and validates the relevance of the human chromosome abnormality. This model is a founder mouse for forward genetics of a developmental brain disorder and an invaluable tool for its therapeutic development. I will show our analyses on these mice towards understanding the molecular pathophysiology of autism spectrum disorder.

2S3-3 De-regulation of translation and synaptic function in rodent models of autism

○**Peter Scheiffele**
Biozentrum of the University of Basel

An imbalance of neuronal protein synthesis is emerging as a pathophysiological hallmark in Fragile X Syndrome and some forms of autism. Disease-associated mutations modifying the function of the eIF4F eukaryotic translation initiation factor complex or ribosome processivity result in elevated translation downstream of metabotropic glutamate receptors (mGluR). However, most autism-associated mutations do not directly impact the translation machinery or its regulators. Thus, it is unknown whether strategies targeting protein synthesis homeostasis are more broadly applicable in neurodevelopmental disorders. We here report that the autism-associated mutation of Nlgn3, a gene encoding the synaptic adhesion molecule, results in an impairment of neuronal mTORC1-dependent signaling, a reduction in eIF4F assembly, and reduced protein synthesis. Pharmacological inhibition of the MAP-kinase pathway, which modulate eIF4F function, restores normal protein synthesis rates in Nlgn3KO mice. Moreover, a brain penetrant inhibitor alleviates several autism-related behavioral deficits in adult Nlgn3KO mice. This work identifies a target for pharmacological intervention in neuronal disorders with perturbed translational homeostasis.

2S3-4 Current status and future perspective of autism from clinical research with oxytocin and neuroimaging

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Autism spectrum disorders, a highly prevalent neurodevelopmental disorder, currently have no established treatment for its core symptoms. The disorders are characterized by two core symptoms including deficits in social communication and interaction, and repetitive and restricted behavior. Since accumulating evidence supports the concept that oxytocin can induce effects on social and affiliative behaviors, the neuropeptide is thought to be a potential therapeutic approach for deficits in social communication and interaction in individuals with autism spectrum disorders. In fact, our previous studies have revealed oxytocin-induced temporal mitigations of autistic behavior and its neural basis such as brain activity. Ongoing studies are further conducting to examine several unresolved issues such as 1) clinically meaningful effects after long-term administrations of oxytocin, 2) biomarkers predicting individual differences in therapeutic effects in advance, and 3) potential genetic and molecular mechanisms of effects of oxytocin on autism spectrum behaviors. In the symposium, integration of previous findings and introductions of ongoing studies will be presented to promote productive interactions with other speakers and audiences from various research fields.

2S4-1 Study on electrically induced responses in visual cortex for visual prosthetics

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Electrical stimulus applied to the extracellular space of visual cortex evokes a spot-like light percept called phosphen, which provoked the research of cortical prosthetics for blind patients. Previous clinical experiments on human volunteer patients revealed that the evoked phosphens changed their appearance depending on temporal patterns of electrical stimulation. However, relation between the stimulus parameters and the evoked percepts is not well understood. To evaluate percepts evoked by cortical prosthetics, spatiotemporal properties of electrically induced cortical activities were studied in terms of stimulation parameters to reconstruct physiologically feasible phosphen patterns. We conducted a voltage-sensitive dye imaging of cortical responses induced by current stimuli applied to V1 in rodents, which allowed one to study how the induced responses propagate in V1 and to higher order cortical areas. All experiments were approved by the Institutional Animal Care and Use Committee of the Graduate School of Engineering, Osaka University. A single current pulse（＞10μA）with biphasic waveform applied with a metal electrode（ME13213, MicroPrpbes）induced responses which resembled the PSPs observed in previous electrical recordings on single cells in mammals. The threshold and the half activating intensity was 5-10μA and around 40μA, respectively. The induced response propagated laterally in V1 via a poly-synaptic transmission. For a low current intensity（＜10μA）, the excitatory response to a repetitive stimulation was cumulative for several hundreds of ms. The response to each stimulus pulse showed synaptic depression, that was more prominent at higher stimulus intensities（＞20 μA）and gradually diminished as the repetitive stimulation continued for ＞1s. The response to the repetitive stimuli declined more rapidly in V2 than in V1, indicated the signal transmission from V1 to V2 is not sustained. According to the present experiments and previous clinical experiments, physiologically feasible phosphen patterns were reconstructed on a head mount display and presented to volunteer subjects to evaluate the visual percepts evoked by cortical prosthetics. The feasible prosthesis were designed and a prototype was implemented for animal experiments.

2S4-2 Monoaminergic/cholinergic modulation of neuronal visual information processing and behavioral contrast detectability.

○**Satoshi Shimegi**（**七五三木聡**）
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Our brain is regarded as a computer processor, but there is a crucial difference between them. The brain can dynamically change the input-output relationship depending on physiological states and behavioral contexts to optimize the information processing while the computer processor can't. Monoaminergic/cholinergic transmitters such as serotonin（5-HT）, noradrenaline（NA）, and acetylcholine（ACh）play central roles in the state/context-dependent modulation of brain functions. In this presentation, I'll talk about how those transmitters modulate visual information processing at the single neuron level and improve behavioral visual ability. Using drifting sinusoidal grating stimuli with various contrast, a contrast-response function（input-output relationship）was estimated for individual V1 neurons of anesthetized monkeys, and the effects of micro-iontophoretic administration of agonists/antagonists of those receptors on the contrast-response functions were tested. The effects was complex but reasonable. For example, each activation of 5-HT1B and 5-HT2A receptors exerted both suppressive and facilitative effects, depending on the firing rate of the recorded neurons. The detailed analysis suggested that 5-HT1B receptors enhance the signal-to-noise（S/N）ratio of visual responses by suppressing spontaneous activity（noise）and facilitating visual response（signal）, and 5-HT2A act as a gain controller by enhancing weak response and suppressing excessive response. ACh also enhanced S/N ratio and modulated the contrast-response function mainly in a manner of response gain control for V1 of monkeys and rats. To examine whether the neuronal modulation contributes to visual ability, we performed behavioral measurements of contrast sensitivity in freely moving rats, and tested the effects of the systemic injection of various drugs on the contrast sensitivity. We found that contrast detectability was improved by donepezil, a cholinesterase inhibitor, suggesting that ACh endogenously released in cognitive behavior controls the contrast detectability by modulating cortical visual information processing. Thus, monoaminergic/cholinergic systems seem to control the fine and elaborated functions of the primate visual system to meet the purposes of behavioral context.

2S4-3 Ocular dominance plasticity regulated by Otx2-inducible molecules

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Understanding molecular mechanisms of experience-dependent plasticity is a subject of intense investigation. For example, inputs from the two eyes first converge in the primary visual cortex, where competitive interactions determine which eye will eventually dominate both functionally and anatomically. It is widely believed that distinct GABAergic circuits drive the critical period of ocular dominance plasticity. Our previous report showed that experience-dependent transfer of Otx2 homeoprotein into parvalbumin (PV)-cells activates this sensitive period. Otx2 deletion results in reduction of mature PV-cells enwrapped by chondroitin sulfate glycosaminoglycans and in disruption of plasticity. This homeoprotein may promote a downstream cascade for plasticity, however its target genes remain obscure. Recently, we found that Otx2 induced chondroitin sulfate surrounding PV-cells and that chondroitin sulfate further promoted Otx2 accumulation. A positive feedback loop between Otx2 and chondroitin sulfate regulates the onset and offset of plasticity. Moreover, Otx2 also induces an actin-binding protein within PV-cells that modulates plasticity. Thus, our results indicate that ocular dominance plasticity is elicited through a well-balanced cooperation of Otx2-inducible molecules inside and outside PV-cells.

2S4-4 Modulation of visual behavior by central thalamic deep brain stimulation

○**Keith Purpura[1], Jonathan Baker[1], Jae-Wook Ryou[1], Xuefeng Wei[2], Christopher R. Butson[3], Nicholas D. Schiff[1]**
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The central thalamus is believed to play an important role in enabling the cerebral cortex to produce behavior and mental states including consciousness. Following the successful use of deep brain stimulation (DBS) to treat movement disorders, it was hypothesized that targeting DBS to the central thalamus of patients with disorders of consciousness (DOC) could help restore cognitive function by modulating activity in the anterior forebrain. The goal of the study described here is to better understand the mechanisms of DBS in the central thalamus and to test methods for improving its effectiveness. We carried out experiments in two normal macaques to test the effects of DBS on visual behavior. Both monkeys were implanted with multiple DBS electrodes in their central thalami. One monkey was trained to perform three tasks：1) a sustained visual attention reaction time task；2) a memory-guided saccade task；3) a visual pattern categorization task. The second monkey was trained on Task 1. Large-scale brain activity was recorded with an array of electrocorticography (EcoG) leads implanted in the skull and with a chronically-implanted array of microelectrodes in the frontal lobe and striatum. High-frequency periodic DBS was turned on and off throughout the behavioral sessions to determine the effect of DBS on task performance and on brain activity. We found that DBS in the central thalamus profoundly modified visual behavior and brain-wide electrical activity. Specific DBS frequencies, when matched with one particular electrode configuration of current injection, were most effective at sustaining performance over many trials, decreasing reaction times and improving pattern categorization. In addition, this method of stimulation also shifted the power spectrum in the EcoG and microelectrode local field potentials：slow rhythmic brain activity decreased and fast rhythmic brain activity increased compared to when DBS was turned off. Our results demonstrate that central thalamic DBS can modulate innate patterns of brain activity and visual behavior, and may provide a means for normalizing impaired cognitive capacity.

2S5-1 The Next Phase of the NIH Center for Regenerative Medicine（NCRM）：Establishment of a Stem Cell Technology Facility

○**Anton Simeonov**
National Center for Advancing Translational Sciences (NCATS), NIH

The field of stem cell technologies has not progressed from basic discovery to therapeutic application as efficiently as many had hoped. Few robust tools, technologies, protocols, and paradigms exist that allow researchers to reproducibly and efficiently maintain a stem cell population in a pluripotent state, produce pure populations of specific cell types, or influence endogenous stem cell populations in vivo. Multiple needs analyses by U.S. and international research bodies have concluded that the lack of such tools and technologies is what is currently limiting the progress of translational and clinical applications of stem cells, and that their creation and provision would be transformative to the stem cell field and to biomedicine. It is this need that the NIH/NCATS Stem Cell Technology Facility（SCTF）, a continuation of the NIH Center for Regenerative Medicine（NCRM）Common Fund Program, will address：the mission of the Facility is to tackle the top methodological or technical problems that currently impede therapeutic use of induced pluripotent stem cells and to rapidly deliver the resulting protocols, standards, data, and tool molecules to the public. The Facility will collaborate with researchers to validate the methods developed within for suitability in regenerative medicine applications. An overview of the facility will be presented, along with emerging small molecule screening technologies that NCATS has developed which can be leveraged to further advance the field of stem cell-derived therapies.

2S5-2 iPSC Non-Clinical Experiments for Nervous System（iNCENS）project-An attempt to evolve the CNS safety pharmacological evaluation by in vitro use of human-induced pluripotent stem cell-derived neurons

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In the process of drug development, the improvement of predictability of the CNS safety pharmacological evaluation is required because the CNS adverse effects cause the drop-outs at the later stages of the developmental process. When human iPSC (hiPSC)-derived neurons were reported in 2007, in vitro use of hiPSC-derived neurons attracted attentions to overcome these issues. However, at present, the majority of the non-clinical CNS safety pharmacological tests are in vivo animal tests and the follow-up in vitro tests are performed when needed. The appearance of hiPSC-derived neurons made us realize that we had not discussed enough about the usefulness or validity of the in vitro evaluations in the CNS safety pharmacological evaluation. We therefore have just launched the "iNCENS"(iPSC non-clinical experiments for nervous system) project. This project has two aims, i.e., the establishment of reliable in vitro biomarkers for the risks of cognitive impairment and epilepsy, and the application of hiPSC-derived neurons to these in vitro systems. So far, we have found good candidates which can be used for the prediction of cognitive impairment and epilepsy. Furthermore, we have established the standard experimental protocol to select hiPSC-derived neurons suitable for the non-clinical CNS safety pharmacological evaluation and have found two lines of hiPSC-derived neurons that express NMDA receptors. We also have succeeded in recording synchronization of spontaneous unit activity of hiPSC-derived neurons across multi-recording channels. In this presentation, we will introduce the progress of our "across-institute" project and discuss about the potentials of the in vitro use of hiPSC-neurons in the CNS safety pharmacological evaluation.

2S5-3 Early stage development of human iPSCs-derived neurons and its application to non-clinical study

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Recent advances in human induced pluripotent stem cells（hiPSCs）offer new possibilities for biomedical research and clinical applications. However, the detail processes of neuronal development from hiPSCs have not been known. In this study we analyzed development of hiPSCs-derived neurons（hiPS-neurons）, particularly focusing on their early developmental stages. We cultured iCell Neuron（Cellular Dynamics International）and compared their development with that of the primary cultured neurons derived from rat hippocampus. In 2 days in vitro（DIV）culture of the rat neurons, we observed three different stages which were stages 1, 2, and 3 in the developmental classification proposed by Dotti et al.（1988）. Most developed stage 3 neurons had several short neurites with one long neurite, which is destined for an axon. In the DIV2 iCell Neurons, we observed neurons in all stages similar to the rat neurons, although the number of stage 3 neurons was few. This result indicates that the hiPS-neurons differentiate slower than rat neurons. In addition, the length of the axons and the speed of axonal elongation were measured. We found iCell Neuron had significantly shorter axons and significantly slower elongation speed of axon. Finally we examined if this slow axonal elongation in iCell Neuron is because of the abnormality of the growth cones, and found no differences. Together, our study shows the growth of hiPS-neuronal axons is slower but its differentiation is comparably to rat neurons. Furthermore, in this symposium, I would like to mention about the use of hiPS-neurons as a tool for non-clinical study. Our preliminary data suggests that evaluation of hiPS-neuronal development could be a useful tool to assess developmental neurotoxicity of medicines.

2S5-4 The iPSC technology to model human neurodevelopmental diseases in vitro

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Intellectual disability（ID）and autism spectrum disorders（ASD）are complex developmental mental disorders characterized by social and communicative deficits, language impairments and repetitive behaviors, with an estimated prevalence in Europe of 1-2%. Although several genetic alterations have been recognized as causal of ID and ASD, the etiopathogenesis of these complex diseases remain largely unknown.We investigated the molecular bases of ID and ASD by using an innovative strategy based on the recently established technology of genetic reprogramming. Because most of proteins encoded by genes involved in ID and ASD are associated to the synaptic junction between neurons and are involved in its function, we focused our attention on two genes that codify for the synaptic proteins Shank3 and IL1 RAPL1. We generated iPS cells, which we induced to differentiation into excitatory and inhibitory neurons, from fibroblast of patient carrying mutations in these genes. Studying the function of these proteins in parallel with knock out mouse models for Shank3 and IL1RAPL1 we will contribute to better understand the molecular mechanisms of synapse formation, plasticity and learning and memory processes, and will open the possibility of future therapeutic approaches for neurodevelopmental diseases like ID and ASD.

2S6-1 Slow inhibitory oscillation in the basolateral amygdala and its alteration by stress load

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Basolateral amygdaloid complex（BLA）is deeply involved in emotional processing and is sensitive to chronic stress. Its abnormality is related to several psychiatric disorders. In the BLA, sensory information from the cortex and the thalamus are evaluated in terms of emotional valence and these signals are transmitted to central nucleus of amygdala. Previously, we showed that projection neurons in BLA receive rhythmic inhibitory inputs which are evoked by synchronous firings of interneurons. Such neurons were mostly distributed in ventral part of BLA. In addition, this inhibitory oscillation requires the glutamatergic transmission within BLA, suggesting that local network activities are essential for the oscillation. In the present study, we examined the effect of sleep deprivation（SD）on the BLA because the frequency of slow inhibitory oscillation（0.1–3 Hz）is similar to that of delta wave（1–4 Hz）observed in LA of animals during slow wave sleep. Thereby we examined the effects of SD on properties of projection neurons or inhibitory interneurons in rat BLA to reveal the physiological significance of the inhibitory network oscillation. We applied acute SD（morning, 3 hours）on juvenile Wistar rats（P14–24）. Rats were held in the cage where 1.5–2 cm height of water was filled. Thereafter, coronal slices were immediately prepared for whole-cell recording. We found that power of the low-frequency（0.1–3 Hz）oscillation（rhythmic inhibitory inputs）was decreased in SD rats. The decline was caused by a reduction of synaptic current amplitude. In addition, spike firing of inhibitory interneurons was attenuated. These results suggest that stress by sleep disturbance modulates the magnitude of network oscillation by reducing the interneuronal activity in BLA.

2S6-2 Metabotropic glutamate receptor sensitive slow calcium oscillations in striatum.

○**Makoto Osanai（小山内　実）**

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The striatum receives inputs from the cortex and is thought to play a crucial role in controlling somatic motor movements, behavioral patterns, cognition, learning, and memory. Ca^{2+} is a universal intracellular messenger, and plays enormous versatile rolls in cells. However, the properties of the Ca^{2+} signaling in the striatum remain less understood.There are many types of metabotropic receptors that may contribute to intracellular Ca^{2+} signaling in the striatum. Group I mGluRs, which are known to modulate the intracellular Ca^{2+} signaling, are densely expressed in the striatum.We have found the long-lasting spontaneous calcium transients（slow Ca^{2+} oscillation）, which lasted up to about 300 s, in the striatal neurons and astrocytes. Neither the inhibition of action potentials nor ionotropic glutamate receptors blocked the slow Ca^{2+} oscillation. Depletion of the intracellular Ca^{2+} store and the blocking IP3 receptors diminished the slow Ca^{2+} oscillations. The application of an antagonist against mGluR5 also blocked the slow Ca^{2+} oscillations in both putative-neurons and astrocytes. Thus, the mGluR5-IP3 signal cascade is the primary contributor to the slow Ca^{2+} oscillation in both putative-neurons and astrocytes.The slow Ca^{2+} oscillations have poor regularity but feature multicellular synchrony. In the condition of blockade of action potentials, the regularity of the Ca^{2+} oscillations was increased, however the cellular correlation of the Ca^{2+} oscillations was reduced. These phenomena were observed only in the corticostriatal slice but not in the striatal slice. Thus, the cortical activities might contribute to the slow Ca^{2+} oscillations.Intracellular Ca^{2+} can modulate various proteins, thus, the slow Ca^{2+} oscillations we found may regulate the cellular functions leading to change the state of cellular networks in the striatum. Though in a simulation study, we found out that the slow Ca^{2+} oscillation could alter the firing rate of the medium spiny neuron via modulation of Ca^{2+}-activated potassium channels.mGluR5 has also been suggested as a therapeutic target for Parkinson's disease and may interact with dopamine signaling via Ca^{2+}. Dopamine signaling is essential for neuronal functioning in the striatum. Thus, the slow Ca^{2+} oscillation is expected to play a role in information processing in the striatum.

2S6-3 Optogenetically-induced seizure and longitudinal hippcampal network dynamics

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Background

Epileptic seizure is a paroxysmal and self-limited phenomenon characterized by abnormal hypersynchrony of a large population of neurons. But our current understanding of seizure dynamics is still limited. Traditional animal model of epileptic seizure by electrical stimulation generates large artifacts which interfere with recordings of neuronal activities in animal model.

Methods/Results

Here we propose a novel in vivo model of epileptic seizures using optogenetics. Repetitive pulse photo-stimulation induced seizures in the hippocampus of Thy1.2-ChR2-Venus transgenic rat. Simultaneous multisite recordings and immunohistochemical study by c-Fos staining revealed the seizure involved the entire hippocampus along the longitudinal（septo-temporal, ST）axis. Granger causality analysis of local field potentials recorded with multi-contact array electrode inserted along the ST axis of hippocampus showed a bidirectional but asymmetric increase in signal flow along the ST direction. State space presentation of the causality and coherence revealed three discrete states of the seizure phenomenon：1）resting state；2）afterdischarge initiation with moderate coherence and dominant septal-to-temporal causality；and 3）afterdischarge termination with increased coherence and dominant temporal-to-septal causality.

Conclusions/Significance

This novel hippocampal seizure model was advantageous in its reproducibility and artifact-free electrophysiological observations. Our results provide additional evidence for the potential role of hippocampal ST interactions alternating in temporal cource in seizure phenomenon.

2S6-4 Electrophysiological temporal property of the striatum

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The striatum is related to motor function, as well as other 1st person cognitive processes such as action selection, action execution and re-evaluation of the executed action. These striatal functions are processed in time, within several seconds, suggesting the necessity of investigating striatal time-domain properties. In order to understand the temporal structure of the striatal activities, we assessed a temporal summation of synaptic potentials of adult rodent medium spiny neurons and a firing probability modulation after the depolarization. We found that the striatal neuron in acute slice was able to respond to low frequency synaptic stimulations and sum up the potentials；the maximum inter-stimulus interval（ISI）to observe the potential summation was 100-ms, while the cortex layer 5 pyramidal neurons have shorter temporal summation ability：the maximum ISI is 25-ms（Reyes and Sakmann 1999）. We also investigated the firing probability modulation after optogenetic stimulation. The striatal neurons showed a prolonged firing response of gradually increasing duration when exposed to repetitive optogenetic photostimulation. The prolonged firing response also recurred after a long intermission of up to 20 sec. These results indicate that the striatum has a characteristic time-domain property of the synaptic input integration, suggesting that the prolonged property could be a new pharmacological target.

2S7-1 Involvement of TRPV1 activation in pain and itch sensation

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The capsaicin receptor, transient receptor potential vanilloid 1（TRPV1）, acts as a polymodal detector of pain-producing chemical and physical stimuli in sensory neurons. Hyperglycemia and hypoxia are two main phenomena in diabetes associated with several complications. Although many studies on streptozotocin-induced diabetic rats indicate that early diabetic neuropathy is associated with potentiation of TRPV 1 activity in dorsal root ganglion neurons, its underlying mechanism and distinctive roles of hyperglycemia and hypoxia have not been completely clarified. In this symposium, we introduce that artificial hypoxic and high glucose conditions in vitro potentiate the TRPV1 activity without affecting TRPV1 expression in both native rat sensory neurons and HEK293 cells expressing rat or human TRPV1. Surprisingly, hypoxia was found to be a more effective determinant than high glucose, and hypoxia-inducible factor-1 alpha（HIF-1α）seemed to be involved. In addition, high glucose enhanced TRPV1 sensitization only when high glucose existed together with hypoxia. The potentiation of TRPV1 was caused by its phosphorylation of the serine residues, and translocation of protein kinase C（PKC）ε was clearly observed in the cells exposed to the hypoxic conditions in both cell types, which was inhibited by 2-methoxyestradiol, a HIF-1α inhibitor. These data suggest that hypoxia is a new sensitization mechanism for TRPV1, which might be relevant to diabetes-related complications, and also for other diseases that are associated with acute hypoxia. In addition to the effect of TRPV1 on the pain sensation in diabetes, we also introduce the involvement of TRPV1 on itch sensation through its activation by chemokines.

2S7-2 Chronic nociceptive stimuli induce “cell memory due to pain” with epigenetic modification

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Neuropathic and inflammatory pain promote a large number of persisting adaptations at the cellular and molecular level, allowing even transient tissue or nerve damage to elicit changes in cells that contribute to the development of chronic pain and associated symptoms. There is evidence that injury-induced changes in chromatin structure drive stable changes in gene expression and neural function, which may cause several symptoms, including allodynia, hyperalgesia, anxiety, and depression. We have shown that a robust increase in MCP-3 protein, which lasts for up to 2 weeks after surgery, in the dorsal horn of the spinal cord of mice with sciatic nerve ligation is seen mostly in astrocytes, but not microglia or neurons. This increase in MCP-3 gene transcription was accompanied by the decreased trimethylation of histone H3 at lysine27（H3K27me3）at the MCP-3 promoter. The increased MCP-3 expression associated with its epigenetic modification observed in the spinal cord was almost abolished in interleukin-6（IL-6）knockout mice with sciatic nerve ligation. It has been reported that a Jumonji domein containing 3（JMJD3）function as transcriptional activators that demethylate H3K27me3. Therefore we performed ChIP assays with antibodies against JMJD3 at the promoter region of MCP-3. As a result, sciatic nerve ligation significantly increased the induction of jmjd3 at MCP-3 promoter. These findings suggest that increased MCP-3 expression associated with IL-6-dependent epigenetic modification at the MCP-3 promoter after nerve injury, mostly in spinal astrocytes, may serve to facilitate astrocyte-microglia-neuron interaction in the spinal cord. In this symposium, we will also discuss the importance of epigenetic changes in another molecular targets for chronic pain.

2S7-3 Neurotransmitters Modulating Pain Inhibitory Pathways

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The spinal dorsal horn is the powerful target of regulation of pain signaling by local and supraspinal mechanisms. Descending control of spinal nociception originate from many brain regions and plays critical roles in determining the experience of both acute and chronic pain. Most clinically available analgesic drugs for acute and chronic pain, like opiates and α2 adrenenoceptor agonists, change the descending pain controlling pathways. In this presentation, I focus on the noradrenaline/serotonin systems, which originate from locus coeruleus（LC）and rostalventrolateral medulla（RVM）respectively, and will discuss about their roles in pain modulation and modulation of other brain functions. In chronic neuropathic pain model, plastic change of the descending pathways of noradrenaline/serotonin are related to the pain processing including the effects of endogenous analgesia and the effects of analgesic drugs.

2S7-4 Microglial transcription factors and neuropathic pain

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In contrast to physiological pain, pathological pain is not dependent on the presence of tissue-damaging stimuli. One type of pathological pain-neuropathic pain-is often a consequence of nerve injury or of diseases. Neuropathic pain can be agonizing, can persist over long periods, and is often resistant to known painkillers. A growing body of evidence indicates that many pathological processes within the CNS are mediated by complex interactions between neurons and glial cells. In the case of painful peripheral neuropathy, spinal microglia react and undergo a series of changes that directly influence the establishment of neuropathic pain states. Results of our laboratory have demonstrated that the transcription factor interferon regulatory factor-8（IRF8）is upregulated in spinal microglia after peripheral nerve injury（PNI）and regulates expression of genes crucial for converting the cells to reactive ones. Furthermore, we recently identify IRF5 as a target of IRF8 and as being required for upregulation of P2X 4 receptors（P2X4Rs；ATP-gated channels essential for producing neuropathic pain）. PNI increased expression of IRF5 in spinal microglia in a cell type-specific manner. The upregulation of IRF5 expression was abolished in IRF8-deficient mice. IRF5 induced expression of P2X4R by directly binding to the promoter region of the P2rx4 gene. Mice lacking Irf5 did not upregulate spinal P2X4R after PNI, and also exhibited substantial resistance to pain hypersensitivity. Thus, an IRF8-IRF5 transcriptional axis contributes to shifting spinal microglia toward a P2X4R-expressing reactive state after PNI. These results may provide a new target for treating neuropathic pain.

3S1

History and future of Japanese Society for Neurochemistry（JSN）and International Society for Neurochemistry（ISN）. JSN/ISN の歴史を探り今後の発展を考える

○**Kunihiko Suzuki**[1]**（鈴木　邦彦）, Mei Satake**[2]**（佐武　明）, Eishichi Miyamoto**[3]**（宮本　英七）, Kazuhiro Ikenaka**[4]**（池中一裕）, Hiroshi Kiyama**[5]**（木山　博資）**

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In this round table discussion, each discussant will present a topic concerning history and future of JSN and ISN, then all participants including audiences from floor discuss it freely. The topics are followings：1）Outline of history of JSN（K. Uyemura）. The JSN from 1958 is the oldest society for Neurochemistry in the world. It developed much and is now the 58th meeting in Oomiya, 2015. 2）The early stage of JSN（M. Satake）. From the beginning, active and friendly discussions were carried out among clinical doctors such as psychiatrists and scientists of basic medicine. And the discussions continued traditionally until now. They also made great efforts to obtain the national grants. 3）History of ISN and J. Neurochem.（K. Suzuki）. As the treasurer of ISN, Suzuki contributed much to establish its financial background. He succeeded to make J. Neurochem. the official journal of ISN and to obtain considerable benefits to ISN from it. Thereafter, ISN are able to several supports to local meetings, travel expenses and so on. Now as a new treasurer of ISN, Ikenaka was elected. 4）Contributions of Japanese to ISN（E. Miyamoto）. Many Japanese contributed to ISN as officers, council members, the members of editorial board of J. Neurochem. In addition, the ISN meeting were invited to Japan twice（4th ISN meeting, 1973, Tokyo, Chairman Y. Tsukada and 15th meeting, 1995, Kyoto, Chairman K. Kuriyama）. The possibility to invite 3rd ISN meeting in Japan will be discussed. Recently, as a new council member of ISN, Hiroko Baba was elected in addition to Hisanaga. Their active contribution to ISN and JSN will be expected. 5）History and future of Asian Pacific Society for Neurochemistry（APSN）（K. Ikenaka）. Under ISN, there are three sub-societies in the world, American Society for Neurochemistry（ASN）, European Society for Neurochemistry（ESN）and Asian-Pacific Society for Neurochemistry（APSN）. Each national society belongs to one of sub-society.

Therefore, JSN belong to APSN. Ikenaka, who contributed to APSN as the treasurer and the president of APSN previously, will talk on topics of APSN. 6）Recent problems of JSN（T. Shirao）. There are several problems on JSN, such as the number of members, financial situation including members fee and supports from other resource, policies to support young scientists and woman scientists. In addition, relationship between JSN and other related societies, such as Japanese Neuroscience Society will be discussed. 7）Hopeful future of JSN（H. Kiyama）. Finally, Kiyama, the president of JSN, will show the future of JSN, after these all discussions.

3S2-1 Regulation of BDNF gene expression and its possible role in neural functions and diseases

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It has been widely accepted that the expression of BDNF gene is controlled in a neuronal activity-dependent manner. We have investigated molecular mechanisms underlying the activity-dependent expression of *Bdnf*. Here we especially focused on the regulation of *Bdnf* expression by stimulation of G protein-coupled receptor（GPCR）, which is a major receptor for neuromodulators such as monoamines and neuropeptides. Using primary culture of cortical cells, we found that the stimulation of PAC1, a Gs/q-coupled GPCR, with pituitary adenylate cyclase-activating polypeptide（PACAP）selectively activated NMDA receptor（NMDAR）-calcineurin pathway, resulting in the induction of *Bdnf* expression through CREB and its co-activator, CREB-regulated transcriptional co-activator 1（CRTC1）. Interestingly, this induction of *Bdnf* was similarly observed when the cells were treated with other Gs- or Gq-coupled GPCR agonists such as SKF38393, isoproterenol, corticotropin-releasing factor（CRF）, and neurotensin. These results suggest that the selective activation of the NMDAR-calcineurin pathway is generally induced by the stimulation of Gs/q-coupled GPCR in neurons. It is well known that CREB, CRTC1, and BDNF contribute to expressing long-lasting changes in neural functions such as learning and memory. Moreover, dysregulation of neuromodulatory systems, such as dopaminergic and serotonergic signaling, is suggested to be related to neural and psychiatric disorders including depression and schizophrenia. Taken together, the GPCR-mediated *Bdnf* expression through the NMDAR-calcineurin-CRTC1-CREB pathway would contribute to plasticity-related phenomena and the disruption of this regulation may be related to pathogenesis of psychiatric disorders.

3S2-2 CAPS2-The positive regulation factor of BDNF secretion and the candidate gene for autism

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Calcium-dependent activator protein for secretion 2（CAPS 2）plays a role in regulation of dense-core vesicle secretion and is associated with autism susceptibility. In this study, we analyzed CAPS2-mediated secretion of brain-derived neurotrophic factor（BDNF）and cellular and behavioral phenotypes of CAPS2 knockout（KO）mice. CAPS2 KO mice had significant reduction in BDNF levels in the hippocampus compared to their wild type（WT）littermates. Time-lapse live cell imaging showed that overexpression of exogenous CAPS2 enhanced quantity and kinetics of activity-dependent BDNF-pHluorin release from cultured hippocampal neurons of CAPS2 KO mice. KO mouse hippocampus also showed decreased number of GABAergic neurons, decreased synaptic vesicle density of inhibitory synapses and decreased frequency and amplitude of mIPSCs. Theta-burst induced long-term potentiation（LTP）at CA3-CA1 synapses was normally induced in KO mice but was reduced in the maintenance phase compared to the WT. The LTP maintenance, however, became indistinguishable between KO and WT in the presence of GABA-A receptor antagonist picrotoxin. In addition, KO mice exhibited augmented autistic and anxiety-like behavior which may be associated with impaired GABAergic transmission. Taken together, our study suggests that CAPS2 is an important regulator of BDNF secretion and thereby influences development of GABAergic inhibitory synapse networks.

3S2-3 New insight in transport and secretion of BDNF：Implications in brain-related diseases

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Brain-derived neurotrophic factor（BDNF）is an essential factor for survival, differentiation, and functions of neurons in the central nervous system（CNS）. BDNF protein is needed to be transported from the cell body to the secretion sites by secretory vesicles to exert its biological functions. Dysfunctions of the transport have been suggested in several psychiatric and neurodegenerative diseases such as Huntington's disease. We examined the impact of a stress-related hormone, glucocorticoid, on BDNF-containing vesicle transport in neurite of cortical neurons. Glucocorticoid treatment enhanced the microtubule-based BDNF vesicle transport and the effect was dependent on increased expression of huntingtin protein. We will discuss the importance of transport and secretion steps in BDNF function, focusing on the interaction between BDNF, huntingtin and glucocorticoids.

3S2-4 Biological roles of the BDNF pro-peptide

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Most growth factors are initially synthesized as precursor proteins and subsequently processed into their mature form by proteolytic cleavage, resulting in simultaneous removal of a pro-peptide. However, as compared to that of mature form, the biological role of the pro-peptide is poorly understood. Here, we investigated the biological role of the pro-peptide of brain-derived neurotrophic factor（BDNF）and first showed that the pro-peptide is expressed and secreted in hippocampal tissues and cultures, respectively. Interestingly, we found that the BDNF pro-peptide directly facilitates hippocampal long-term depression（LTD）, requiring the activation of GluN2B-containing N-methyl-D-aspartate（NMDA）receptors and the pan-neurotrophin receptor p75NTR. The BDNF pro-peptide also enhances NMDA-induced alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor endocytosis, a mechanism crucial for LTD expression. Thus, the BDNF pro-peptide is involved in synaptic plasticity that regulates a mechanism responsible for promoting LTD. The well-known BDNF polymorphism Val66Met affects human memory function. Here, the BDNF pro-peptide with Met mutation completely inhibits hippocampal LTD. These findings demonstrate functional roles for the BDNF pro-peptide and a naturally occurring human BDNF polymorphism in hippocampal synaptic depression.

3S3-1 Epigenetic alterations in neuronal cells of patients with bipolar disorder and schizophrenia

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Despite the extensive efforts, genetic factors involved in major psychiatric disorders such as bipolar disorder（BD）and schizophrenia（SZ）can explain only a part of their pathophysiology. Accumulating evidence suggests that epigenetic factors such as cytosine and histone modifications are involved in not only fundamental and higher brain functions but also the pathophysiology of psychiatric disorders. We comprehensively analyzed DNA methylation profiles of neuronal and non-neuronal nuclei derived from frozen prefrontal cortex samples of patients and controls. Bioinformatic analysis of differentially methylated regions revealed the propensity of promoter-wide hypomethylation in addition to hypermethylation of neuronal function-related genes in BD and SZ. Using cell culture and animal models, we systematically assessed the effect of mood stabilizers and antipsychotics, and found that they may account for only minor fraction of DNA methylation changes detected in brain. Our results suggest that further epigenetic analyses in the brain will contribute to understand the pathophysiology of psychiatric disorders. Our ongoing analyses focusing on various cytosine modifications in more specific cell types, and comparative neuronal epigenomics for understanding the role of epigenetics on neuronal functions will be discussed.

3S3-2 A role for inefficient RNA editing in the amyotrophic lateral sclerosis（ALS）pathogenesis

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Motor neurons of the majority of amyotrophic lateral sclerosis（ALS）cases express abnormal GluA2 that possesses glutamine（Q）instead of arginine（R）at the Q/R site due to failure of adenosine to inosine（A-to-I）conversion. This molecular abnormality results from down-regulation of the RNA editing enzyme adenosine deaminase acting on RNA 2（ADAR 2）that specifically catalyzes A-to-I conversion at the Q/R site of GluA2 pre-mRNA. AMPA receptors containing unedited GluA2 are highly permeable to Ca^{2+}, and resultant exaggerated Ca^{2+} influx causes slow death of motor neurons in conditional ADAR2 knockout（AR2）mice. Notably, ADAR2-lacking motor neurons in the AR2 mice exhibit TDP-43 pathology, the most reliable neuropathological hallmark of ALS. Furthermore, TDP-43 pathology and ADAR2 down-regulation are concomitantly observed in the same motor neurons of ALS patients. These lines of evidence indicate that ADAR 2 down-regulation is involved in the ALS pathogenic mechanism and a molecular target for ALS therapy. Therefore, we developed a gene therapy for ALS by the delivery of the ADAR2 gene to the mouse motor neurons using an adeno-associated virus（AAV）vector. A single intravenous injection of AAV-ADAR2 successfully prevented AR2 mice from developing clinical and pathological ALS phenotype. In this symposium, I would like to present that failure of RNA processing causes human disease and normalization of the RNA processing is a possible therapeutic strategy.

3S3-3 Epigenetic studies in Alzheimer's disease

○Katie Lunnon, Rebecca Smith, Eilis Hannon, Philip De Jager, Gyan Srivastava, Manuela Volta, Claire Troakes, Safa Al-Sarraj, Joe Burrage, Ruby Macdonald, Daniel Condliffe, Pavel Katsel, Vahram Haroutunian, Zachary Kaminsky, Catharine Joachim, Lorna Harries, John Powell, Simon Lovestone, David A. Bennett, Leonard Schalkwyk, Jonathan Mill
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Background

Increasing knowledge about the biology of the genome has implicated an important role for epigenetic variation in human health and disease, and recent methodological advances mean that epigenome-wide association studies (EWAS) are now feasible for complex disease phenotypes including Alzheimer's disease. Epigenetic epidemiology is a relatively new endeavor, however, and there are important considerations regarding study design, tissue-type, analysis strategy and data interpretation. Here we describe two systematic cross-tissue EWAS analyses of DNA methylation in AD using a powerful sequential replication design, with the goal of identifying disease-associated methylomic variation across pathologically-relevant regions of the brain.

Methods

We used the Illumina Infinium Human Methylation 450K BeadChip to assess genome-wide methylation at >485,000 CpG sites in a discovery cohort of 117 individuals from the London MRC Brain Bank. We profiled multiple brain regions (prefrontal cortex, entorhinal cortex, superior temporal gyrus and cerebellum) representing the spectrum of AD pathology and matched samples collected pre-mortem. Differentially methylated positions (DMPs) of interest were validated in a second independent cohort of 144 individuals from the Mount Sinai Brain Bank. Data was analysed using various R packages to identify differentially methylated loci important in disease, including network analyses, pathway analyses and a sliding window approach to identify differentially methylated regions (DMRs) spanning multiple DMPs.

Results

Data was analysed using various R packages to identify differentially methylated loci important in disease, including network analyses, pathway analyses and a sliding window approach to identify differentially methylated regions (DMRs) spanning multiple DMPs. In our discovery cohort we identified a number of differentially methylated regions (DMRs) in cortical regions, which were associated with neuropathological measures of Alzheimer's disease. Many of these were validated in our independent replication cohort.

Conclusions

This study provides compelling evidence for an association between epigenomic dysfunction and AD-related neuropathology. This study represents the first epigenome-wide association study (EWAS) of AD employing a sequential replication design across multiple tissues, highlighting the power of this strategy for the identification of disease-associated DMRs.

3S3-4 Behavioral consequences of dysmyelination

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Myelin is required for proper nerve conduction and has an important role in normal neuronal function. Polymorphisms in myelin genes are associated with neurologic and psychiatric diseases. However, little is known about the neuronal and behavioral consequences of myelin disruption and the role of myelin genes in pathology. To address the contribution of myelin function to behavioral and cognitive deficits, we investigated behavior in the proteolipid protein (PLP)-null mouse. These mice generate myelin but they exhibit progressive myelin dysfunction and subsequent axonal degeneration. We tested 3 and 8 month-old PLP knockout PLP (-/Y) male mice in several behavioral tests. No motor deficits were observed in 3 and 8 month old PLP (-/Y) mice on the Rotarod, a classical test of motor function. In an open field test, 8 month PLP (-/Y) mice spent less time in the center of the open field, while exploration of the walls was increased. PLP (-/Y) mice had decreased motivation to bury marbles in the marble burying task, in which the number of marbles buried in 10 minutes is quantified. This is an instinctive behavior, but 3 month PLP (-/Y) mice buried fewer marbles and by 8 months they buried almost none. Their performance on the Y maze, a test of spatial memory and hippocampal function, was normal at both ages. However, their behavior in the Puzzle Box, a test of problem-solving and executive function, suggested deficits in problem solving. The Puzzle Box involves moving from a lighted box into a darkened goal box. The entry to the goal box becomes increasingly difficult, initially by covering it, then putting sawdust into it and finally covering the sawdust-filled entry. The 3 and 8 month PLP (-/Y) mice displayed cognitive deficits evidenced by longer latency to reach the goal box when presented with the new challenges. Intriguingly, 3 and 8 month PLP (-/Y) mice exhibit a significant increase in immobility and a lack of coordinated swimming behavior when placed in water. We are currently investigating why there is a swimming deficit in PLP (-/Y) mice. These behavioral results indicate that myelin dysfunction prior to significant axonal degeneration results in targeted behavioral deficits and cognitive dysfunction. Ongoing investigation aims at refining the characterization of these deficits and linking them to structural alteration of myelin in specific areas of the brain. Supported by NS 25304.

3S4-1 Optogenetic manipulation and imaging of CaMKII–Rho GTPase signaling pathway during synaptic plasticity

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National Institute for Physiological Sciences（生理学研究所）

Ca2＋/Calmodulin–dependent kinase II（CaMKII）is one of the most important signaling molecules for synaptic plasticity underlying learning and memory. Here, we developed a photo–activatable CaMKII（paCaMKII）. Light–induced spine specific CaMKII activation successfully induced the structural plasticity, which suggesting that CaMKII activation is sufficient for the plasticity. In addition, we imaged the activity of Rho GTPases, RhoA and Cdc42, by using 2pFLIM and found that these molecules are activated via CaMKII. Furthermore, since the loss of function assay suggests that RhoA and Cdc42 works for triggering and maintaining the structural plasticity, respectively, these molecules may cooperatively work for establishing the spine structural plasticity.

3S4-2 Reward action of dopamine on the structural plasticity of dendritic spines

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Animal behaviors are reinforced by subsequent rewards following within a narrow time window. Such reward signals are primarily coded by dopamine, which modulates the synaptic connections of medium spiny neurons in the striatum. However, it has been difficult to understand why dopamine reinforces preceding, but not subsequent, sensorimotor events if dopamine always activates downstream molecules such as protein–kinase A（PKA）. In acute slices of mouse brain, we selectively stimulated dopaminergic and glutamatergic inputs on D1R–MSNs by optogenetic stimulation of dopaminergic fibers and two–photon uncaging of caged–glutamates paired with APs（STDP）. We found that dopamine markedly potentiated spine enlargement, but this only occurred within a narrow time window（0.3–2 s）closely following STDP, which is consistent with behavioral conditioning findings. FRET imaging of Ca^{2+}/calmodulin–dependent protein kinase II（CaMKII）and protein–kinase A（PKA）revealed that the sequence detection involved molecular signaling upstream of PKA activation：Sufficient generation of cAMP for PKA activation occurred only when spikes preceded dopamine to prime adenylyl–cyclase（AC1）, otherwise cAMP was effectively removed by a potent phosphodiesterase（PDE）activity in thin distal dendrites of MSNs due to a large surface–to–volume ratio. Therefore, PKA was activated only within the specific timing for reinforcement, which then activated CaMKII through the dopamine– and cAMP–regulated phosphoprotein 32 kDa（DARPP–32）. Thus, these intracellular mechanisms can explain the reward action of dopamine. We are now studying how D1R–MSNs are involved in a Pavlovian conditioning task using head–fixed mice.

3S4-3 Phospho-proteomic analysis enables discovery of reward signals

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It is well known that dopamine（DA）is necessary for motor function, motivation, working memory and the reward system. The principal target of DA is medium spiny neurons（MSNs）, which are divided into two distinct classes, DA type 1 receptor（D1R）-or type 2 receptor-expressing neurons（D1R-MSNs or D2R-MSNs, respectively）, within the striatum. D1R-MSNs in the striatum form direct projections to the substantia nigra pars reticulata, whereas D2R-MSNs form indirect projections to the substantia nigra pars reticulata via the pallidum and subthalamic nuclei. These two pathways control the dynamic balance in the basal ganglia-thalamocortical circuit. DA is believed to regulate membrane properties acting through D1R-protein kinase A（PKA）for controlling reward-related behaviors. However, how PKA regulates the D1R-MSN excitability and reward-related behaviors remains largely unknown. To elucidate PKA-dependent reward signaling in D1R-MSNs, we performed phospho-proteomic analyses to comprehensively identify the PKA substrates downstream of D1Rs in the striatum. We also attempted to characterize neurochemical and behavioral significance of these phosphorylated substrates. We will introduce our recent findings in this symposium.

3S4-4 Genetic manipulation of memory engram

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It is a fundamental question how memories are represented in the brain. A prevailing hypothesis suggests that memory is encoded by a cooperative activity of specific subset group of neurons. However, identifying these neurons supporting a given memory is challenging because these neuronal ensembles are likely sparsely distributed within the brain. To circumvent this difficulty, we have previously developed a transgenic system in mice that allows us to manipulate neurons activated during a relevant behavior. In the system, the expression of a given transgene is regulated by neuronal activity via the promoter of c-fos gene, whose expression is rapidly and transiently induced in response to neuronal activity, and is also dependent on a tetracycline inducible expression system. I will introduce approaches trying to manipulate the memory engram to address many unanswered questions about memory. I will also discuss about the possibility to manipulate a molecular activity in those neuronal ensembles.

3S4-5 Computational modeling of dopaminergic actions on striatal medium spiny neurons

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The striatum is the input structure of the basal ganglia and involved in various aspects of reward evaluation and incentive-based learning. Since it was suggested that dopamine encodes the reward prediction error, the neural substrates of this type of learning has often been discussed in the context of reinforcement learning theory, in which the dopamine-dependent corticostriatal plasticity is thought to be a critical element for linking sensory inputs and motor actions via reward signals, resulting in goal-directed behavior. How can this plasticity be interpreted as an outcome of intracellular molecular signaling cascades? What dysfunction of molecular signaling could lead to the breakdown of the plasticity? What extracellular signal could modulate the plasticity? In this talk, we present our studies to seek for possible answers of these questions from a viewpoint of "dynamical systems."
The majority of neurons in the striatum is constituted by medium spiny neurons (MSNs), whose population is divided into two subpopulations：one expresses dopamine D1-like receptors (D1Rs) and the other subpopulation expresses dopamine D2-like receptors (D2Rs). In the first half, we present a kinetic signal transduction model of D1R-expressing MSN constructed based on existing literature and database, and show that the bistability of the positive feedback loop constituted by PKA, PP2A and DARPP-32 (pThr75) could play an important role in reverting long-term depression to long-term potentiation in dopamine-dependent plasticity. In the second half, we present our on-going study on a kinetic signal transduction model of D2R-expressing MSN, which is based on observations that dopamine exerts pharmacological actions opposite to that of D1R-expressing MSN and counteracts adenosine signaling, which facilitates PKA activity via adenosine A2A-like receptors (A2ARs). The model predicts that the bidirectional plasticity of the MSN could be maintained through the balance between dopamine and adenosine signals.

3S4-6 Comparative genetic analysis of autism and schizophrenia：Focus on rare variants

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There is strong evidence that genetic factors make substantial contributions to the etiology of autism spectrum disorder (ASD) and schizophrenia, with heritability estimates being at least 80%. In recent years new molecular genetics findings, particularly from the application of genome-wide association studies (GWASs), have implicated risk factors for ASD and schizophrenia, and have suggested the possibility of a genetic overlap between them. Earlier, we conducted low resolution copy number variation (CNV) screening using affimetrix 5.0 array in order to catalog CNVs that may increase the schizophrenia susceptibility in the Japanese population. The current study is an extension of previous project. For the CNV detection, we are using high resolution comparative genomic hybridization array (aCGH) with 720k probes and 4,000 bps resolution. In addition we are conducting whole genome next generation whole genome sequencing for the patients in whom large psychiatric CNVs have been detected.Besides the known large CNVs that are previously reported to be associated with schizophrenia we found hundreds of small to medium size novel, exon disrupting sequence variations in more than 10% of patients with developmental disorders. Many of those are in functionally relevant protein domains, with the potential to affect physiological function of the affected gene productIn summary, these findings point to the number of genomic variants that may be relevant to the pathoetiology of schizophrenia and ASD were below the detection threshold of last generation CNV typing technologies. In addition, much future work is required, and this work should not be constrained by current categorical diagnostic systems. Such studies should explore the relationship of genes and genetic risk factors to symptomatology across current diagnostic categories.

3S5-1 AGE-DEPENDENT SPECIFIC CHANGES IN AREA CA2 OF THE HIPPOCAMPUS AND SOCIAL MEMORY DEFICIT IN THE 22Q11.2 MOUSE MODEL OF SCHIZOPHRENIA

○**Vivien Chevaleyre[1], Rebecca Piskorowski[1], Anastasia Diamantopoulou[2,4], Steven Siegelbaum[3], Kaoutsar Nasrallah[1], Jun Mukai[2], Joseph Gogos[2]**
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An imbalance between excitation and inhibition is observed in several pathologies. Post-mortem studies have shown a reduction in interneuron number in hippocampal area CA2 with schizophrenia. We examined transmission in area CA2 of Df（16）A+/− mice, a mouse model of the 22q11.2 deletion syndrome, which presents the highest known risk for developing schizophrenia. These mice recapitulate many of the behavioral deficits and neuroanatomical changes observed in humans. We found that Df（16）A+/− mice have fewer Parvalbumin-expressing interneurons in area CA2, with no differences found in areas CA1 and CA3. Second, we found that the level of feed-forward inhibitory transmission is also reduced in area CA2 of these mice. As a consequence, excitatory transmission from Schaffer collaterals is larger in basal conditions. Third, these differences did not manifest until the mice reached adulthood. Fourth, we also found an age-dependent hyperpolarization of the resting membrane potential of CA2 pyramidal cells in Df（16）A+/− mice. CA2 pyramidal cells in Df（16）A+/− mice displayed fewer action potentials in response to proximal and distal excitatory input stimulation. Long-term depression at inhibitory synapses is reduced in Df（16）A+/− mice, resulting in impaired action potential firing in Df（16）A+/− mice. Finally, Df（16）A+/− mice display a deficit in social memory, a phenotype similar to the one observed after complete silencing of CA2 pyramidal neurons. Thus, our results shed new light onto a potential mechanism underlying the social memory impairment observed during schizophrenia.

3S5-2 Pioneer discovery of the CA2 function in the hippocampus

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Among subregions of the *Cornu Ammonis*（CA1/CA2/CA3）in the hippocampus, the CA2 region has remained unexplored in detail for long time, although a huge amount of studies have been performed on the CA3, CA1 and CA3-CA1 synapses. In early 1990's, the optical device with sufficiently high resolution for time and spatial information has been developed. The device enabled us to observe spread of depolarization along the CA3-CA2-CA1 pathway in the rat hippocampal slices, suggesting the distinguishable function of the CA2（Sekino, et al 1997）. This report is the first description of CA2 activity in the hippocampus. Additionally we have immnohistochemically shown that adenosine A1 receptors are highly expressed in the CA2 neurons（Ochiishi, et al 1999）. Inputs from the suprammamillary nucleus, which provides the classical definition of the CA2, is involved in the spread of epileptic activity in the hippocampus（Saji, et al 2000）. We have also shown that number of C-Fos expressed neurons in the CA2 of rats placed in the open field is reduced（unpublished data）. These studies have added much information on the cytoarchitectural information of the CA2 by Lorente de Nov（1934）, but further precise functional roles of the CA2 have not been elucidated until recent technical break through, the discovery of the genetic molecular markers defining the CA2 neurons. I will introduce our pioneer discovery and insights of the CA2 function.

3S5-3 An examination of the local circuitry and impact on network activity by supramammillary nucleus inputs to area CA2 of the hippocampus

○**Rebecca Piskorowski, Vincent Robert, Ludivine Therreau, Vivien Chevaleyre**
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Area CA2 of the hippocampus differs from areas CA1 and CA3 in many aspects. Recent findings indicate that this region is unlikely to code spatial information, but is critical for social memory. Neurons in area CA2 form a reciprocal connection with the supramammillary nucleus (SuM), a hypothalamic structure activated by stress and reward. We are using targeted viral vectors in combination with transgenic mouse lines in order to selectively express channelrhodopsin in SuM neurons and selectively excite projections from these neurons in transverse hippocampal slices. We have found that SuM fibers form excitatory synapses onto both pyramidal cells and interneurons in the deep portion of the somatic layer in area CA2. An inhibitory post-synaptic potential is evoked in CA2 pyramidal cells following SuM stimulation, which is entirely abolished after blocking excitatory transmission. These results reveal that SuM fiber stimulation effectively evokes action potentials in interneurons that feed-forward onto CA2 pyramidal cells. In contrast, the direct glutamatergic transmission between SuM and CA2 pyramidal cells is quite weak and unable to evoke firing. Furthermore, SuM activity results in the release of neuropeptide, allowing for an indirect modulation of inhibitory transmission in this area. We are examining the properties and axonal projections of the interneurons that receive inputs from SuM fibers in order to better understand how SuM activity alters the local circuitry in the hippocampus. We postulate that strong recruitment and modulation of perisomatic inhibition in this area may influence network oscillatory activity.

3S5-4 The role of CA2 in regulating information flow in the hippocampus

○**Thomas J. McHugh**
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Understanding the flow and processing of spatial and contextual information across the subregions (CA1/CA2/CA3/DG) of the hippocampus has been a long standing goal of neuroscience and has provided key insights to the formation, consolidation and expression of declarative memory. Key to this understanding has been the combination of in vivo recording of the spatially selective place cells and local field potential oscillations across the structure with modern genetic tools that allow interventions in neuronal function at specific nodes of the circuit. While this approach has yielded insights into the contributions of CA1, CA3 and the DG, the role of the small, yet highly connected CA2 region to spatial processing and circuit function remain largely unexplored. CA2 is unique in its pattern of synaptic plasticity and its anatomy, possessing multiple bidirectional connections with areas both within and outside the hippocampus, including layer II of the entorhinal cortex, CA3, the supramammillary nucleus of the hypothalamus, and the medial septum. Using in vivo recordings in awake behaving mice we have found that while many aspects of CA2 physiology are similar to the neighboring CA 3 region, the consequences of CA2 silencing are quite unique. Using multiple genetically encoded systems we have investigated the consequences of both transient and chronic silencing of CA2 synaptic transmission on hippocampal physiology. Surprisingly, our data suggests that CA2 may serve a crucial role as a regulator of interhippocampal information flow.

3S6-1 Glial assembly：gliotransmission and pathophysiological consequences

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Glial cells form functional “assembly”, by which they control a broad range of brain functions, especially in the pathophysiological conditions. To assess the operating principle of glial assembly, we have taken two strategies；(1) visualization of glial functions at the interphase of astrocytes, (2) spatiotemporal control of gliotransmission. (1) For visualization of interphase astrocyte, we expressed genetically encoded calcium indicator (GECI), Lck-GCaMP3 or GCaMP3, into astrocytes. To introduce GECI into astrocytes, we injected adeno-associated viruses with GFAP minimal promoter into the brain or used transgenic mice generated by Cre-loxP mediated recombination. GECI successfully reports numerous calcium signals at processes in acute brain slices. By using these, we found that even very mild ischemic episode (preconditioning；PC) could increase an Ca2+ excitability in interphase glia, which was mediated via P2X7 receptors. This was responsible for induction of ischemic tolerance, a phenomenon that a mild ischemic episode induces resistance to a subsequent severe ischemic injury. We also discuss molecular mechanisms underlying astrocyte-mediated ischemic tolerance. (2) For spatiotemporal control of gliotransmission, we used transgenic mice to suppress or overexpress P2Y1 receptors (or VNUT) in astrocytes. ATP/P2Y1 receptor has a central role in regulation of gliotransmission in astrocytes. Astrocytes overexpressing P2Y1 receptors showed ～3 fold increase in calcium excitability in astrocytes from the dentate gyrus of acute brain slices, which is responsible for synchronization of astrocytic Ca2+ excitabilities. In addition, as for pathophysiological consequences of these, we found that ATP/P2Y1 receptor-mediated signals was required and sufficient for scar formation after traumatic brain injury, leading to neuroprotection against secondary damages of TBI. These strategies should shed light on the unique and novel functions of glial assembly.

3S6-2 Disease-associated modification of hereditary demyelinating disorder-related protein dynamics

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Oligodendrocytes and Schwann cells contribute to producing myelin sheaths in the central nervous system (CNS) and peripheral nervous system (PNS), respectively. The myelin sheath consists of morphologically differentiated plasma membranes of myelin-forming glial cells. Myelin sheaths not only insulate axons to increase their nerve conduction velocity but also protect them from various external stresses such as physical stress. For this reason, myelin sheaths play essential roles in homeostasis of the nervous system. Therefore, the diseases that affect them, triggering dismyelination and repeated demyelination, cause nerve damage and, in turn, severe CNS or PNS neuropathies. One such disease is Pelizaeus-Merzbacher disease, a rare X-linked recessive disease. This disease is the prototypic hereditary hypomyelinating leukodystrophy (HLD) and is now designated as HLD1 (OMIM No. 312080). The responsible gene is plp1, and the disease can be caused by a variety of alterations to it such as missense mutations and gene multiplication. Through technological advances, including next-generation sequencing technology, different somatic genes have been identified to date as the HLD responsible genes (from the hld2 to hld9 or hld10 genes)；nevertheless, it still remains to be understood how their alterations affect the properties of their protein products. Herein, we are going to talk about our recent studies of whether or how some HLD-associated mutated proteins cause their diseases. Also, we will discuss about their possible therapeutic drug targets.

3S6-3 Emerging concept of primary microgliopathy in the pathogenesis of neurological diseases

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Microglia are derived from primitive macrophages in the yolk sac and ubiquitously distributed in the brain. They are critical effectors and regulators of changes in CNS homeostasis during development and in healthy and pathological conditions. Numerous studies over the last decade have suggested that microglia activation reactively induced by neurodegeneration and neuroinflammation substantially modulate disease progression and severity in various neurological disorders including Alzheimer's disease and amyotrophic lateral sclerosis（ALS）. Several lines of evidence has recently suggested that primary microglial dysfunction essentially contributes to the pathogenesis of the neurological diseases predominantly affecting the cerebral white matter. This condition is now recognized as primary microgliopathy. Hereditary diffuse leukoencephalopathy with spheroids（HDLS）and Nasu–Hakola disease（NHD）predominantly are considered as primary microgliopathies. The causative genes for HDLS and NHD are *colony stimulating factor–1 receptor*（*CSF–1R*）and *DAP12/TREM2*, respectively, both of which are strongly expressed in microglia. We previously showed that HDLS is caused by haploinsufficiency of CSF–1R and loss of CSF–1R–mediated signaling. The neuropathological examination revealed that density of microglia decreased in HDLS brain. Moreover, individual microglia in HDLS brain demonstrated their characteristic morphology with thin processes and many knotlike structures. These findings have suggested that microglia dysfunction associated with loss of CSF–1R signaling play an essential role in the phathogenesis of HDLS. Considering that microglia are important players in the maintenance and plasticity of neuronal circuits, contributing to the protection and remodeling of synapse, microglial disability and dysfunction may be relevant to the axonal and myelin damages characteristically observed in the white matter. These pathological events in primary microgliopathies may shed new light on our understanding of unrecognized physiological role（s）of microglia.

3S6-4 Analyses of neuronal and glial cell phenotype of *dystonia musculorum* mice

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Mutation of the *Dst* gene encoding dystonin, a cytoskeletal linker protein, in mice results in a movement disorder, termed *dystonia musculorum*（*dt*）, which shows dystonia and cerebellar ataxia, in addition to sensory neuron degeneration. Both the pathological feature and the molecular basis for the *dt* phenotype are not fully understood. In the present study, we investigated neuronal and glial phenotypes in the central nervous system（CNS）of the *dt* mice. We found abnormal staining pattern of neurofilaments（NFs）, densely immunoreactive cell bodies and thick axons in the CNS of *dt* mice, such as in the vestibular and reticular nucleus of brainstem, some of which are responsible for motor functions. We also found reduced glial cell proliferation in the CNS of *dt* mice. We will discuss how much these abnormalities contribute to the *dt* phenotype.

3S7-1 Neuroinflammatory features of the cytokine-induced animal model for schizophrenia；implication of the regional specificity

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Cytokine-mediated mild chronic inflammation is implicated in the pathogenesis of schizophrenia. Many genetic studies of GWAS identified the major histocompatibility locus as a major risk component for schizophrenia and suggest the contribution of immune inflammatory processes to this illness. However, the etiological and neuropathological mechanisms underlying verbal hallucination and its age-dependent onset of schizophrenia remain to be clarified. To test the immune inflammatory hypothesis for schizophrenia, we administered a variety of cytokines to rodent pups, juveniles and adults and characterized neurobiological, pathological and behavioral consequences. In the neonatal stage but not the juvenile and adult stages, subcutaneously-injected factors penetrated the blood-brain barrier and acted on brain neurons, which later resulted in persistent behavioral impairments associated with schizophrenia endophenotypes. Neonatally-EGF-treated animals exhibited persistent hyperdopaminergic abnormalities mainly in the nigro-pallido-striatal system. Once mal-development of the dopaminergic system is established during early development, dopamine-associating behavioral deficits become irreversible and manifest at post-pubertal stages. The EGF-treated model rats also exhibited mild gliosis and white matter shrinkage without apparent neuronal loss during aging. Among several neocortical regions, auditory cortex exhibited the most apparent signs of mild chronic inflammation as observed with protein increases in a microglia marker and activated astrocyte markers. These neuropathologic changes were concomitant with the auditory abnormality of this animal model. These findings suggest that the development and maintenance of the auditory system are also vulnerable to the inflammatory cytokine circulating in the pre- and peri-natal periphery. The pathologic link of the dopaminergic mal-development with the cortical specificity of inflammation remains to be illustrated, however.

3S7-2 Stress behaviors and the innate immune system in the brain

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Stress is a physical and psychological strain induced by aversive stimuli, and precipitates psychiatric disorders. Various kinds of stress in rodents, such as forced swim, restraint and social defeat, induce morphological changes in neurons of multiple brain areas, associated with emotional and cognitive changes. We and others reported that inflammation-related molecules are highly expressed in microglia and are critical for repeated stress-induced behavioral changes in mice. However, since none of these studies manipulated functions or gene expressions selectively in microglia, the direct evidence for a role of microglia in repeated stress-induced behavioral and neuronal changes is lacking. Whether and how repeated stress activates microglia also remains elusive. In chronic diseases associated with physical tissue damage, the concept of sterile inflammation has emerged, in which innate immune molecules, such as Toll-like receptors (TLRs), sense cellular damage or stress to induce inflammation. Here we have shown that the loss of TLRs and their adaptor molecule MyD88 abolishes social avoidance induced by repeated social defeat stress in mice. TLRs and MyD88 are highly expressed in microglia, and the loss of TLRs impairs repeated stress-induced microglial activation as well as dendritic atrophy and attenuation of stress-induced response in prefrontal neurons. Furthermore, we developed methods to manipulate microglial functions in a brain region-specific manner, and have shown a causal role of TLR-mediated microglial activation in the prefrontal cortex in repeated stress-induced behavioral changes. In this symposium, I will highlight the importance of neuron-microglia crosstalk mediated by innate immune molecules in repeated stress, and will discuss the implications of these findings to therapeutic interventions of psychiatric disorders.

3S7-3 Neuroinflammation and sensation of fatigue

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Fatigue is defined as difficulty in initiating or sustaining voluntary activities, and is thought to be accompanied by deterioration of performance. Fatigue can be caused by many factors such as physical and mental stress, disturbance in the circadian rhythm, and various diseases. For example, following the flu or other types of infections, we experience a sense of fatigue that can last for days or weeks. The fatigue sensation is thought to be one of the signals for the body to suppress physical activity in order to regain health. The mechanism of induction of the fatigue sensation following viral infection has not been well understood. We obtained such a fatigue model in rats by administration of polyriboinosinic：polyribocytidylic acid（poly I：C), double-stranded RNA, which mimics viral infection. Intraperitoneal administration of poly I：C induced transient fever and suppression of locomotor activity in rats. The animals overexpressed interleukin（IL）-1β and IL-1 receptor antagonist in the brain including the cerebral cortex. Blocking the IL-1 receptor in the brain by intracerebroventricular（i.c.v.）infusion of recombinant IL-1 receptor antagonist completely blocked the poly I：C-induced suppression of spontaneous activity and attenuated amplification of brain interferon（IFN）-α expression, which has been reported to produce fatigue-like behavior by suppressing the serotonergic system. Furthermore, i.c.v. infusion of neutralizing antibody for IL-1 receptor antagonist prolonged recovery from suppression of spontaneous activity（PLOS ONE, 9, 2014）. Our findings indicated that IL-1β is the key trigger of fatigue-like behavior and that IL-1 receptor antagonist prevents the neuroinflammation entering the chronic state. The balance of IL-1β and the endogenous antagonist in the brain possibly regulate neuroinflammation and fatigue sensation（Neural Regen. Res., 10, 2015）. Although IL-1β is known to be produced mainly in microglia, cells engaging in suppression of neuroinflammation are poorly understood. We also discuss a new concept that glial progenitor cells maintain neuronal function by controlling local immune reactions and anti-apoptotic pathways.

3S7-4 Immaturity of the brain cells and mild chronic inflammation：Candidate endophenotype of neuropsychiatric disorders

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Adequate maturation and integration of the adult-generated neurons into the circuit of the hippocampus would be crucial for normal cognitive functions and emotional behaviors. Disruption of the process could result in some disturbance in mental health. Previously, we reported that mice heterozygous for a null mutation of αCaMKII, a key molecule in synaptic plasticity, have profoundly dysregulated behaviors including hyper-locomotor activity and a severe working memory deficit, which are endophenotypes of schizophrenia and other psychiatric disorders. In these mice, almost all the neurons in the dentate gyrus（DG）of the mutant mice failed to mature at molecular, morphological and electrophysiological levels, causing severe deficit in the synaptic plasticity at mossy fiber-CA3 synapses. By using a simple real-time PCR assay using iDG markers, we identified five other strains of mutant mice that have a phenotype strikingly similar to iDG, including the forebrain specific calcineurin knockout mice, the mice lacking Neurogranin, a well-established schizophrenia susceptibility gene, and the mice lacking Schnurri-2, an NF-kappa B site-binding protein, that tightly binds to the enhancers of major histocompatibility complex（MHC）class I genes and inflammatory cytokines.
We also found that chronic fluoxetine treatment or single pilocarpine administration can induce “dematuration” resulting in iDG-like phenotype in wild type mice. Most of the mice showing iDG-like phenotype seem to have increased adult neurogenesis in DG. Gene and protein expression patterns in the brains of these mice are strikingly similar to those found in the post-mortem brains of the patients of psychiatric disorders, such as schizophrenia and bipolar disorder. The brains of iDG mice show mild chronic inflammation, distinct from typical acute inflammation, as revealed by bioinformatics analyses of gene expression data. Anti-inflammatory drugs can reverse the iDG phenotype as well as some behavioral abnormalities in a subset of the mice showing iDG. I will discuss the potential implication of these findings in elucidating the pathophysiology of those neuropsychiatric disorders.

1G1-01 Extracellular vimentin interacts with insulin-like growth factor 1 receptor to promote axonal growth

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Vimentin, an intermediate filament protein, is generally recognised as an intracellular protein. Previously, we reported that vimentin was secreted from astrocytes and promoted axonal growth. The effect of extracellular vimentin in neurons was a new finding, but its signalling pathway was unknown. In this study, we aimed to determine the signalling mechanism of extracellular vimentin that facilitates axonal growth. We first identified insulin-like growth factor 1 receptor（IGF1R）as a receptor that is highly phosphorylated by vimentin stimulation. IGF1R blockades diminished vimentin- or IGF1-induced axonal growth in cultured cortical neurons. IGF1, IGF2 and insulin were not detected in the neuron culture medium after vimentin treatment. The combined drug affinity responsive target stability method and western blotting analysis showed that vimentin and IGF1 interacted with IGF1R directly. In addition, immunoprecipitation and western blotting analyses confirmed that recombinant IGF1R bound to vimentin. The results of a molecular dynamics simulation revealed that C-terminal residues（residue number 330-407）in vimentin are the most appropriate binding sites with IGF1R. Thus, extracellular vimentin may be a novel ligand of IGF1R that promotes axonal growth in a similar manner to IGF1. Our results provide novel findings regarding the role of extracellular vimentin and IGF1R in axonal growth.

1G1-02 GRAB, a GEF of Rab8, regulates axonal outgrowth in a Cdk5 phosphorylation-dependent manner

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Cyclin-dependent kinase 5（Cdk5）is a neuron specific Ser/Thr protein kinase that is activated by binding a p35 regulatory subunit. It plays an important role in a variety of neuronal functions including neurite elongation through the supply of membrane components to neurite tip. However, it is not fully understood how Cdk5-p35 regulates the membrane transport in growing axons. Membrane transport is regulated by Rab small GTPases, whose activity is in turn regulated by guanine nucleotide exchange factors（GEFs）. Among many GEFs, we were interested in GRAB, which is a GEF for Rab8A and also known as a binding protein for Rab 11A/B, because GRAB could be a potential Cdk5 substrate with（S/T）PX（R/K）consensus phosphorylation sequences. Here we show that GRAB was phosphorylated at Ser169 and Ser180 by Cdk5-p35 and their phosphorylation inhibited the interaction with dominant negative Rab8A-T22N, indicating that the phosphorylation of GRAB suppresses its Rab8 activation ability. Phosphorylated GRAB colocalized with Rab11 A but not with Rab8A in primary culture neurons. Live image analysis revealed that GRAB was transported on Rab11 A-positive endosomes in axon. We examined axonal elongation activity of Rab8A and GRAB. Both of them stimulated axonal outgrowth when overexpressed. Further, the non-phosphorylation mutant of GRAB（GRAB-S169/180A）promoted axonal outgrowth more than its phosphomimic mutant（GRAB-S169/180D）. We would like to propose a novel Rab cascade, Rab11-GRAB-Rab8, in regulation of axonal outgrowth, in which GRAB activity is controlled by phosphorylation with Cdk5.

1G1-03 Dephosphorylation of CRMP2 enhanced recovery after spinal cord injury

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The presence of inhibitory molecules and the lack of neurotrophic factor are two major difficulties to central nervous system（CNS）regeneration. Common mediator of these two has been not found yet. Collapsin response mediator protein 2（CRMP2）was originally identified as a mediator of Semaphorin3A-induced repulsive response. CRMP2 directly binds and stabilizes cytoskeletal microtubule polymerization and transport tyrosine kinase B（TrkB）to axonal tip, the receptor for brain-derived neurotrophic factor（BDNF）, to promote axonal elongation. Meanwhile, inhibitory molecules-induced signals phosphorylate CRMP2 to decrease its affinity to cytoskeleton proteins, leading to axonal growth inhibition. However, the role of CRMP2 phosphorylation after CNS injury *in vivo* remains unknown. Here we investigate the role of CRMP2 phosphorylation after spinal cord injury（SCI）using CRMP2 knock-in（KI）mouse where CRMP2 phosphorylations by Cdk5 and GSK3β are eliminated by replacing serine 522 with alanine residue. Elevated level of pCRMP2 was observed in injured spinal cord. Inhibition of CRMP2 phosphorylation exhibited neuroprotective effect against SCI by suppressing depolymerization of microtubules and fibrous scar formation. This permissive environment for enhanced axon growth of 5-HT-positive raphe-spinal tract induced locomotor recovery in CRMP2KI mice. To examine the signaling cascades involving CRMP2 phosphorylation, we cultured dorsal root ganglion（DRG）neurons. Suppressed axonal growth inhibition by chondroitin sulfate proteoglycan（CSPG）and enhanced axonal elongation with BDNF were observed in CRMP2KI neurons. Therefore, dephosphorylation of CRMP2 could be a unique approach to repair injured CNS by reduced inhibitory responses and enhanced sensitivity to neurotrophin.

1G1-04 Involvement of SRF cofactors in BDNF-induced Arc gene expression.

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One of the neuronal immediate early genes, Arc（activity-regulated cytoskeleton-associated protein）, is a representative SRF（serum response factor）-target gene which plays a key role in the endocytosis of AMPA receptors and regulation of dendritic spine morphology. Arc gene is rapidly activated in response to various stimuli, such as synaptic activity and BDNF（brain-derived neurotrophic factor）. SARE（synaptic activity-responsive element）, which contains the binding sites for transcription factor CREB（cAMP-response element binding protein）and MEF2（myocyte enhancer factor 2）and SRF, is located at -7 kbp upstream of the Arc gene transcription start site. The SARE has been shown to be deeply involved in synaptic activity-regulated Arc gene expression in rat cortical neurons. However, little is known about how SRF cofactors regulate Arc expression.In this study, we have demonstrated the functional roles of SRF cofactor MKL（megakaryoblastic leukemia）on Arc gene expression after BDNF stimulation in rat cortical neurons. Arc mRNA and protein were immediately and transiently increased after BDNF stimulation. The mutation of SRF-binding site but not the mutation of SRF cofactor, ternary complex factor（TCF）-binding site, on the SARE decreased BDNF-induced Arc gene transcriptional activity. Overexpression of dominant negative SRF mutant inhibited BDNF-induced Arc gene transcription. Knockdown of MKL2 but not MKL1 inhibited minimum promoter activation of SARE induced by BDNF. On the other hand, double knockdown of MKL1 and MKL2 increased BDNF-induced gene promoter activity including the 7 kbp promoter region. Taken together, the complex of MKL2-SRF-SRE on SARE might be important to Arc gene activation. In contrast, the TCF-binding site of SARE might be involved in the repression of Arc gene activity at the basal level. We speculate that the regulation of Arc expression is highly complicated because of the involvement of not only SRF cofactors but also other transcription factors.

1G2-01 Analysis of axon selective myelination depending on neuronal subtypes and neuronal activity

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Oligodendrocyte（OL）myelinates multiple axons in the central nervous system. Recent reports have revealed that depolarization of myelinating OL increases conduction velocity of axons. OLs are also known to transfer exosomes to neuronal axons that respond to neurotransmitters. These findings suggest OLs modulate functions of multiple neurons, because OLs ensheath a lot of axons. If one OL selectively ensheaths particular neuronal axons, it is possible that the OL comprehensively modulate functions of those neurons. We examined whether OL preferentially myelinate a particular type of axons depending on neuronal subtypes or neuronal activity. We previously developed a novel method to observe interaction between each OL and neuronal axons. In this method, injection of rabies virus harboring the gene encoding GFP sparsely labels OLs in the targeted white matter, adeno-associated virus type2 labels axons projecting to the white matter massively. By the method, we found 25.9% of callosal OLs preferentially ensheath axons derived from particular brain areas and 50% of chiasmal OLs dominantly ensheath axons derived from either of two eyes. In addition, unilateral eyelid suturing does not impact on myelination by each chiasmal OL. This study revealed that some parts of OLs selectively myelinate axons depending on neuronal subtypes, and suggesting OLs do not select axons depending on neuronal activity.

1G2-02 Bergmann glia alignment along the Purkinje cell layer is important for the proper translocation of climbing fiber synapses from the Purkinje cell soma to dendrites

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The climbing fiber（CF）is a specific type of projection providing a powerful input to the cerebellum from the inferior olive. CF to Purkinje cell（PC）synapses have been well studied as a model for the process of synapse elimination in the central nervous system. Although increasing reports suggest that astrocytes contribute on synapse pruning in other brain regions, role of Bergmann glia（BG）, a type of astrocyte specific to the cerebellum, in CF synapse elimination is poorly understood. BG align their soma in the Purkinje cell layer（PCL）and have a fiber attaching with pia matter via their endfeet. In our previous study, transgenic mice overexpressing an astrocyte specific membrane protein MLC1 in astrocyte lineage cells showed abnormal alignment of BG in the molecular layer. MLC1 is one of the causative genes for leukoencephalopathy. Further analysis on the effect of MLC1 overexpression in cerebellar development revealed that MLC1 overexpression disturbed BG alignment only after birth. In mature BG, MLC1 expression was partially associated with Purkinje cell soma. Interestingly, a marker for climbing fiber synapse（vGluT2）and MLC1 had their own distinct territories in PCL. In the MLC1 overexpressing mice, MLC1 appeared to tightly wrap the Purkinje cell soma. Moreover, elimination of CF synapses to their dendrites was impaired. Though PC dendrites existed, translocation of CF synapses to them was seemed to be inhibited. These results suggest that BG alignment along the PCL is important for the proper translocation of CF synapses from the PC soma to dendrites.

1G2-03 *In vivo* Ca^{2+} imaging reveals that spinal astrocytes respond to nociceptor stimulation

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Astrocytes, a major type of glial cells in the CNS, respond to neuronal activity, elevate intracellular Ca^{2+} levels and modulate synaptic transmission by astrocytic factors released in a Ca^{2+}-dependent manner. The spinal dorsal horn（SDH）directly receives sensory inputs from the periphery through primary afferent fibres, but there is no report showing that astrocytes in the SDH respond to sensory inputs through primary afferents *in vivo*. In this study, we utilized an *in vivo* Ca^{2+} imaging technique and monitored activity of mouse SDH astrocytes that had expressed the ultrasensitive calcium sensor protein GCaMP6 by microinjecting with an AAV encoding this protein. We found that intracellular Ca^{2+} levels in SDH astrocytes were clearly increased soon after an intraplantar injection of capsaicin, a TRPV1 agonist that stimulates nociceptors. SDH astrocytes also responded to noxious mechanical stimulation（pinch）, but not innocuous stimuli（touch and acetone）. The capsaicin and pinch-induced astrocytic Ca^{2+} elevations were diminished by ablation of TRPV1-positive primary afferent fibers using resiniferatoxin, indicating that TRPV1-positive fibers transfer noxious information to SDH astrocytes. Furthermore, we also found that the noxious stimulation-evoked astrocytic Ca^{2+} signals disappeared in mice lacking inositol 1,4,5-trisphosphate（IP_3）receptor type 2（IP_3R2）. Taken together, SDH astrocytes are activated following noxious stimulation in a manner that requires IP_3R2 and may contribute to pain processing in the SDH.

1G2-04 Cereblon accumulates in aggresome and shows cytoprotective effect against proteasome inhibition

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A nonsense mutation of the gene *cereblon* was found in a large kindred associated with intellectual disability（Higgins J.J. *et al. Neurology* **63**, 1927-1931（2004））. The gene product cereblon（CRBN）is reported as a component of E3 ubiquitin ligase complex（Ito T *et al. Science* **327**, 1345-1350（2010））. E3 ubiquitin ligase is known as a key factor to form aggresome. Aggresome formation is one of the defense mechanisms in living cells under various stress conditions（Olzmann J.A. *et al. Biochem. So. Trans.* **38**, 144-149（2010））. Furthermore, it is reported that the aggregated protein is degraded by autophagy after aggresome formation at the perinuclear region. Here we examined whether CRBN accumulates in aggresome under proteasome inhibition. CRBN overexpressing PC12 cells were treated with proteasome inhibitor MG132. Exogenous CRBN localized at perinuclear regions, and co-localized with several aggresome markers under MG132 treatment. Moreover, we examined whether CRBN overexpressing PC12 cells shows cytoprotective effects against protease inhibition. Cell death induced by MG132 were significantly decreased in CRBN overexpressing PC12 cells compared with that of control cells. We propose that CRBN accumulates in aggresome and shows cytoprotective effects against protease inhibition.

1G2-05 Mitochondrial-targeted cereblon suppressed stress-induced cell death

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Cereblon is a candidate gene for autosomal recessive form of mild mental retardation (Higgins JJ *et al., Neurology* **63**, 1927-1931 (2004)). The product Cereblon (CRBN) contains a conserved Lon domain, which is a characteristic domain found in Lon protease (Higgins JJ *et al., Neurology* **63**, 1927-1931 (2004)). Lon protease is one of the stress response proteins in mitochondria in mammalian cells (Venkatesh S. *et al., Biochimica et Biophysica Acta-Molecular Cell Research* **1823**, 56-66 (2012), Jenny K. Ngo *et al., Free Radical Biology and Medicine* **46**, 1042-1048 (2004)). Under various extracellular stresses, such as oxidative stress and nutrient starvation, Lon protease is up-regulated and supports the cell viability (Jenny K. Ngo *et al., Free Radical Biology and Medicine* **46**, 1042-1048 (2004)). Although CRBN contains a highly conserved, large Lon domain, whether CRBN has Lon protease-like functions remains unknown. Here, we investigated whether CRBN, similar to Lon protease, plays a protective role against extracellular stresses. Firstly, using western blot and confocal microscopy analysis, we reported that CRBN was present in various subcellular compartments including mitochondria. Next, to focus on the mitochondria-specific function of CRBN, we constructed an expression vector of mitochondria targeting sequence (MTS)-fused CRBN. Using this vector, we constructed stable human neuroblastroma SH-SY5Y cell lines expressing MTS-CRBN. Finally, we confirmed that the cell lines showed suppression of neuronal cell death induced by hydrogen peroxide and serum starvation. Taken together, these results indicate that mitochondrial-targeted CRBN could be responsible for the protective functions against extracellular stresses, as Lon protease does.

1G2-06 Nuclear cereblon modulates Ikaros-mediated transcription

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The gene coding for *cereblon* (*CRBN*) was originally identified in genetic linkage analysis for mild autosomal recessive nonsyndromic mental retardation. CRBN was also identified as a thalidomide-binding protein and a component of cullin-4-contaning E3 ubiquitin ligase complex (Ito *et al., Science* **327**, 1345-50 (2010)). CRBN has broad localization in both the cytoplasm and the nucleus. However, the significance of nuclear CRBN remains unknown. In the present study, we aimed to elucidate the role of CRBN in the nucleus. Firstly we generated a series of CRBN deletion mutants and determined the regions responsible for the observed nuclear localization. Only CRBN protein lacking the N-terminal region (1-119 a.a.) was localized outside the nucleus, suggesting that the N-terminal region is important for its nuclear localization. Thalidomide has been reported to be involved in the regulation of a transcription factor, Ikaros, with CRBN mediated degradation (Lu *et al., Science* **343**, 305-9 (2014); Kronke *et al., Science* **343**, 301-5 (2014)). To further investigate the nuclear function of CRBN, we performed co-immunoprecipitation experiments and evaluated the association between CRBN and Ikaros. We showed that CRBN is associated with Ikaros protein, and the N-terminal region of CRBN was required for Ikaros binding. Using luciferase reporter gene experiments, we showed that CRBN modulates transcriptional activity of ikaros. These results suggested that CRBN regulates transcriptional activity of Ikaros, and may play an important role in the neuronal development involved in this transcription factor.

1G3-01 ATP supplementation therapy for ALS with SIGMAR1 mutation

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The dominant missense mutation（p.E102Q）by SIGMAR1 gene mutation was discovered in the patients of juvenile amyotrophic lateral sclerosis（ALS）. The sigma-1 receptor（Sig-1R）is a chaperone protein localizing in the mitochondrial-associated endoplasmic reticulum（ER）membrane in where the receptor regulates Ca2+ transport from ER to the mitochondria through IP3 receptor（IP3R）. When Sig-1R mutant（Sig-1RE102Q）overexpression in neuroblastoma neuro2A cells, Sig-1RE102Q dissociated from the IP3R and formed aggregations in the cytosol（Biochem Biophys Acta 2014；1840：3320）. Mitochondrial Ca2+ transport induced by IP3R stimulation was also disturbed by Sig-1RE102Q expression, thereby reducing mitochondrial ATP production. The Sig-1RE102Q mutant also reduced the mitochondrial membrane potential and promoted mitophagy. Moreover, the ATP reduction caused the decreased proteasome activity and in turn TAR DNA binding protein（TDP-43）accumulation in the cytosol. These events were recapitulated by pharmacological inhibition of either proteasome or mitochondrial Ca2+ transport. We tried to rescue the ATP reduction by supplementation of mitochondrial TCA cycle substrate, methyl pyruvate. The methyl pyruvate treatment rescued the Sig-1RE102Q-induced ATP reduction, thereby restoring the proteasome activity with concomitant inhibition of cytoplasmic accumulation of TDP-43. Taken together, ATP supplementation with methyl pyruvate can rescue the mitochondrial injury associated with ALS caused by Sig-1RE102Q mutation.

1G3-02 Determination of the key domains of CHRNA7 in the interacting actions of Arctic mutant Aβ

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Amyloid β protein（Aβ）plays a central role in the pathogenesis of Alzheimer's disease（AD）. Point mutations within the Aβ sequence that are associated with familial AD（FAD）are clustered around the central hydrophobic core of Aβ. Several types of mutations within the Aβ sequence have been identified, and the 'Arctic' mutation（E22G）has a purely cognitive phenotype typical for AD（Nilsberth, C. et al., *Nat. Neurosci.* **4**（2001））. Previous studies showed an increased formation of Aβ protofibrils as a primary result of the 'Arctic' mutation. However, the molecular mechanism underlying this effect remains un-clarified. Previous reports suggested that Aβ42 binds to one of the neuronal nicotinic acetylcholine receptor's subunits, neuronal acetylcholine receptor subunit alpha-7（CHRNA7）, with high affinity and thus, may be considered to relate to AD（Wang, H. Y. et al., *J. Biol. Chem* **275**（2000）；Wang, H. Y. et al., *J. Biol. Chem* **75**（2000））. Our previous study indicated that Arctic β binds to CHRNA7 with high affinity, enhances its aggregation further when co-incubated with CHRNA7 and destabilizes the function of CHRNA7 via the inhibition of the Ca^{2+} response and activation of ERK1/2（Ju, Y. et al., *J Neurochem.* **131**（2014））. This study aims at addressing the key domains of CHRNA7 potentially interacting with Arctic Aβ. Site-directed mutagenesis was carried out to study the key domains. We performed an *in vitro* binding assay using purified mutant CHRNA7 and synthetic Arctic Aβ to search the critical domains for Arctic Aβ-CHRNA7 binding. Furthermore, we are currently over-expressing mutant CHRNA7 in neuronal cells in order to investigate the key domains in CHRNA7 potentially interact with Arctic Aβ to regulate the functions of this receptor.

1G3-03 Function of Cathepsin C and Cystatin F during demyelination

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Cystatin F, a papain-like lysosomal cysteine proteinase inhibitor, and its main target, cathepsin C, have been demonstrated to be crucial factors in demyelinating diseases. It is found that the expression of cathepsin C and cystatin F are profoundly elevated and matched with ongoing demyelination/remyelination. However, their accurate functional role in demyelinating diseases is still unclear. To clarify their function in the pathological process of demyelination, we used a spontaneous chronic demyelination mouse model, named heterozygous PLP transgenic 4e (PLP4e/−) mouse. Meanwhile, Flexible Accelerated STOP-Tetracycline Operator Knock in (FAST) system is applied to up or down regulate cathepsin C or cystatin F gene expression. Cystatin F as the inhibitor of cathepsin C is predicted as a protective factor. But higher severity of demyelination was observed in cystatin F overexpressing PLP4e/− mice. Additionally, microglia showed highly activated morphology in cystatin F overexpressing mice. Together with the in situ hybridization results that in PLP4e/− mice conditional knock down of cystatin F gene in microglia lead to the down regulation of cathepsin C mRNA level, we predicted that cystatin F induce cathepsin C gene expression in addition to their protein level interaction during demyelination. In order to prove this hypothesis, we plan to check cathepsin C and cystatin F gene and protein expression by using real time PCR and western blot.

1G3-04 Time-lapse imaging of migrating new neurons in the injured adult cerebral cortex

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In the adult rodent brain, new neurons are constantly generated from neural stem cells in the ventricular-subventricular zone (V-SVZ). Under physiological conditions, these new neurons form chains and migrate along the blood vessels in the rostral migratory stream (RMS) toward the olfactory bulbs, where they are integrated into pre-existing neural network. After brain injuries, V-SVZ-derived new neurons also migrate in chains along blood vessels toward the injured sites and differentiate into mature neurons, suggesting the potential of endogenous neural stem cells for neuronal regeneration. Here we show the patterns and dynamics of neuronal migration in the injured adult mouse brain. In the photothrombotic stroke model, we observed new neurons migrating in chains or individually frequently associated with blood vessels in the injured corpus callosum and cerebral cortex, suggesting that new neurons utilize blood vessels as a migratory scaffold. To analyze dynamics of neuronal migration toward the injured sites, we performed time-lapse imaging of migrating new neurons in the cultured adult brain slices. Chain-forming new neurons migrated along the blood vessels from the V-SVZ toward the injured sites. Similar to the neuronal migration in the RMS, new neurons in the injured brain showed saltatory movement, executed by repeated extension of the leading process followed by the advancement of the soma. However, migration speed was significantly slower in the injured brain. These observations provide insights into similarity and difference in neuronal migration between physiological and pathological conditions.

1G3-05 Histamine N-methyltransferase deficiency induced the abnormal sleep-awake cycles and aggressive behavior in mice

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Histamine plays as a neurotransmitter in various physiological events such as sleep-awake cycle and appetite regulation. In order to maintain the homeostasis of histaminergic neuronal activities, the excessive histamine should be inactivated. The previous studies suggested that histamine N-methyltransferase（HNMT）was important for histamine inactivation. However, the role of HNMT in vivo remains almost unclear. In the present study, we generated and investigated the Hnmt knockout mice（KO）to elucidate the importance of HNMT. First, we generated KO by inserting LacZ gene into HNMT gene. LacZ reporter assay revealed that Hnmt were highly expressed in cortex, amygdala, locus coeruleus and Raphe nucleus. Histamine content in the brain lysate of KO was 6 times as abundant as that in wild type mice（WT）. The extracellular histamine in the hypothalamic area was also increased in KO. These results cleared that HNMT was essential for brain histamine clearance. Most of KO was wounded by fighting in home-cage, suggesting the increase of aggressive behavior in KO. We confirmed the aggressive behavior of KO in the resident-intruder test and aggressive biting behavior test. The sleep analysis revealed that the sleep duration of KO in dark period was longer than that of WT, and the KO showed an increase in slow-wave EEG in wakefulness, suggested that Hnmt deficiency caused the dysfunction of sleep-awake cycles. These results indicated that Hnmt was involved in sleep-awake cycles and aggressive behavior through the regulation of histamine concentration and histamine neuronal activities.

1G3-06 The acute immediate effect of X-irradiation and Carbon ion-irradiation on synaptic function and fear memory formation.

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Carbon-ion therapy becomes widely known and is used as the advanced therapy for cancer. Carbon-ion irradiation is thought to show a higher biological effectiveness compared to X-irradiation, but still the acute immediate effect on the brain function is poorly understood. In this study, we compared the acute effects of X-ray and carbon-ion irradiation on fear memory formation and accumulation of a synaptic protein. We used 10-12-week old male mice, and administered a single dose of 10 Gy of either X-ray or carbon-ion beam to whole brains. Then fear conditioning was conducted 7 hrs and 24 hrs after the irradiation. We found that the mice irradiated by either X-rays or carbon-ion beam 7 hrs before training did not retrieve the contextual and auditory memories, whereas those irradiated 24 hrs before training did retrieve the both memories. We analyzed drebrin, a marker for synaptic function, immunohistochemically in neuropil of the dentate gyrus of hippocampus. We found there were significant decreases of drebrin intensities 2 hrs and 8 hrs after the irradiation and it returned to the former level 24 hrs after the irradiation. Interestingly, the number of drebrin clusters also decreased with a similar time course in in vitro study. When we analyzed the number of drebrin clusters after we irradiated mature primary hippocampal neurons, it decreased significantly 2 hrs and 8 hrs after X-irradiation and returned to the former level after 24 hrs. Similarly, the number of drebrin clusters significantly decreased 2 hrs after carbon-ion irradiation and tended to return 24 hrs after the carbon-ion irradiation. These results suggest that there are transient effects on the synaptic function of both X-irradiation and carbon-ion irradiation and these may cause fear memory deficits.

2G1-01 The role of blood flow in neuronal turnover in the adult olfactory bulbs

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In the adult mouse olfactory bulbs（OBs）, new neurons generated from neural stem cells in the ventricular–subventricular zone are continuously supplied whereas old ones are eliminated, suggesting that olfactory interneurons are replaced throughout life. We have previously reported that olfactory input promotes reiterated use of the same positions by new neurons in the adult OB. However, mechanisms underlying the spatiotemporal regulation of neuronal turnover remain unknown. Here we show the relationship between neuronal turnover and blood flow in the adult OB. By performing in vivo two–photon imaging, we found that neuronal addition and elimination occur in the vicinity of blood vessels, suggesting that blood vessels provide preferable microenvironment for efficient neuronal turnover. To test the possibility that neuronal addition and elimination are correlated with the blood flow, we labeled blood plasma with fluorescent dye and measured capillary blood flow by in vivo two–photon line–scan imaging. Interestingly, newly added neurons were observed more frequently in the vicinity of blood vessels with high blood flow as compared with those with low flow. These data suggest that blood flow promotes neuronal turnover in the perivascular regions of adult OB.

2G1-02 Drebrin knockout mice show olfactory dysfunction by impairment of adult neurogenesis and cell survival

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Drebrin is an F–actin–binding protein which plays an important role in regulation of spine morphology. Drebrin consists of two major isoforms, drebrin A and E. We have previously examined the functional role of drebrin using drebrin A specific knockout mice（DAKO）. DAKO shows behavioral abnormality in context dependent fear conditioning test（Kojima et al., 2010）. To better understanding of the role of drebrin for brain function, we generated drebrin null–knockout mice（DXKO）. DXKO showed abnormal behavior in buried food test and three–chamber social interaction test which were olfactory bulb（OB）related behavior. In Golgi staining, we observed normal dendritic spines in the OB of DXKO, suggesting that the olfaction disorder was not caused by spine abnormality. We then immunohistochemically analyzed the number of dying cells and mature neurons in the OB. In the OB of DXKO, the number of cell death decreased compared to that of wild–type mice（WT）, whereas the number of mature neurons did not change. Since newly generated neurons migrate from subventricular zone（SVZ）to OB, we next examined the number of arriving newly generated neurons in the OB. One day after injection of BrdU, the number of newly generated neurons in the OB of DXKO was smaller compared to that of WT. At 1 week, however, there was no difference in the cell number, and the cell number was larger in DXKO at 7 weeks. These results suggest that the adult neurogenesis decreases whereas the neuronal survival in OB increases in DXKO. These abnormalities might cause olfaction disorder in DXKO.

2G1-03 Modeling and analysis of intercellular adhesion between cells from the developing cerebral cortex

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The mammalian neocortex has a highly organized 6-layered structure of neurons. Cortical neurons are generated within the ventricular zone (VZ) or subventricular zone (SVZ), and migrate along radial fibers toward the pial surface. Newly born excitatory neurons migrate radially into the cortical plate (CP) past the earlier-born neurons, resulting in the birth-date-dependent "inside-out" alignment of neurons in the CP. Although the Reelin-deficient mouse, *reeler*, has been studied for more than 60 years and Reelin is indispensable for the establishment of the "inside-out" neuronal layers, cellular and molecular functions of Reelin for layer formation are still largely unknown.

Reaggregation culture is a tool for studying intercellular adhesion. In the previous study, several clusters of MAP2-positive neurons were abnormally observed in the reaggregates of the *reeler* cerebral cortical cells. This result suggests the possibility that intercellular adhesion is altered in the *reeler* cerebral cortex. In the present study, to uncover how Reelin controls the intercellular adhesion among cortical cells, we performed Reelin stimulation experiments using reaggregation culture of the cells from the *reeler* cerebral cortex. We transfected an expression vector for Reelin into part of the cortical cells in the *reeler* reaggregates. Overexpression of Reelin unexpectedly caused clustering of nestin-positive cells in the inner part of the *reeler* reaggregates. To understand the mechanism of this cell clustering, we made mathematical models of cell aggregation, and examined the factors important for recapitulating the cell clustering patterns in the presence or absence of Reelin.

The reaggregation culture and the mathematical model of cell sorting suggest that transient increase in neuronal adhesion is required for nestin-positive cluster formation in the inner part of the *reeler* reaggregates. Transient but not persistent increase in cell-cell adhesion might be necessary for the highly organized layered structure of neurons in the mammalian neocortex.

2G1-04 Ergothioneine promotes neuronal differentiation via induction of neurotrophin 5 in cultured neural stem cells.

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Ergothioneine (ERGO) is a food-derived hydrophilic antioxidant, distributed to the brain, and taken up into neural stem cells (NSCs) via carnitine/organic transporter OCTN1/SLC 22A4. OCTN1-mediated ERGO uptake in mouse NSCs promoted neuronal differentiation accompanied with induction of Math1, one of the basic helix-loop-helix (bHLH) transcription factors, via unidentified mechanisms different from antioxidant action (Ishimoto et al., PLOS ONE 9, e89434, 2014). In the present study, we focused on neurotrophins (NTs), which promote neuronal differentiation by induction of bHLH transcription factors, as one of the candidate mechanisms. Some NTs are known as important factors related with pathogenesis of neuropsychiatric disorders. Since NTs are not distributed to the brain across the blood-brain barrier unlike ERGO, induction of NTs by ERGO may be a novel therapy for neuropsychiatric disorders. Exposure of NSCs to ERGO at 500 μM for 9 days significantly increased mRNA expression of Math1 and neurotrophin 5 (NT-5), and tended to increase expression of brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3). Short term exposure of NSCs to ERGO also increased mRNA expression of NT-5, BDNF and NT-3 depending on the exposure time of ERGO until 12 hours, followed by increase in expression of Math1 at 24 hours. NT-5, BDNF and NT-3 activate neurotrophic tyrosine kinase receptor type2 (TrkB). To clarify the intracellular signaling pathway related with induction of Math1 by ERGO exposure, NSCs were incubated with ERGO in either the presence or absence of the inhibitor of TrkB or its three downstream signaling pathways, PI3K/Akt, PLCγ and MAPK/ERK signaling, and expression of Math1 was examined. As a result, all inhibitors of TrkB, Akt, PLCγ or Erk suppress induction of Math1 by ERGO. These results suggest that ERGO may promote neuronal differentiation at least partially by activation of TrkB signaling via autocrine/paracrine action of NT-5. Further studies are required in order to clarify upstream mechanisms underlying induction of NT-5 by ERGO.

2G2-01 Effect of a novel cognitive enhancer ST101 on decreased CaMKII activity in schizophrenia model rats.

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［Background］ Development of cognitive enhancer is essential to improve quality of life for schizophrenia patients. We used neonatal ventral hippocampus（NVH）-lesioned rats as schizophrenia model animal, in which risperidone improves sensory motor gating deficits. However risperidone, a typical anti-psychotics fails to improve cognition assessed by novel object recognition test. Notably, cognitive impairment of NVH-lesioned rats is associated with the decreased CaMKII activity in the medial prefrontal cortex（mPFC）and hippocampus（Yabuki et al., Neuroscience 2013；234：103-115）. Therefore, we here investigated the effect of a novel cognitive enhancer ST101（piro［imidazo［1,2-a］pyridine-3,2-indan］-2（3H）-one）on the cognitive impairment of NVH-lesioned rats and its mechanism.［Methods］ To prepare NVH-lesioned rats, ibotenic acid was injected into the bilateral ventral hippocampus on the postnatal day（PD）7. After PD 70, animals were administrated with ST101（0.01, 0.1, or 0.5 mg/kg, p.o.）once a day for 2 weeks and were subjected to cognition tests. CaMKII activity was measured using immunohistochemical and western blotting analyses.［Results］ The chronic administration of ST101 rescues the decreased CaMKII activity in the mPFC and hippocampus. The cognitive impairment of NVH-lesioned rats was significantly improved by ST101 administration.［Conclusion］ NVH-lesioned rats are potential animal model of schizophrenia because the animals show abnormal sensory motor function only after post-pubertal. A novel cognitive enhancer ST101 which is T-type calcium channel stimulator restores cognitive impairment by stimulationg CaMKII activity in the mPFC and hippocampus. Taken together, ST101 is attractive candidate therapeutics for cognitive impairment in schizophrenia patients.

2G2-02 Brain-Active Herbal Metabolites for the Treatment of Alzheimer's Disease

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Our previous studies have shown that the water extract of a crude drug DR＊（1, 10 μg/ml）could reverse Aβ（25-35）-induced axonal atrophy in cultured mouse cortical neurons. Administration of DR extract（p.o., for 21-31 days）to Alzheimer's disease（AD）model, 5×FAD mice, improved deficits of object recognition memory and spatial memory. In this study, we aimed to clarify effects of DR extract on axonal degeneration and AD pathologies in the 5×FAD mice brain. In addition, we tried to identify active compounds detected in the brain, which were distributed constituents or metabolites in DR extract.Oral administration of DR extract（500 mg/kg）for 31 days to 5×FAD（male and female, 6-8 months old）significantly reduced amyloid β plaques in the medial prefrontal cortex, perirhinal cortex and hippocampus. Abnormally swollen degenerated axonal terminals were also significantly decreased in the DR-treated group. To identify DR-derived compounds transferred into the brain, the plasma and cerebral cortex were isolated from DR- or vehicle-treated 5×FAD mice at 0.5 and 5 h after the administration. We had already known about main constituents in DR extract by chemical analyses. Three metabolites in the cortex and thirteen metabolites in the plasma were detected by LTQ-Orbit trap FT-MS/MS analysis. Axonal regeneration activities of those metabolites in the brain are under investigation. To investigate starting point in the signaling mechanism of DR extract, Drug Affinity Responsive Target Stability（DARTS）analysis was performed. By DARTS method using DR extract and mouse cortical lysate, CRMP2 and RKIP were suggested as candidates for direct target proteins of DR constituents. Signaling pathways of DR-derived real active compounds working in the brain are now investigated. Those information may provide a new viewpoint of memory improvement pathway. ＊A name of the crude drug is not open due to patent matters.

2G2-03 Bruton's tyrosine kinase (BTK) inhibitor has a protective effect on ischemic brain injury.

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Stroke causes ischemic brain injury, which is a leading cause of neurological disability and death worldwide. The therapeutic time window of t-PA is only 4.5 hours after stroke onset. There is a need for an efficacious therapy that can be administered beyond this time window. Post-ischemic inflammation is a hallmark of ischemic stroke pathology. IL-1β promotes brain tissue injury and is therefore potential targets for therapy after ischemic stroke. Activation of IL-1β is required for caspase-1 activation by formation of inflammasome. We used a transient middle cerebral artery occlusion (MCAO) model (60 min) induced by means of an intraluminal suture. In this study, we demonstrates that The FDA-approved BTK inhibitor ibrutinib (PCI-32765) significantly suppressed infarct volume growth and neurological damage in the brain ischemia/reperfusion model. Ibrutinib suppressed caspase-1 activation and IL-1β secretion by inhibiting the formation of inflammasome.

2G2-04 Immediate or delayed administrations of matrine improve motor dysfunction in spinal cord injured mice

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In spinal cord injury (SCI), descending neuronal tracts are disrupted, which induce motor dysfunction. Although reconstruction of spinal tracts by axonal growth must be effective for improvement of motor dysfunction, inhibitory factors for axonal extension, such as chondroitin sulfate proteoglycan (CSPG), increase in the lesion site. We previously found that the water extract of dried roots of *Sophora flavescens* (SF) promoted axonal extension even on inhibitory CSPG *in vitro* and improved the axonal density and motor dysfunction in SCI mice. In this study, we aimed to identify active constituents in SF extract and investigate effects of the active constituents in SCI mice. Axonal extension activities of compounds in SF extract were evaluated in cultured cortical neurons (ddY mice, E14) on the CPSG. Four days after the treatment, axonal length was quantified by immunostaining for phosphorylated neurofilament-H. Although axonal growth was inhibited on the CSPG, matrine (10 μM) extended axons even on the CSPG. Consecutive oral administrations of matrine (100 μmol/kg/day, for 30 days) or vehicle solution to SCI mice (ddY, female, 8 weeks old) were started from 1 h after the injury. Matrine significantly recovered motor function of hindlimbs. Furthermore, effects of matrine on the motor function in SCI mice by delayed administration were investigated. Matrine (100 μmol/kg/day) was administrated to SCI mice for 154 days from 28 days after the injury. As a result, motor function was significantly recovered by matrine treatment. This study suggests that matrine is one of the active constituents in SF extract and effective for recovery of motor function of SCI mice in chronic phase as well as acute phase. Investigating mechanisms underlying matrine effects is ongoing.

2G2-05 Novel candidate compounds identified by in silico screening activate TrkB and attenuate depressant-like behavior in mice

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BDNF is a ligand for its receptor TrkB and contributes to neuronal survival, differentiation and synaptic plasticity in central nervous system. Recent reports have indicated that dysfunction of BDNF-TrkB is related with depression. We previously performed in silico screening to find new anti-neuroblastoma（NB）therapeutics targeting BDNF binding domain of TrkB. From 3 million low molecular weight compounds, we finally discovered 7 compounds that induce apoptosis（Nakamura et al., 2014）. In the study, we have also identified 2 distinct compounds（48 and 56）harboring the effects to enhance cell survival in TrkB expressing NB cells. Therefore, we hypothesized that these compounds act as TrkB agonist. We first examined in vitro and in vivo whether the compounds induce TrkB phosphorylation. Western blot analysis in SH-SY5Y/TrkB cells revealed that the treatment of compound 48 and 56 increased phosphorylation of TrkB and its downstream molecules, AKT and ERK. Intraperitoneal injection of compounds 48 and 56 in C57BL/6J mice caused higher phosphorylation of TrkB in hippocampus and cerebral cortex compared with vehicle-injected controls. In SH-SY5Y/TrkB cells treated with the compounds, the phosphorylation of TrkB was blocked by the pretreatment of a Trk inhibitor, K252a. Finally, to investigate the antidepressant effects of the compounds, we carried out the forced swim test（FST）, a model for assessing antidepressant-like behavior. As the result of the FST in C57BL/6J mouse, the both compounds showed reduced immobility compared with vehicle-injected controls. These data suggest that the compounds 48 and 56 could cross the blood brain barrier, activate TrkB and have potential ability of novel antidepressants.

2G2-06 Effects of coffee on vascular endothelial growth factor expression in human neuroblastoma SH-SY5Y cells.

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Objectives Coffee is one of the most world-widely consumed beverage on a daily bases. Recent epidemiological studies have reported that amyotrophic lateral sclerosis（ALS）patients are less frequent and prolonged to coffee intake than healthy persons. However, the precise molecular mechanisms of the effects of coffee are yet uncertain. Vascular endothelial growth factor（VEGF）is known to have protective effects on ALS in development of symptoms and prolongation of life. Therefore, we investigated the effects of coffee on VEGF expression in human neuroblastoma SH-SY5Y cells. Methods SH-SY5Y cells were cultured in Ham's F-12/DMEM（1：1）medium supplemented with 15% FBS. The cells were exposed to coffee or coffee extracts up to at 2.0%（v/v）. After 4 hours, the whole cell lysates were isolated and subjected to immunoblotting for HIF-1α. VEGF gene expression was monitored by qPCR using RNAs isolated from the cells treated with coffee for 8 hours. After 12 hours, the amount of VEGF in the culture medium was measured with an ELISA kit（eBioscience）. Results Coffee induced VEGF expression in dose-dependent manner whereas decaffeinated coffee or caffeine（100 μM）showed no effects. The induction profile of VEGF was corresponding to that of an activation of HIF-1α by coffee. The active constituents of coffee were produced by roasting process of coffee beans and were extractable with n-butanol.Conclusion Coffee induced VEGF expression via the HIF-1α activation in SH-SY5Y cells. This activity may contribute to the preventive effects of coffee on ALS. Further study to identify active components and to elucidate the mechanism of the effects is needed to clarify the molecular basis of neuroprotective effect associated with daily coffee consumption.

1O1-01 Novel intracellular D2LR signaling is critical for dendritic spine formation.

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Abnormalities in activity of the dopamine D2 receptor（D2R）are associated with neuropsychiatric disorders, making it a target for antipsychotic drugs. Here, we report that novel signaling through the intracellular D2R long isoform（D2LR）elicits persistent extracellular signal-regulated kinase（ERK）activation and dendritic spine formation through Rabex-5/Rab5-mediated endocytosis. D2LR directly binds and activates Rabex-5, promoting early endosome formation. Endosomes containing D2LR and platelet-derived growth factor receptor-beta（PDGFRbeta）are then transported to the Golgi apparatus, where those complexes trigger Galphai3-mediated ERK signaling. Loss of intracellular D2LR-mediated ERK activation decreases neuronal activity and dendritic spine density in striatopallidal medium spiny neurons（MSNs）. Taken together, novel intracellular D2LR signaling is critical for prolonged ERK activation and dopamine-regulated synaptic activity in striatopallidal MSNs.

1O1-02 Rbfox1, an autism causal gene, plays an essential role in cortical development

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Rbfox1（aka Fox1 or A2BP1）is an RNA-binding protein necessary for regulation of alternative splicing. Critical neurological functions for Rbfox1 have been approved by human mutations in RBFOX1 gene that cause neurodevelopmental disorders including autism spectrum disorder（ASD）. To elucidate the pathophysiological relevance of Rbfox1, we here performed cell biological analyses of the neuron-dominant Rbfox1 isoform 1（Rbfox1-iso1；A2BP1-A016）during mouse cerebral development. Knockdown of Rbfox1-iso1 by in utero electroporation method caused abnormal neuronal distribution during corticogenesis. Rbfox1-iso1 knockdown did not affect cell proliferation in the neural progenitor/stem cells. Confocal laser microscope-associated live-imaging analyses revealed that migration defects occurred during radial migration and terminal translocation. While Rbfox1-iso1-deficient neurons did not show any morphological abnormality during migration, they could not efficiently enter the cortical plate and were prevented from smooth migration in the cortical plate, perhaps due to impaired nucleokinesis. Indeed, the distance between nucleus to centrosome was abnormally elongated in Rbfox1-iso1-deficient neurons during radial migration. Rbfox1 was also found to regulate neuronal network formation in vivo since interhemispheric axon extension and dendritic arborization were suppressed in Rbfox1-iso1-deficient neurons. Aberrant morphology was further confirmed in in vitro analyses；Rbfox1-iso1-silencing in primary cultured hippocampal neurons resulted in the reduction of primary axon length, total length of dendrites, spine density and mature spine number. Taken together, aberrant phenotypes observed in this study may relate to structural and functional defects of the cerebral cortex, leading to the emergence of the clinical symptoms of neurodevelopmental disorders.

1O1-03 Dysregulation of fear memory and CaM kinase II activity in NCX1 heterozygous mice

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Na^+/Ca^{2+} exchangers（NCXs）are mainly expressed in the plasma membrane and mediate the electrogenical exchange of one Ca2＋ for three Na＋, depending on the electrochemical gradients across the plasma membrane. NCXs have three different isoforms（NCX1, NCX2, NCX3）encoded by distinct genes in mammals. We here report that mutant mice lacking NCX1（NCX1-KO）exhibit impaired fear-related memory. NCX1-KO showed significantly impairment of fear-related behaviors measured by elevated-plus maze, light-dark task, open-field task and marble burying tasks. In addition, NCX1-KO mice show abnormality in fear-related tone memory but not in contextual memory with fear-conditioning task. In immunohistochemical analyses, NCX1-KO mice revealed significant increase in the number of cFos positive cells in the lateral amygdala but not the central amygdala following fear-related tone stimuli. The cFos expression peaked at 1 hr. Furthermore, enhancement of CaM kinase II or IV activities in the latelal amygdala were observed in NCX1-KO mice by immnoblot analyses. By contrast, CaM Kinase II null mice failed to increase in the number of cFos positive cells in the lateral amygdala without changes in CaM Kinase IV null mice. Taken together, the increased CaM kinase II activitiy and in turn cFos expression likely account for the dysregulation of fear memory in NCX1-KO mice.

1O1-04 A newly identified stres hormone responsive molecule, Hit, regulates nuclear transport of Glucocorticoid Receptor

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Maternal stress during pregnancy increases secretion of stress hormones such as glucocorticoid（GC）. A numbers of studies indicate high level exposure to GC during prenatal period affects neural development and stress response after birth. Moreover, recent studies suggest it causes psychiatric disorders such as depression（Front Neurosci. 8：420, 2015）, schizophrenia（Psychopharmacology 214：89-106, 2011）and autism（Neuroscience and Biobehavioral Reviews 32：1519-1532, 2008）. Although detail molecular mechanisms that link prenatal stress and these psychiatric disorders are not well understood, molecules responding to GC must have critical roles.

Here, we show a newly identified GC responsive molecule, Hit. Treatment with GC agonist, DEX, or restraint stress on pregnant mice resulted in reduction of Hit mRNA expression in the brain of their embryos. Similar to effects of DEX, Hit RNAi/over-expression affects proliferation, differentiation and migration of neuron-like PC12 cells and also neural cells in primary culture. We also found Hit RNAi facilitates nuclear transport of GC receptor（GR）, which is a critical step for GR function to regulate various genes transcription. Thus, our data suggest Hit has critical roles on prenatal development by negatively regulating the GR transport to nuclear.

Interestingly, recent studies show molecules involved in the GR transport to nuclear（FKBP5 and HSP90 for example）are closely related to psychiatric disorders such as depression（Neurosci Biobehav Rev. 37：2375-97, 2013）and PTSD（Nat Neurosci 16：33-41, 2013）. Hit may have interaction with these molecules and potentially link to these psychiatric disorders.

1O2-01 Rer1 and calnexin regulate endoplasmic reticulum retention of a peripheral myelin protein 22 mutant that causes type 1A Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth disease (CMT1) is the most commonly inherited neurological disorder of the peripheral nervous system with an estimated frequency of 1/2,500. Approximately 70% of patients with CMT1 harbor a genetic abnormality (e.g. duplication and mutation) of a membrane protein, PMP22. Although the accumulation of misfolded PMP22 in the endoplasmic reticulum (ER) correlates with pathogenic mechanism, the molecular mechanisms in the ER accumulation of PMP22 are largely unknown. Here, we studied the quality control mechanisms for the PMP22 mutants L16P and G150D, which were originally identified in mice and patients with CMT. We found that the ER-localised ubiquitin ligase Hrd1/SYVN1 mediates ER-associated degradation (ERAD) of PMP22 (L16P) and PMP22 (G150D), and another ubiquitin ligase, gp78/AMFR, mediates ERAD of PMP22 (G150D) as well. We also found that PMP22 (L16P), but not PMP22 (G150D), is partly released from the ER by loss of Rer1, which is a Golgi-localised sorting receptor for ER retrieval. Rer1 interacts with the wild-type and mutant forms of PMP22. Interestingly, release of PMP22 (L16P) from the ER was more prominent with simultaneous knockdown of Rer1 and the ER-localised chaperone calnexin than with the knockdown of each gene. These results suggest that CMT disease-related PMP22 (L16P) is trapped in the ER by calnexin-dependent ER retention and Rer1-mediated early Golgi retrieval systems and partly degraded by the Hrd1-mediated ERAD system.

1O2-02 Multivesicular body is formed after endoplasmic reticulum stress.

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A number of cellular stress conditions lead to the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) lumen, named as ER stress. Prolonged ER stress results in a fundamental threat to the cell. Accumulation of malfolded proteins in the ER triggers the unfolded protein response (UPR) to avoid cell damages. The UPR consists of at least three distinct components, namely transcriptional induction of ER-resident chaperones, translational attenuation, and ER-associated degradation (ERAD). A number of studies indicate that ER stress and its stress response are associated with pathophysiology of neurodegenerative disorders including Alzheimer's disease and Parkinson's disease.
In this study, we found that multivesicular body (MVB) was formed in response to ER stress. MVBs are a type of late endosome containing intraluminal small vesicles which include secretary proteins, and the vesicles in MVBs are secreted as exosomes. Treatment of human neuroblastoma SK-N-SH cells and human glioma U251MG cells with ER stressors, such as thapsigargin and tunicamycin, enhanced formation of MVBs that are detected by a MVBs marker, GFP-TSG101. We also investigated whether three major ER stress transducers, IRE1, PERK, and ATF6 are involved in the formation of MVBs. In PERK knockout mouse embryonic fibroblasts (MEFs), MVBs were not formed by the treatment with thapsigargin, whereas MVBs were formed in IRE1αβ knockout or ATF6αβ knockdown MEFs after treatment with thapsigargin. Activation of eIF2α downstream of PERK pathway by a chemical compound salubrinal also increased MVB formation, suggesting ER stress facilitates the formation of MVBs through the activation of PERK-eIF2α pathway. Inhibition of MVB formation by the treatment with manumycinA which is a neutral sphingomyelinase inhibitor induced the expression of UPR related genes such as BiP, EDEM, and CHOP. Our findings suggest that MVB formation in response to ER stress is regulated via PERK-eIF2α signaling and might function to attenuate ER stress.

1O2-03 Sigma 1 receptor deficiency is involved in motor neuronal degeneration through calcium deregulation at mitochondria-associated membrane.

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A homozygous mutation in the gene coding sigma 1 receptor (Sig1R) is causative for juvenile inherited amyotrophic lateral sclerosis (ALS). Sig1R specifically localizes at an interface of mitochondria and endoplasmic reticulum called as mitochondria-associated membrane (MAM). In this study, we aimed to elucidate the mechanism that a loss-of-function of Sig1R causes ALS. First, ALS-linked Sig1R mutant was unstable and unable to bind to inositol triphosphate receptor type 3 (IP_3R3). Loss of Sig1R resulted in mislocalization of IP_3R3 and deregulation of intracellular calcium flux. In ALS-linked mutant Cu/Zn superoxide dismutase (SOD1) transgenic mice, mutant SOD1 proteins were accumulated in MAM, inducing depletion of Sig1R and IP_3R3 from MAM. Moreover, onset of the disease for $SOD1^{G85R}$ mice was markedly accelerated in the absence of Sig1R with over-activation of calpain. Our findings suggest that the loss-of-interaction between Sig1R and IP_3R3 causes motor neuronal degradation through calcium deregulation in MAM, and restoring the function may be a promising therapeutic strategy.

1O2-04 Dextran sulfate sodium inhibits amyloid-β oligomer binding to cellular prion protein

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Amyloid-β peptide (Aβ), especially its oligomeric form, is believed to play an important role in the pathogenesis of Alzheimer's disease (AD) and the binding of Aβ oligomer to cellular prion protein (PrP^C) plays an important role in synaptic dysfunction in a mouse model of AD. In this study, we have screened for compounds that inhibit Aβ oligomer binding to PrP^C from medicines already used clinically, and identified dextran sulfate sodium (DSS). In a cell-free assay, DSS inhibited Aβ oligomer binding to PrP^C but not to ephrin receptor B2, another endogenous receptor for Aβ oligomers, suggesting that the drug's action is specific to inhibition of the binding of Aβ oligomer to PrP^C. Dextran on the other hand did not affect this binding. DSS also suppressed Aβ oligomer binding to cells expressing PrP^C but not to control cells. Furthermore, while incubation of mouse hippocampal slices with Aβ oligomers inhibited the induction of long-term potentiation (LTP), simultaneous treatment with DSS restored the LTP. Since DSS has already been approved for use for patients with hypertriglyceridaemia, and its safety in humans has been confirmed, we propose further analysis of this drug as a candidate for AD treatment.

1O2-05 Endocytic pathology in astrocytes：dynein dysfunction disrupts Abeta clearance in astrocytes via disturbed endosome trafficking

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A large number of studies suggest that endocytic disturbance is involved in Alzheimer's disease（AD）pathogenesis, especially for β-amyloid protein（Aβ）pathology. Our previous studies showed that aging affects retrograde motor protein dynein in cynomolgus monkey brain, and dynein dysfunction reproduces age-dependent endocytic pathology, resulting in the accumulation of intracellular β-amyloid precursor protein（APP）and Aβ. These findings suggest that dynein dysfunction may be one of the responsible factors for age-dependent endocytis distrbance leading to AD pathogenesis. On the other hand, it remains unclear whether such age-dependent endocytic disturbance also occurs in glial cells. Here, we show that intracellular accumulation of enlarged endosomes occurs even in astrocytes of aged monkey brains, and we confirmed that Aβ accumulates in those enlarged endosomes. RNA interference studies demonstrated that dynein dysfunction reproduces astroglial endocytic pathology and disrupts Aβ clearance in astrocytes via disturbed endosome trafficking. Interestingly, dynein dysfunction did not affect Aβ uptake itself. These findings suggest that endocytic disturbance in astroglial cells may also be involved in age-dependent Aβ pathology.

1O2-06 Identification of domains of FUS required for the regulation of genes with conserved introns.

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FUS is an RNA binding protein and known as a causative factor of Amyotrophic Lateral Sclerosis, ALS. Many mutations linked to familial cases of ALS have been identified in the C-terminal end, around its nuclear localization signal, and most of them abnormally localized in cytoplasm. Since FUS wild type strictly localizes in nucleus, it is believed that the mislocalization of FUS mutants is critical to pathogenesis of ALS. However, it is still unclear how mutations of FUS cause ALS. Because the normal function of FUS was not clear, which might be important to reveal the mechanism of FUS mutant effects, we employed HITS-CLIP and RNA-seq experiments to reveal the physiological function of FUS in neurons. We found that FUS bound to many RNAs through their introns and 3'UTR regions, which was observed in both human brains and mouse ES cell derived neurons. However, almost no significant difference of expressions of target RNAs was observed in FUS knock down neurons compared to control siRNA treated neurons. Thus far, it is still unclear what the normal function of FUS in neurons is. Among these FUS target introns, we found that FUS preferentially bound to introns conserved among species. Moreover, the expressions of these RNAs with conserved introns were affected significantly in FUS knock down neurons. Interestingly, many RNA binding protein-coding genes have these conserved introns, suggesting that FUS regulates the expressions of RNA binding proteins by regulating their RNA levels through their conserved introns. To reveal the molecular mechanism of this regulation, deletion constructs of FUS were utilized and the effects on target RNAs were analyzed. FUS has six domains including SYGQ-rich, Gly-rich, RNA recognition motif（RRM）, Arg-Gly-Gly-rich1（RGG1）, Zn-finger and RGG2. When these domains were deleted one by one, all of mutant localized to the nucleus, indicating that these domains were not involved in the regulation of its localization in neurons. In this presentation, I will show the results of further analyses and discuss how FUS regulates the expressions of RNAs with conserved introns.

1O3-01 Brain dopamine D1 receptor bindings in young adults with autism spectrum disorder

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Autism spectrum disorder（ASD）is characterized by repetitive and/or obsessive interests and behavior and by deficits in sociability and communication. A previous neuroimaging study using positron emission tomography（PET）indicated that dopamine transporter bindings were significantly higher in the orbitofrontal cortex of adults with ASD, although the details remain unknown.

In this study, we measured the binding of dopamine D1-like receptors with the radio-ligand ^{11}C-SCH23390 in the brain of subjects with ASD（n＝20）and age- and sex-matched control subjects（n＝20）. Whole-brain voxel-based analyses as well as regions of interest-based methods were used for between-subject analysis and within-subject correlation analysis with respect to clinical variables.

Both voxel- and region of interest-based analyses revealed significantly higher ^{11}C-SCH23390 binding potentials, in the orbitofrontal cortex of subjects with ASD than in those of controls（corrected P＜．05）. There was no statistically significant correlation between orbitofrontal ^{11}C-SCH23390 binding potential and any of ASD symptoms evaluated.

The results suggest that a dopaminergic dysregulation in the orbitofrontal cortex, which may play a role in pathophysiology of ASD, can be observed in adult subjects with ASD.

1O3-02 Differential roles of dopamine D1 and D2 receptor-containing neurons of the nucleus accumbens shell in behavioral sensitization

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The nucleus accumbens（Nac）mediates the reinforcing and motor stimulating properties of psychostimulants. It receives dopaminergic afferents from the ventral midbrain and is divided into two distinct subregions：shell and core. Each of these contains two subtypes of medium spiny neurons, which express either dopamine D1（D1R）or D2（D2R）receptors. However, functional dissociation between the two subtypes in psychostimulant response remains to be elucidated. We performed selective ablation of each subtype in the Nac shell in mice, using immunotoxin-mediated cell targeting, and examined the behavioral sensitization evoked by repeated administration of methamphetamine（METH）. The D1R cell-targeted mice exhibited delayed induction of sensitized locomotion compared to control mice, whereas the D2R cell-targeted mice showed a mildly enhanced rate of induction of sensitization. In vivo microdialysis revealed a marked blockade of the increase in extracellular dopamine in the Nac of the D1R cell-targeted animals in response to METH, indicating that the observed delay in behavioral sensitization in these mice involves an impairment in accumbal dopamine release. Our results reveal differential roles of D1R- and D2R-containing accumbal shell neurons in the development of behavioral sensitization to psychostimulants.

1O3-03 Molecular Mechanisms of Fear Memory：Hippocampal Interneurons and their Relevance for Post Traumatic Stress Disorder

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Pavlovian fear conditioning is an established learning paradigm that allows to study neural mechanisms of fear and anxiety in various species and that may be employed to emulate specific aspects of anxiety disorders. We investigated, in mice, molecular and physiological processes in the amygdalo-hippocampal system that are involved in the consolidation and reconsolidation of such long-term fear memory. GABAergic interneurons are of critical importance for these processes, as indicated by the hyperarousal, fear generalization and deficit in fear extinction of mice deficient for the key enzyme in GABA synthesis, glutamic acid decarboxylase 65. We found that a single reactivation of fear memory in mice is sufficient to trigger long lasting changes in contextual fear, anxiety and endogenous corticosterone levels. With high resolution gene expression we identified changes in gene expression of GABAergic and glutamatergic receptors the CA3 subfield of the hippocampus under these conditions. These molecular changes are accompanied by corticosterone-sensitive alterations in gamma frequency oscillations as well as sharp wave ripple activities measured in hippocampal slice preparations of behaviorally stimulated animals. Both types of network activity patterns are generated by a group of parvalbumin-positive basket cells in the hippocampus. Indeed, genetic enhancement of the activity of these fast spiking interneurons resulted in increased sharp wave ripple propagation in hippocampal slice preparations and enhanced reconsolidation of contextual fear memory at the expense of fear extinction. Together, our data demonstrate the role of a group of GABAergic interneurons in the hippocampus in the development of pathological fear memory and its physiological network correlates.

1O3-04 Prenatal administration of valproic acid or SAHA alters the development of Purkinje cell dendrites and network formation in rat cerebellum

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Valproate（VPA）, the popular antiepileptic drug, is known as an inducer of autism. It has many kinds of physiological properties, including the inhibition of histone deacetylase（HDAC）. Now we investigated the effects of administration of VPA or other HDAC inhibitors to fetus and observed their postnatal cerebellar development. Each HDACi drug was administrated to embryonic day 16 p.o.（VPA；600mg/kg of mother weight, trichostatin A, TSA；0.05mg/kg, MS-275；4 mg/kg）or i.p.（suberoylanilide hydroxamic acid, SAHA；60 mg/kg）.
In cerebellar development, the soma of Purkinje cells form a single layer and elongate their dendrites with synapses during the first two weeks. ATP release was drastically increased from P9 in response to synaptogenesis between Purkinje cells and granule cells. In VPA administrated rat, the elongation of Purkinje cell dendrites started earlier and reached all over the molecular layer even in P12. It was observed also in SAHA administrated rat, while it was obscure in MS-275 or TSA administrated rat. In addition, in VPA or SAHA administrated rat, ATP release became earlier and larger than normal cerebellar development. Some network components would be changed by prenatal HDACi administration. It was suggested that HDACi-induced epigenetic effects would change the developmental progress in immature cerebellum.

1O3-05 Analyses of the pathological roles of the altered brain cytoarchitectures with ectopic neurons

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Altered brain cytoarchitectures have been pointed out as one of the neuropathological features of neuropsychiatric disorders, such as schizophrenia and autism, but their pathological roles have not yet been completely understood. To reveal the pathological mechanisms of the altered brain cytoarchitectures, we generated mouse models with ectopic neurons by inducing focal heterotopias with the in utero electroporation technique. The mice with focal heterotopias in the somatosensory cortex exhibited spatial working memory deficit and low competitive dominance behavior, which have been shown to be related to the activity of the medial prefrontal cortex（mPFC）in rodents. Analysis of the mPFC activity revealed that the immediate-early gene expression was decreased and the local field potentials（LFPs）of the mPFC were altered in the mice with heterotopias as compared to the control mice. Moreover, activation of these ectopic and overlying sister neurons mitigated the deficits observed in the mice with heterotopias. These findings suggest that cortical regions containing focal heterotopias can affect distant brain regions and give rise to behavioral abnormalities. Based on these observations, the pathological mechanisms of the altered brain cytoarchitectures with ectopic neurons will be discussed.

1O3-06 Multivariate consideration with social, thermal ambient and bio-molecular interactions suggested new developmental models between common marmosets and humans

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Statistical development in multivariate analyses is potentially accompanied by a large variety of challenging application to imply neuronal complex systems, such as psycho-cognitive functions. In the real world, not only ideal mechanisms studied in laboratories but also multiple kinds of factors interactively influence on one another. To explore computational approaches to infer neuropsychological mechanisms, we have examined several kinds of multivariate analyses whether they could suggest any interplay information or not, particularly in our focusing on emotional development through behavioral and physiological dynamics in two primate species, humans and common marmosets. Given the global rise in autism and other developmental disorders, we hypothesized that critical periods of social learning must be key roles and that parent-infant interactions and socializing with peers shape fundamental nervous system processes, including cognition and emotion. Furthermore, we paid attention what affect the development are not only social environments but together ambient factors for instance, thermal regulation, around individuals every day. We derived the seasonal and gravity factors to be considered. In the trials with multivariate analyses including the principal component analysis, we obtained expressive models visualizing developmental regression curves with repeatedly observed inflection at the similar age stage in common marmosets（Callithrix jacchus）. The application to human children also seemed to suggest some essential changes in the summarized complex analytic information dependently on biological and physical environments. They might be able to translate non-verbal descriptions of psycho-cognitive neuronal modulations as understandable complex systems.

2O1-01 Muller cell regulates axon elongation of retinal ganglion cells via $P2Y_6$ receptor signals

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Muller cells, retina-specific glia, are known to support various functions of retinal ganglion cells（RGCs）, one of the retinal neurons which transmit visual information from the eye to the brain. Precise axon outgrowth is critical to forming functional neuronal circuits but primary cultured RGCs do not extend their axons by default, so extrinsic signals supporting the elongation are required. Here we show that Muller cells enhance axon elongation of RGCs via nucleotide-mediated gliotransmission. Cultured RGCs significantly enhanced their axon outgrowth when they were co-cultured with Muller cells. This effect was abolished by nucleotide-degrading enzyme apyrase. The enhancement was mimicked by exogenously applied nucleotides on RGC monocultures. Pharmacological analysis revealed that $P2Y_6$ receptor in RGCs was responsible for the axon elongation. $P2Y_6$ receptor was expressed in ganglion cell layer of retina and in cultured RGCs. High performance liquid chromatography revealed that Muller cells constitutively release uridine triphosphate（UTP）, a precursor of endogenous $P2Y_6$ receptor agonist. Similarly, RGCs obtained from $P2Y_6$ receptor knockout mice showed only short axons even though they were co-cultured with Muller cells. Furthermore, RGCs exposed to an *in vitro* glaucoma exhibited only short axons associated with down-regulated $P2Y_6$ receptor expression. Taken together, our data highlight the role of purinergic gliotransmission between Muller cells and RGCs as a key factor for regulating axon outgrowth and sensing glaucomatous conditions.

2O1-02 Atypical myosin drives dendritic growth cone splitting to create complex arbor branching patterns.

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Neurons require highly branched dendritic arbor morphologies that underlie circuit organization. Yet the intricate cell biological mechanisms by which complex dendritic arbor architectures are established remain largely unknown. We developed in vivo time-lapse imaging in to study this process, and created a customized algorithm for automated recognition and tracing of different neuronal features over the imaging series. This algorithm facilitated analysis of the large and complex time-lapse data sets created through live imaging of Drosophila sensory neurons. From this we identify novel in vivo dynamic dendritic growth cone-like structures that undergo splitting in order to create branching. From an in vivo time lapse imaging screen we identify Myosin VI, the atypical myosin, as driving the splitting process. Myosin VI is targeted to the cortical lamellipodia of the growth cone and we demonstrate that Myosin VI directs promotes stabilization of polymerized-actin concentration at internal base of growth cone filopodia, and directs microtubule polymerization to these sites in order to drive growth cone splitting in order to generate new major branches.

2O1-03 The region specific stabilization of branched axons mediated by the axonal transport dependent system

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The maintenance of cellular morphology is especially important for neurons to make connection with specific targets. The mechanisms by which neurons locally control cellular nanostructures, such as F-actin/microtubules remained unsolved. Our aim is to demonstrate molecular systems by which neurons process spatial information and regulate cellular structure at right position at right time. We focused on the axonal branch morphology and tested the possibility that axonal transport might play roles to regulate the axonal branch pattern. Previous studies have revealed that the motor domain of kinesin heavy chain（K5H）is accumulated in axon in hippocampal neurons. By using dissociated cerebellar granule neurons, we found that there is a positive correlation between signal intensity of K5H-GFP and axonal branch length, suggesting the possibility that axonal branch pattern is regulated via axonal transport. We further performed long-term multipoint time-lapse imaging of branched axons. By quantitative analysis of growth/retraction, we found that axonal branch which contain high ratio of K5H-GFP show lower retraction value.

2O1-04 Development of axon collaterals as the inter-areal connections in the cerebral cortex.

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Cerebral neocortex integrates different sensory inputs with internal status to elicit an appropriate behavior. Direct neuronal connections between functional areas within a cerebral hemisphere should play an important role in this process. Long association fibers（LAFs）are the long-range connections between distant areas located in different cortical lobes. Recent studies reported that the LAFs are aberrant in the mental/developmental diseases like schizophrenia and autism spectrum disorders, suggesting the importance of LAFs in cognitive functions. However, the detailed axonal structure of long association neurons（LANs）that constitute the LAFs and how its final structure is established during cortical development are yet to be revealed. To study the structure and development of the LANs, we searched for the mouse genes expressed in LANs. In our retrograde tracing from the primary motor cortex（M1）, the LANs in the primary somatosensory area（S1）were located in the layers 2/3, 5a, and 6b. Therefore, we supposed that the genes expressed in the LANs should be found among the known marker genes for these layers. By combining in situ hybridization using probes for the layer marker genes and retrograde tracing from M1 to S1, we found a candidate gene that was expressed in the LANs in the layers 2/3 and 5a of S1. We induced the EGFP expression in the LANs in the layer 2/3 using the promoter of this gene and visualized the axons projecting from S1 to M1. Imaging of the entire axonal structure revealed that the labeled LANs project their axons to both ipsilateral M1 and contralateral S1. Interestingly, the projection to M1 was one of the collaterals branching at layer 5. We report the developmental changes of the axonal structure of the labeled LANs.

2O2-01 Regulation of post-ischemic inflammation by DAMPs

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Inflammation is an essential step for the pathology of ischemic stroke. Because brain is a sterile organ, the inflammation is triggered by some endogenous molecules. High mobility group box 1（HMGB1）is the well-known danger associated molecular patterns（DAMPs）which exaggerate the disruption of blood brain barrier. Here, we have identified peroxiredoxin（Prx）family proteins as previously unknown DAMPs in the ischemic brain. Prx activates infiltrating immune cells and induces the inflammatory cytokine production through TLR2 and TLR4 signaling pathway. Both the extracellular release of Prx and the infiltration of immune cells reach the peak within 1 to 3 days after the onset of ischemic stroke and thereafter they decrease. This will lead to the resolution of post-ischemic inflammation. Indeed, the gene expression profile of infiltrating immune cells in the late phase shows the phenotype for anti-inflammation and tissue repair. Our results indicate that macrophage and microglia contribute to the resolution of post-ischemic inflammation independently. DAMPs regulate not only the induction but also the resolution of post-ischemic inflammation. The novel neuroprotective strategy for ischemic stroke will be developed by promoting the resolution of post-ischemic inflammation.

2O2-02 Prothymosin-alpha implicates microglial TLR4 for the prevention of ischemic damages in retina

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Toll-like receptor（TLR4）is one of the well-discussed components in neurobiology owing to its dual roles, harmful and beneficial, in ischemia. TLR4 generally contributes to ischemic damages in the central nervous system including brain and retina, whereas the ischemic- or lipopolysaccharide-preconditioning provides TLR4-mediated neuroprotection against severe ischemia. Prothymosin-alpha, a nuclear protein, is implicated in multiple functions including the protection of brain and retina from ischemic damages. Although prothymosin-alpha contributes to TLR4-mediated immunopotentiation against virus, the beneficial effects of prothymosin-alpha-TLR4 signaling against ischemia remain to be elucidated. In the present study, preconditioning treatment with prothymosin-alpha 48 h before retinal ischemia prevented the cellular damages estimated by histology and immunohistochemical analyses, and functional deficits of retina evaluated by electroretinography. Prothymosin-alpha preconditioning prevented the ischemia-induced loss of ganglion, bipolar and photoreceptor cells, but not amacrine cells. Prothymosin-alpha treatment in the absence of ischemia caused the mild activation, proliferation and migration of retinal microglia, whereas the ischemia-induced microglia activation was inhibited by prothymosin-alpha preconditioning. All of these preventive actions by prothymosin-alpha preconditioning against ischemia were abolished in TLR4 knock-out mice, and by pretreatments with anti-TLR4 antibodies or minocycline, a microglial inhibitor, which themselves had no effects on the ischemia-induced damages or microglia activation. Taken together, the present study suggested that TLR4 mediates prothymosin-alpha preconditioning-induced prevention through microglia in the retinal ischemia model.

2O2-03 Essential role for STAT3-dependent reactive astrocytes in maintenance of chronic itch

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Chronic itch is a debilitating symptom of skin diseases, such as atopic and contact dermatitis. Recent studies have revealed neuronal pathways selective for itch, but the mechanisms by which itch turns into a pathological chronic state are poorly understood. Using mouse models of atopic and contact dermatitis, we demonstrate a long-term reactive state of astrocytes in the dorsal horn of the spinal segments corresponding to the lesioned, itchy skin. We further found that STAT3 was selectively activated in dorsal horn astrocytes and that conditional disruption of astrocytic STAT3 activation prevented reactive astrocytes and chronic itch without affecting acute physiological itch. Pharmacological inhibition of spinal STAT3 alleviated fully developed chronic itch. Moreover, atopic dermatitis mice exhibited a striking enhancement of scratching evoked by intrathecal GRP, an itch-inducing neuropeptide, and this phenotype was normalized by suppressing astrocytic STAT3. Our findings indicate that STAT3-mediated reactive astrocytes in the spinal dorsal horn are necessary for the maintenance for chronic itch by inducing spinal sensitization of itch, providing a previously unrecognized target for treating chronic itch.

2O2-04 Translational research of chronic pain patients using human blood-induced microglia-like（iMG）cells

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Backgrounds：
Chronic pain is known to be one of the most intractable diseases with frequent and various physical symptoms. Unremitting pain restricts activities of daily live, and these situations tend to affect psychosocial conditions, and may easily induce various psychiatric conditions such as depression and anxiety. The underlying biological mechanisms of chronic pain have not been well clarified, while recent rodent studies using the models of chronic pain have suggested the abnormalities of microglia, immune cells in the CNS. Dynamic actions of microglia in living humans have not been clarified due to a lack of studies dealing with in situ microglia. Recently, we developed a novel technique to induce microglia-like（iMG）cells from human peripheral blood（Ohgidani et al. Sci Rep 2014）.
Methods：
Herein, we compared the responses of iMG cells against external stimuli between healthy controls（HC：n＝10）and chronic pain patients（CPP：n＝14）.
Results：
Basal mRNA expression level of TNF-α was significantly lower in CPP-iMG cells compared to HC-iMG cells. Interestingly, TNF-α expression after ATP stimulus was significantly higher in CPP-iMG cells compared to HC-iMG cells.
Discussion：
These data have suggested that microglial response against external stimulus could be supersensitive in CPP compared to HC. TNF-α from microglia may be a key player in the pathophysiology of chronic pain.

2O3-01 Entry of circulating molecules is restricted by alternative barrier in sensory circumventricular organs of adult mouse brain

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Fenestrated capillaries of the sensory circumventricular organs (CVOs), including the organum vasculosum of the lamina terminalis, the subfornical organ, and the area postrema, lack completeness of the blood–brain barrier (BBB) to sense a variety of blood–derivedmolecules and convey the information into other brain regions. In the present study, we investigated the restriction of parenchymal entry of circulating molecules in the sensory CVOs. Previous and present tracer assays revealed that molecules more than or equal to molecular weight (MW) 10,000 stayed in the perivascular space between endothelial and parenchymal basement membranes. On the other hand, low MW tracers such as fluorescein isothiocyanate (MW：389) entered into parenchyma but did not pass beyond the area of dense astrocytic network in the lateral part of the sensory CVOs. Immunoreactivity of tight junction proteins of claudin-1, occludin, and zonula occludens-1 appeared at the parenchyma of the sensory CVOs, suggesting that parenchymal cells, probably astrocytes express tight junction proteins and have barrier functions, especially for low MW molecules. The present study demonstrates that high MW tracer is trapped in perivascular space and diffusion of low MW tracer into parenchyma is limited, indicating that there are alternative barrier that protect neurons or adjacent brain area from toxic molecules in the sensory CVOs.

2O3-02 GlcNAc6ST-1 regulates sulfation of N-glycans and myelination in the peripheral nervous system.

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Myelin is a multilamellar, tightly compacted membrane that surrounds axons in the peripheral nervous system (PNS) and central nervous system (CNS). Because glycoproteins are prominent components of plasma membranes, a growing number of glycoproteins have been identified and characterized in myelin. In this study, we found that PNS myelin had many anionic N-glycans, especially sulfated N-glycans, harbored on glycoproteins in pigs and mice at a much higher rate than CNS myelin. Major sulfated N-glycans in porcine and mouse PNS myelin were identified. The sulfation at the 6-O-GlcNAc position on glycoproteins was highly conserved in PNS myelin between these species. P0 protein, the most abundant glycoprotein involved in PNS myelin compaction, had 6-O-sulfated N-glycans abundantly. Mice deficient in N-Acetylglucosamine 6-O-Sulfotransferase-1 (GlcNAc6ST-1) were impaired in the elaboration of 6-O-sulfated N-glycans in PNS myelin. Further, GlcNAc6ST-1 deficiency in mice caused hypomyelination and axonal degeneration. Taken together, these results indicate that GlcNAc6ST-1 plays critical roles in PNS myelination through the elaboration of 6-O-sulfated N-glycans.

2O3-03 The role of myelin sheaths in the regulation of axonal homeostasis

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In vertebrates, most axons are insulated with myelin sheaths, and the action potentials that enable the rapid salutatory propagation of the nerve impulses are regenerated at the nodes of Ranvier. Myelin sheaths are not only important for the salutatory propagation, but are also involved in many aspects of neural functioning. However, little is known about the contribution of myelin sheaths to axonal homeostasis such as the regulation of Ca^{2+}. The current study examines the distribution of type 1 inositol 1,4,5-trisphosohate receptor（IP_3R1）in Purkinje axons in developmental mice and cerebroside sulfotransferase（CST；a sulfatide synthetic enzyme）deficient mice, which partially lack paranodal axo-glial junctions（PNJs）in both the CNS and PNS. IP_3R1 is a Ca^{2+} channel on the endoplasmic reticulum（ER）and is a predominant isoform in the brain among the three types of IP_3Rs. At 8 days of age before myelination, IP_3R1 was stained throughout Purkinje axons. After formation of complete PNJs, by 21 days of age IP3R1-positive areas had gradually concentrated into myelinated internodes. In CST-deficient mice, IP_3R1 formed focal small swellings in Purkinje internodal axons at 12 days of age, and the number and the size of the swellings increased with age. Although CST-deficient mice do not display any neurological symptoms until 4 to 6 weeks of age, the alteration of the distribution of IP_3R1 was already observed when compact myelin formed. Contrary to in the cerebellum, in the sciatic nerves, the distribution of vesicular ER at nodes of Ranvier differed between CST mutant and wild control mice. These results suggest that the state of myelin sheaths plays a role in the distribution of IP_3R1 and ER in axons and in the regulation of axonal homeostasis.

2O3-04 Upregulation of TN-C and GFAP in reactive astrocytes in injured brain and in primary culture is dependent on aquaporin-4 expression

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We have previously reported that one of the main water channel family, aquaporin 4（AQP4）is exclusively expressed in the endofoot of astrocytes in the brain, and its expression is upregulated after the stab wound to mouse brains or injection of MeHg in common marmosets. Moreover, glial activation was induced by the stab wound injury enhanced by a neuroimmunological function of AQP4 involving osteopontin, which is an inflammatory cytokine inducer. It is already reported that expression of glial fibrillary acidic protein（GFAP）and tenascin-C（TN-C）is prominently upregulated in reactive astrocytes around injury site of the brain by us and other researchers, however the functional roles of these molecules are poorly known. Since AQP4 is highly expressed not only at the membrane of endofoot of astrocyte but also in the cytoplasm of activated astrocytes, we analyzed the functional correlation among GFAP, TN-C and AQP4 using wild type（WT）and AQP4-deficient mice（AQP4/KO）. By the immunohistochemistry and Western blot analysis, high levels of GFAP and TN-C expression were observed in activated astrocytes in WT mice brain；however, insignificant in AQP4/KO mice. Furthermore, lipopolysaccharide（LPS）stimulation activated the primary culture of astrocytes and upregulated GFAP and TN-C expression in the cells from WT mice, while it was slightly upregulated in the cells from AQP4/KO mice. Moreover, mRNA expression level of inflammatory cytokines was examined in primary culture of astrocytes or microglial cells treated with or without LPS, and found that inflammatory cytokines were upregulated in the cells from WT mice, while modest increases were observed in the cells from AQP4/KO mice. Here, we propose that upregulation of GFAP and TN-C in reactive astrocytes induced by stab wound in mouse brain and LPS-stimulated primary culture of astrocytes is dependent on upregulation of AQP4 expression.

2O3-05 Mechanism of process tip localization of astrocytic glutamate transporters

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Following synaptic activation, excitatory neurotransmitter glutamate released to synapses must be removed to terminate the signal and to protect neurons from excitotoxicity. Astrocytes are mostly responsible for the clearance of glutamates. They extend thousands of thin cellular processes among the networks of neurons to approach synapses for this purpose.In this study, we found that the trimeric transmembrane transporter domain of glutamate transporters has a property to localize to the tips of filopodia, while their N– and C–terminal cytoplasmic tails are not required. A transporter domain fragment of a neutral amino acid transporter ASCT1, another trimeric transporter family member, similarly localized to the filopodia tips. Neither transporter activity nor astrocyte specific protein was required for this filopodia tip localization. We also found that the transporter core within filopodia tips strengthens the attachment of filopodia to external substrates, thereby stabilizing the filopodia. The process tip localization of glutamate transporter is hyaluronan dependent. However, CD44, a representative hyaluronan receptor, was not required. Instead, hyaluronan synthase showed hyaluronan dependent interaction with glutamate transporters.

2O3-06 Calcium imaging method for the visualization of subtle and local activity of astrocytes in intact brain

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Astrocytes generate dynamic changes in the intracellular Ca^{2+} level（Ca^{2+} signals）that are thought to regulate their function. In vivo analysis of Ca^{2+} signals with high spatiotemporal resolution may be instrumental in unveiling enigmatic functions of astrocytes. Here we report a method for in vivo astrocytic Ca^{2+} imaging using transgenic mice expressing the ultrasensitive ratiometric Ca^{2+} indicator YC–Nano50 in astrocytes. Using the method, we succeeded in detecting a previously unidentified pattern of spontaneous Ca^{2+} signals（Ca^{2+} twinkles）, which occur predominantly in the fine processes but not the cell body. Upon sensory stimulation, astrocytes initially responded with Ca^{2+} signals at the fine processes, and the Ca^{2+} signal subsequently propagated to the cell body. Ca^{2+} twinkles and evoked Ca^{2+} signals were partially and fully dependent on the Ca^{2+} release via the type 2 IP_3 receptor, respectively. These results suggest that astrocytic fine processes function as a high–sensitivity detector of neuronal activities, and indicate the importance of intracellular Ca^{2+} stores in the regulation of astrocytic functions. Thus, the current method provides a useful tool to uncover the functions of astrocytes in the intact brain.

3O1-01 Molecular mechanism of monoamine deficiency in the mouse lacking an enzyme for recycling of tetrahydrobiopterin

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Quinonoid dihydropteridine reductase（QDPR）regenerates tetrahydrobiopterin（BH4）, which is a cofactor for monoamine synthesis and phenylalanine metabolism. Patients who have a genetic mutation in the *QDPR* gene develop hyperphenylalaninemia and severe neurological symptoms including dystonia, convulsion, and hyperthermia due to the depletion of catecholamines and serotonin. We examined a *Qdpr*-deficient mouse model in order to reveal the physiological significance of the BH4 recycling reaction. The *Qdpr*-deficient mice showed mild hyperphenylalaninemia and monoamine deficiency, although the BH4 contents in the liver and brains were not decreased. The blood phenylalanine levels were dropped off by the intraperitoneal injection of BH4, indicating the lack of BH4 in the liver. The serotonin contents in the brain were slightly increased after the administration of BH4, whereas the dopamine and noradrenaline contents in the brain were unchanged. Then, we treated the *Qdpr*-deficient mice with a phenylalanine-restricted diet. The brain monoamine levels were restored by the diet. Because the high concentration of phenylalanine can competitively inhibit transportation of tyrosine and tryptophan into the brain through L-type amino acid transporter 1, and inhibit the activity of tyrosine hydroxylase and tryptophan hydroxylase, the monoamine deficiency in the *Qdpr*-deficient mice were thought to be caused by hyperphenylalaninemia, not but a deficiency of BH4. The present study suggested that the monoaminergic neurons in the brain have ability to synthesize monoamine-neurotransmitters without any help of Qdpr.

3O1-02 Sex difference in hippocampal synapses and hormones

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Sex difference in the brain is a very attractive problem. For example, the hypothalamus, the brain region responsible for reproductive behavior, exhibits a clear sex difference in the size of nerve nucleus and the number of neurons.
In contrast, the hippocampus, a center for learning and memory, does not have sex difference at the anatomical level including the volume and the number of neurons. Nevertheless, the significant sex difference in the performance of hippocampus-dependent task such as spatial memory using Morris water maze or radial arm maze task exists.
We hypothesized that the sex difference in the hippocampal structure exists at more subtle level, that is, synaptic level. A novel software, Spiso-3D, which we developed, allowed us to reveal the sex difference in the density of spines（post synaptic region）and the fluctuation of spines in female with a period of 4 days（estrous cycle）.
What generates the sex difference in the hippocampal synapses? So far, sex difference in the hippocampus has been attributed to the level of sex hormones in the blood. We revealed, however, that the hippocampal neurons themselves synthesized sex hormones including estradiol（E2）, testosterone（T）, dihydrotestosterone（DHT）and progesterone（PROG）in both sexes. The levels of sex hormones in hippocampus were higher than that in plasma, suggesting that hippocampal sex hormones had more impact to hippocampal functions than circulating ones did.
The levels of sex hormones exhibited a clear sex difference, and in female, especially, fluctuated across estrous cycle. Surprisingly, no sex difference in the mRNA level and the localization pattern of steroidogenic enzymes and receptors was observed in the hippocampus. The estrous cycle-dependent fluctuation of the spine density in female rat hippocampus had a good correlation with the cyclic fluctuation of hippocampal levels of E2 and PROG.
This clear sex difference in hormonal profile in hippocampus may generate the sex difference in the hippocampal structure at more subtle level, that is, synaptic level, resulting in the sex difference in the performance of hippocampus-dependent task.

3O1-03 Reduced axonal localization of a Caps2 splice variant impairs axonal release of BDNF and causes autistic-like behavior in mice

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Ca2＋-dependent activator protein for secretion 2（CAPS2 or CADPS2）potently promotes the release of brain-derived neurotrophic factor（BDNF）. A rare splicing form of CAPS2 with deletion of exon3（dex3）was identified to be overrepresented in some patients with autism. Here, we generated Caps2-dex3 mice and verified a severe impairment in axonal Caps2-dex3 localization, contributing to a reduction in BDNF release from axons. In addition, circuit connectivity, measured by spine and interneuron density, was diminished globally. The collective effect of reduced axonal BDNF release during development was a striking and selective repertoire of deficits in social- and anxiety-related behaviors. Together, these findings represent the first mouse model of a molecular mechanism linking BDNF-mediated coordination of brain development to autism-related behaviors and patient genotype.

3O1-04 Rational design of a novel high-affinity, ultrafast, red calcium indicator R-CaMP2

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Fluorescent Ca^{2+} reporters are widely used as readouts of neuronal activities. Here, we designed R-CaMP2, a novel high-affinity red genetically encoded calcium indicator（GECI）with a Kd for Ca^{2+} < 70 nM, and with a Hill coefficient near 1. Use of the calmodulin-binding sequence of CaMKK-α/β in lieu of a M13 sequence resulted in three fold faster kinetics than R-CaMP1.07 in rise and decay time of Ca^{2+} transients. These features allowed to resolve single action potential（AP）and fast AP trains up to near 20-40 Hz in acute cortical slices. *In vivo* imaging of the barrel cortex layer 2/3 neurons revealed reliable recording of single APs in R-CaMP 2-expressing neurons, while synaptic Ca^{2+} transients were robustly detected in individual dendritic spines with similar efficacy as previously reported ultrasensitive green GECIs. R-CaMP2 exhibits a linear relationship between AP trains and fluorescence dynamics *in vivo*. Combining green and red GECIs, we successfully achieved dual-color monitoring of neuronal activities of distinct cell types, in the mouse cortex and in free-moving *C. elegans*. Together, R/G-CaMP imaging using R-CaMP2 provides a powerful means to interrogate orthogonal and hierarchical active ensembles, thus significantly enhancing our current capacity for functional mapping of neuronal circuits *in vivo*.

3O2-01 Impaired late endosomal/lysosomal lipid trafficking attenuates oligodendrocyte differentiation and myelination in Niemann-Pick disease type C

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Niemann-Pick disease type C (NPC) is a childhood-onset autosomal recessive disorder exhibiting progressive neurodegeneration and myelin defects caused by mutations in either the NPC1 or the NPC2 genes. These mutations affect the late endosomal/lysosomal (LE/Lys) lipid trafficking, resulting in the abnormal intracellular accumulation of unesterified cholesterol and glycosphingolipids ; however, the underlying pathophysiology is still poorly understood. Here we examined impact of impaired LE/Lys lipid trafficking on the differentiation of oligodendrocyte (OL), a myelin-forming cell which critically regulates CNS myelination, *in vivo* and *in vitro*. In the developing NPC1-deficient (NPC1-/-) mouse brain, extensive dysmyelination, and abnormal LE/Lys cholesterol accumulation in OLs and oligodendrocyte precursor cells (OPCs) were observed from early postnatal age, as well as in neurons. In primary cultures of OPCs, treatment of U 18666A, a type-II amphiphile which causes intralysosomal accumulation of cholesterol by inhibiting LE/Lys lipid trafficking and NPC-like pathology, affected OL differentiation. Collectively, our results suggest that uptake and intercellular transport of cholesterol plays a critical role for OL differentiation and myelination in the CNS.

3O2-02 Prostaglandin F2α FP receptor inhibitor reduce demyelination and motor dysfunction in a cuprizone-induced multiple sclerosis mouse model

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Previously, we have demonstrated that prostamide/PGF synthase, catalyzes the reduction of prostaglandin (PG) H2 to PGF2α, is constitutively expressed in myelin sheaths and cultured oligodendrocytes, suggesting that PGF2α has functional significance in myelin-forming oligodendrocytes. To investigate the effects of PGF2α/FP receptor signaling on demyelination, we administrated FP receptor agonist and antagonist to cuprizone-exposed mice, a model of multiple sclerosis. Mice were fed a diet containing 0.2% cuprizone for 5 weeks, which induces severe demyelination, glial activation, proinflammatory cytokine expression, and motor dysfunction. Administration of the FP receptor antagonist AL-8810 attenuated cuprizone-induced demyelination, and glial activation in the corpus callosum, and also improved the motor function. These data suggest that during cuprizone-induced demyelination, PGF2α/FP receptor signaling contributes to glial activation, neuroinflammation, and demyelination, resulting in motor dysfunction. Thus, FP receptor inhibition may be a useful symptomatic treatment in multiple sclerosis.

3O2-03 2-Carba-cyclic phosphatidic acid, a chemically synthesized cyclic phosphatidic acid derivative, is a novel drug candidate for multiple sclerosis

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Multiple sclerosis（MS）is a chronic demyelinating disease of the central nervous system characterized by recurrent and progressive demyelination／remyelination cycles, neuroinflammation, oligodendrocyte loss, and axonal pathology. The cuprizone model of demyelination is characterized by apoptotic death of mature oligodendrocytes, and is accompanied by neuroinflammation and motor dysfunction. Cyclic phosphatidic acid（cPA）has a unique structure consisting of a cyclic phosphate ring at the sn-2 and sn-3 positions of its glycerol backbone. cPA elicits a neurotrophin-like action and protects hippocampal neurons from ischemia-induced delayed neuronal death. We previously reported that the administration of cPA reduced cuprizone-induced demyelination. In this study, we investigated the effects of 2ccPA, a chemically synthesized cPA derivative, on the cuprizone-induced demyelination. Mice were fed a diet containing 0.2% cuprizone for 5 weeks, which induces severe demyelination, glial activation, and motor dysfunction. Simultaneous administration of 2ccPA effectively attenuated cuprizone-induced demyelination, glial activation, and motor dysfunction. These data indicate that 2ccPA may be a useful treatment to reduce the extent of demyelination and the severity of motor dysfunction in multiple sclerosis. 2ccPA is a potential lead compound in the development of drugs for multiple sclerosis.

3O3-01 Cell-permeable p38 MAP kinase promotes migration of adult neural stem/progenitor cells

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Endogenous neural stem/progenitor cells（NPCs）can migrate toward sites of injury, but the migration activity of NPCs is insufficient to regenerate damaged brain tissue. In this study, we showed that p38 MAP kinase（p38）is expressed in adult NPCs. Inhibitor experiments using the compound SB203580 revealed that endogenous p38 participates in NPC migration. To enhance NPC migration, we prepared a cell-permeable dominant-active version of p38（PTD-DA）consisting of the HIV protein transduction domain（PTD）fused to the N-terminus of p38. Treatment with PTD-DA protein significantly promoted the random migration of adult NPCs without disturbing cell survival or differentiation；this effect depended on the cell permeability and kinase activity of the fusion protein. These findings indicate that PTD-DA is a novel and useful tool for unraveling the roles of p38, and that PTD-DA provides a reasonable approach for regenerating injured brain by enhancing NPC migration.

3O3-02 Netrin-5 is highly expressed in neurogenic regions of the adult brain

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Mammalian netrin family proteins are involved in targeting of axons, neuronal migration, and angiogenesis and act as repulsive and attractive guidance molecules. Netrin-5 is a new member of the netrin family with homology to the C345C domain of netrin-1. Unlike other netrin proteins, murine netrin-5 consists of two EGF motifs of the laminin V domain（LE）and the C345C domain, but lacks the N-terminal laminin VI domain and one of the three LE motifs. We generated a specific antibody against netrin-5 to investigate its expression pattern in the rodent adult brain. Strong netrin-5 expression was observed in the olfactory bulb, rostral migrate stream（RMS）, the subventricular zone（SVZ）, and the subgranular zone（SGZ）of the dentate gyrus in the hippocampus, where neurogenesis occurs in the adult brain. In the SVZ and RMS, netrin-5 expression was observed in Mash1-positive transit-amplifying cells and in Doublecortin（DCX）-positive neuroblasts, but not in GFAP-positive astrocytes. In the olfactory bulb, netrin-5 expression was maintained in neuroblasts, but its level was decreased in NeuN-positive mature neurons. In the hippocampal SGZ, netrin-5 was observed in Mash1-positive cells and in DCX-positive neuroblasts, but not in GFAP-positive astrocytes, suggesting that netrin-5 expression occurs from type 2a to type 3 cells. These data suggest that netrin-5 is produced by both transit-amplifying cells and neuroblasts to control neurogenesis in the adult brain.

3O3-03 The role of Cdk5 in cell cycle arrest and neural differentiation

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Cell cycle arrest occurs in advance of neural differentiation, however their coordination is not fully elucidated. Cyclin-dependent kinase (Cdk) is a Ser/Thr protein kinase family regulating cell cycle progression. Cdk5, an atypical Cdk, is solely activated in postmitotic neurons, while cell cycle machineries including other Cdks are inactivated in neural differentiation, suggesting that Cdk5 may have inhibitory activity for cell cycle progression. Here we examined the role of Cdk5 in cell cycle arrest and neural differentiation.Firstly, we selected SH-SY5Y human neuroblastoma cell, which is frequently used and differentiated by stimulation with retinoic acid (RA), as model system. PI3K/Akt pathway is important for regulating cell growth and differentiation and also known to be activated in SH-SY5Y cells by RA stimulation. When SH-SY5Y cells were stimulated with RA, the up-regulation of p35, an essential activator for Cdk5, and Akt activation were observed. Next, we examined the expression of inhibitor of differentiation genes (IDs), which are transcriptional suppressors for differentiation and down-regulated in the early step of neural differentiation. Stimulation by RA induced down-regulation of expression of Id1 and Id3 in transcriptional level in SH-SY5Y cells. Overexpression of p35 also induced both activation of Akt and down-regulation of Id1 without neurite outgrowth as was observed by RA stimulation. These results suggest that Cdk5 may induce early events in neural differentiation. Now, we are examining the pathway from Cdk5 activation to Akt activation or Ids down-regulation in both SH-SY5Y cells and neurons.

3O3-04 NRG1-ErbB4 signaling promotes generation of neurons from neural progenitor cells in the developing brain.

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Post-mitotic neurons are generated from neural progenitor cells (NPCs) at the expense of their proliferation. Molecular and cellular mechanisms that regulate neuron production temporally and spatially should impact on the size and shape of the brain. While transcription factors such as neurogenin1 (neurog1) and neurod govern progression of neurogenesis as cell-intrinsic mechanisms, recent studies show regulatory roles of several cell-extrinsic or intercellular signaling molecules including Notch, FGF and Wnt in production of neurons/NPCs from neural stem cells/radial glial cells (NSCs/RGCs) in the ventricular zone (VZ). However, it remains elusive how production of post-mitotic neurons from NPCs is regulated in the sub-ventricular zone (SVZ). Here we show that newborn neurons accumulate in the basal-to-apical direction in the optic tectum (OT) of zebrafish embryos. While NPCs are amplified by mitoses in the apical VZ, neurons are exclusively produced through mitoses of NPCs in the sub-basal zone(SBZ), later in the SVZ, and accumulate apically onto older neurons. This neurogenesis depends on Neuregulin 1 type II (NRG1-II)-ErbB signaling. Treatment with an ErbB inhibitor, AG1478 impairs mitoses in the SVZ of the OT. Removal of AG1478 resumes sub-ventricular mitoses without precedent mitoses in the apical VZ prior to basal-to-apical accumulation of neurons, suggesting critical roles of ErbB signaling in mitoses for post-mitotic neuron production. Knockdown of NRG1-II impairs both mitoses in the SBZ/SVZ and VZ. Injection of soluble human NRG1 into the developing brain ameliorates neurogenesis of NRG1-II-knockdown embryos, suggesting a conserved role of NRG1 as a cell-extrinsic signal. From these results, we propose that NRG1-ErbB signaling stimulates cell divisions generating neurons from NPCs in the developing vertebrate brain.

3O3-05 Involvement of novel mammalian transmembrane ubiquitin ligases in neuronal differentiation and function

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The ubiquitin–proteasome system (UPS) is an essential process that regulates protein homeostasis, and is involved in the regulation of various cellular processes including cell proliferation, differentiation and survival. To date, it has been suggested that dysregulations in this system are implicated in the pathogenesis of neurodegenerative diseases, cancer and immune system disorders. In the UPS, ubiquitin ligases play an important role in the final step in the ubiquitination cascade for protein degradation. It has been shown that ubiquitin ligases are key molecules for neuronal differentiation and function. Recently, we identified 44 types of novel mammalian ubiquitin ligases which have the RING finger domain and the transmembrane domains. In this study, we performed gene expression profiling using human and mouse tissues and showed that several types of the ubiquitin ligases were predominantly expressed in the embryonic brain, compared with the adult brain. To identify novel ubiquitin ligases which are important for neuronal differentiation, maturation and function, we examined expression levels of ubiquitin ligases abundant in the brain during retinoic acid–induced neural differentiation of mouse embryonal carcinoma P19 cells. As a result, 3 types of ubiquitin ligases, RNF150, RNF 152 and RNF182, were markedly up–regulated during neuronal differentiation. These genes have different expression patterns：RNF150 expression was highest at the neurosphere stage；RNF152 and RNF182 were predominantly expressed at the neuronal stages. These results suggested that these ubiquitin ligases may be involved in the regulation of neuronal differentiation and function, and provided further insights into mechanisms of brain development regulated by protein ubiquitination.

3O3-06 Neurogenesis from dying neurons by deregulated DNA repair pathway activation

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The cell cycle exit of progenitor cells must be temporally coordinated with the initiation of neuronal differentiation to control the number and ratio of different neuronal subtypes in the CNS. Once progenitor cells exit the cell cycle, their daughter neurons enter the post–mitotic G0 phase for terminal differentiation and lose their proliferative potential. This inability of differentiated neurons to undergo proliferation is one of the major reasons brain tissue cannot regenerate following injury. When mature neurons re–enter the S phase in pathological situations such as neurodegeneration, they undergo cell death. Thus, the regulatory networks that drive cell proliferation and maintain neuronal differentiation are tightly controlled. Tumor suppressor gene Rb and its family members (p107 and p130) are essential for regulating cell cycle in neuronal progenitors and neurons. Neuronal progenitor cells which lack all Rb family members initiate differentiation without exiting cell cycle and these proliferating neurons develop tumors in some cases. In contrast, when differentiating neurons lose all Rb family members after cell cycle exit, they undergo S phase but do not divide. We recently found the molecular mechanism by which differentiating neurons are tightly protected from cell division even in the absence of Rb family members. In this oral presentation, we show these data and discuss how neurons maintain post–mitotic and non–dividing feature.

1P-01 The 5-HT3 receptor is essential for exercise-induced hippocampal neurogenesis and antidepressant effects

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Exercise has a variety of beneficial effects on brain structure and function, such as hippocampal neurogenesis, mood and memory. Previous studies have shown that exercise enhances hippocampal neurogenesis, induces antidepressant effects, and improves learning behavior. Brain serotonin（5-hydroxytryptamine, 5-HT）levels increase following exercise, and the 5-HT system has been suggested to play an important role in these exercise-induced neuronal effects. However, the precise mechanism remains unclear. In this study, analysis of the 5-HT type 3A receptor subunit-deficient（htr 3a-/-）mice revealed that lack of the 5-HT type 3（5-HT3）receptor resulted in loss of exercise-induced hippocampal neurogenesis and antidepressant effects, but not of learning enhancement. Furthermore, stimulation of the 5-HT3 receptor promoted neurogenesis. These findings demonstrate that the 5-HT3 receptor is the critical target of 5-HT action in the brain following exercise, and is indispensable for hippocampal neurogenesis and antidepressant effects induced by exercise. This is the first report of a pivotal 5-HT receptor subtype that plays a fundamental role in exercise-induced morphological changes and psychological effects.

1P-02 Involvement of calcium-activated potassium channel in the inhibitory network oscillation in the rat basolateral amygdala

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Basolateral amygdaloid complex（BLA）is deeply involved in emotional processing and is sensitive to chronic stress. Its abnormality is related to several psychiatric disorders. In the BLA, sensory information from the cortex and the thalamus are evaluated in terms of emotional valence and these signals are transmitted to central nucleus of amygdala. Previously, we showed that projection neurons in BLA receive rhythmic inhibitory inputs which are evoked by synchronous firings of interneurons. Such neurons were mostly distributed in ventral part of BLA, and the inhibitory oscillation requires the glutamatergic transmission within BLA, suggesting that local network activities are essential for this phenomenon. Moreover, we demonstrated that gap-junctions, several receptors and ion-channels including the low-threshold Ca channel are involved in the generation and/or maintenance of the oscillation, also suggesting the potential role of the calcium-activated potassium channels. In fact, SK channel is involved in amygdala function, suggested by the study that an injection of its activator contributed to recovery from electrophysiological and behavioral alterations by chronic restraint stress. In the present study, we examined the effect of modulators of the calcium-activated potassium channel on inhibitory network oscillation in rat BLA. In BLA projection neurons in vitro, the power of the low-frequency（0.1-3 Hz）oscillation was enhanced by an SK channel blocker, apamin, and was attenuated by an SK channel activator, 1-EBIO, respectively. On the other hand, the oscillation power was insensitive to a BK channel blocker, iberiotoxin. These results suggest that the slow network oscillation is regulated by SK channel activity.

1P-03 Regulation of IP3 Receptor by Transglutaminase

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Reversible and repetitive structural changes are essential for ligand-gated ion channels to mediate biological signaling. Here we show a new mode of posttranslational modification that chronically controls the structural changes in the ligand-gated ion channels. The inositol 1,4,5-trisphosphate receptor (IP3R) in the endoplasmic reticulum assembles ligand-gated ion channels that mediate calcium signaling. IP3Rs are allosteric proteins comprising four subunits that form an ion channel activated by binding of IP3 at a distance. Defective allostery in IP3R is considered crucial to cellular dysfunction, but the specific mechanism remains unknown. We demonstrate that a pleiotropic enzyme transglutaminase type 2 (TG2) targets the allosteric coupling domain of IP3R type 1 (IP3R1) and negatively regulates IP3R1-mediated calcium signaling and autophagy by locking the subunit configurations. The control point of this regulation is the covalent posttranslational modification of Gln2746 residue which TG2 tethers to the adjacent subunit. Modification of Gln2746 and IP3R1 function was observed in Huntington's disease models, suggesting a pathological role of this modification in the neurodegenerative disease. Our study reveals that cellular signaling is regulated by a new mode of posttranslational modification that chronically and enzymatically blocks allosteric changes in the ligand-gated channels which relate to disease states. This is the first demonstration of transglutaminase-catalyzed posttranslational modification in ligand-gated ion channel allostery and provides a new framework for enzymatic regulation of ligand-gated ion channels.

1P-04 Roles of acid-sensing ion channel-1a in hippocampal adult neurogenesis

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ASIC1a (acid-sensing ion channel-1a) is a neuronal acid-activated cation channel located in the postsynaptic membrane. The channel receives synaptic protons, contributing to synaptic plasticity, learning and memory. It is well known that adult neurogenesis is enhanced after ischemic brain injury accompanied with local tissue acidosis. However, the relationship between adult neurogenesis and ASIC1a remains to be elucidated. To examine the potential roles of ASIC1a in mouse adult hippocampal neurogenesis, we used an onco-retrovirus-mediated approach to genetically label and manipulate newborn dentate granule cells (DGCs) in vivo. Three-dimensional reconstruction of confocal Z-stucks was applied to morphologically characterize the dendritic arborization and spine formation of ASIC1a-deficient newborn DGCs labeled with EGFP. We found that newborn DGCs of ASIC1a KO mouse at 28dpi (days post injection) had significantly shorter dendrites and smaller spine heads. Furthermore, retroviral shRNA knockdown of ASIC1a in newborn DGCs induced the same phenotype as the ASIC1a KO mouse. Our results indicate that ASIC1a is necessary for dendritic development and synaptic organization of mouse hippocampal newborn neurons.

1P-05 Study about the mechanisms of DHA-induced enhancement of glial excitatory amino-acid transporter EAAT2 function

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EAAT2 is a predominant astrocytic L-Glutamate（L-Glu）transporter in the forebrain. EAAT2 removes L-Glu from synaptic cleft and maintain efficient synaptic transmissions. Recent studies have clarified that poly-unsaturated fatty acids（PUFAs）regulate the functions of the membrane proteins of neural cells. Omega-3 fatty acid docosahexanoic acid（DHA；C22：6）is a major constituent of astrocyte membrane phospholipids and is released after L-Glu stimulation. However, its effects on EAAT2 is largely unknown. In this study, we investigated the effects of DHA on EAAT2 currents using two-electrode voltage clamp technique in Xenopus oocytes expressing EAAT2 isoform 1. Exogenously-applied DHA（30-300 microM）increased the amplitude of L-Glu-induced currents of EAAT2 but not of EAAT 1 in a dose-dependent manner and these effects were reversible. Exogenously-applied DHA-CoA, a membrane impermeable DHA-analog, increased EAAT2 currents, suggesting that DHA regulate EAAT2 from extracellular side of membrane. PUFAs are also reported to regulate membrane protein functions by changing the elasticity of the lipid bilayer. However, transient application of Triton X-100（200 microM）, which increases membrane fluidity, had little effects on EAAT2 currents, suggesting that the DHA-induced enhancement of EAAT2 currents is independent of the membrane elasticity. The enhancement of EAAT2 was not prevented by the inhibitors of cyclooxygenase or lipoxygenase, suggesting that DHA may exert the effects through direct interaction with EAAT2 not through signal transduction pathways. DHA has a carboxyl group that is protonated（uncharged）, or deprotonated（negatively charged）in a pH dependent manner. When the extracellular pH increased, the enhancement of EAATs by DHA was increased, suggesting that the charge of the DHA carboxyl group is important for the effects of DHA. In accordance with this, DHA-methyl ester（200 microM）, an uncharged DHA-analog, had little effects on EAAT2 currents. Currently, we are identifying which structure or domain of EAAT2 is important for the enhancement of EAAT2 currents using various chimeras of EAAT2 and EAAT1.

1P-06 Phosphorylation of serotonin 1A receptor（5 HT1AR）by Cdk5 activity.

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Mental disorders including depression are one of urgent issues to be addressed. To prevent the onset and develop the treatment, it is important to understand a mechanism of diseases at molecular level. It is generally considered that dysregulation of neuronal activities is an underlying mechanism. Cyclin-dependent kinase 5（Cdk5）is a neuron-specific Ser/Thr kinase, which is activated by regulatory subunit p35 or p 39. Recent reports suggest its function in synaptic activity and association with anxiety and depression. I investigated here a role of Cdk5-p35 in mental disorders by focusing on serotonin 1A receptor（5HTlAR）. 5HTlAR is expressed highly in central nervous system and is thought to be involved in psychiatric activity. 5HT1AR is a seven transmembrane G-protein-coupled receptor, which binds to Gi or Go of trimeric G proteins to inhibit adenylyl cyclase or open K + channels in neurons. Dysfunction of the serotonin signal is considered as the cause of many mental diseases. So, 5HT1 AR has been a target of drug development for anxiety and depression. It is not fully known, however, how 5HTlAR is regulated. There are three possible Cdk5 phosphorylation sites in 5HT1AR. We examined phosphorylation of 5HT1AR by Cdk5-p35. Expression level of 5HT1AR was decreased by co-transfection with Cdk5-p35, but not with kinase negative Cdk5-p35, in COS-7 cells. 5HT1AR was indeed phosphorylated by Cdk5-p35. We constructed non-phosphorylatable Ala mutants, T149A, S245A, and T314A, and examined their phosphorylation. Thr314 was identified as a phosphorylation site in 5HT1AR. These results suggest that Cdk5 controls the serotonin signal through phosphorylation-dependent down regulation of 5HT1AR.

1P-07 Melanin-concentrating hormone-mediated signaling induces reduction of the primary cilium length

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Primary cilia are microtubule-based organelles present on nearly every cell in the mammalian body. The cilium has an important chemosensory function in many types of cells and ciliary dysfunction is associated with ciliopathies such as polycystic kidney disease and obesity. Although the ciliary membrane is contiguous with the plasma membrane, ciliary localization of protein is tightly regulated and only certain molecules are permitted to traffic there. Melanin-concentrating hormone（MCH）is the natural peptide ligand for two G-protein-coupled receptors（GPCR）, MCHR1 and MCHR2. The MCH-MCHR1 system has been implicated in the regulation of feeding and emotional processing in rodents. Recently, MCHR1 expression was detected in primary cilia of the central nervous system. However, the underlying function and signaling pathway via MCHR1 located in primary cilia is unclear. Here we show that treatment of MCH significantly reduces cilia length in hTERT-RPE1 epithelial cells（hRPE1）transfected with Flag-MCHR1. Quantitative analysis indicated that the rate of MCH-induced cilia shorting progressed in time-dependent manner during the first 3 hour with an EC50 value of 1.6nM, and the process was significantly inhibited by pretreatment with Gi/o-selective inhibitor pertussis toxin. In addition, a series of receptor mutagenesis experiment showed that distinct amino acid residues in the second intracellular loop were responsible for MCH-mediated shorting of receptor-positive cilia. These data suggests that MCH-MCHR1 governs the sensitivity by controlling the length of the cell's sensory organelle. Further characterization of MCHR1 as a ciliary GPCR provides a potential molecular mechanism to link defects in cilia with obesity.

1P-08 Identification and appreciation of novel antagonists of GPR173

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G protein-coupled receptors（GPCRs）are integral membrane proteins in the cell surface, and are known to be targets of approximately 60% of the present drugs. Thus, identification of novel synthetic ligands for GPCRs, especially for orphan GPCRs of which endogenous ligands are unknown, is important for not only to our understanding of human physiology but also to the development of novel drugs. We have been developed the ligand screening system for GPCRs using receptor-Gα fusion proteins and［35S］GTPγS binding assay. One of the orphan GPCR subfamily, Super conserved Receptor Expressed in Brain（SREB）, is consisted of GPR27, GPR86, and GPR173, and specifically express in central nervous system. To examine SREB physiological functions, we performed screening of chemical compound using a RIKEN chemical library containing approximately 12,000 compounds. The binding of［35S］GTPγS to GPR173-Gsα fusion proteins expressed in Sf9 cells was measured, and resulted a few potential candidates of a GPR173 antagonist. These compounds are also antagonist for other SREBs, GPR27 and GPR 85. These results also indicated that GPR173 was possible to couple Gs type G proteins, and had significant constitutive activity. These results also indicated that GPR173 was possible to couple Gs type G proteins, and had significant constitutive activity. Then, physiological significance of these compounds on the synaptic transmission was examined by whole cell patch clamp technique. Bath application of these compounds significantly increased the amplitude of EPSC elicited in cerebellar Purkinje cells after electrical stimulation to parallel fibers. In addition, the ratio of paired pulse facilitation of the parallel fiber-mediated EPSC was significantly decreased. These results suggest that constitutive active SREBs regulate the synaptic transmission at parallel fiber to Purkinje cells by modulating the glutamate release from parallel fiber terminals.

1P-09 Functional characterization of the phosphorylation sites of rat melanin-concentrating hormone receptor 1

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Melanin-concentrating hormone（MCH）receptor 1 belongs to the rhodopsin family of G protein-coupled receptors（GPCR）. One of the main pharmacological interests of MCH-MCHR1 system resides in its ability to regulate feeding and energy homeostasis. Phosphorylation of intracellular residues is the most extensively studied post-translational modification regulating GPCR activity. However, until now, only little information concerning the role of MCHR1 phosphorylation is available. In this study, we performed a comprehensive site-directed mutagenesis to analyze the predicted phosphorylation site of the rat MCHR1 in receptor expression, signaling, internalization, and trafficking in a HEK293T cell line. We identified the phosphorylation sites responsible for internalization（S151 and S158）at the second intracellular loop of the receptor, and additional sites involved in internalization（S246 and T251）at the third intracellular loop of the receptor. Although these four amino acid residues of MCHR1 play a critical role for promoting optimal internalization, they are not essential for signal transduction in calcium mobilization. A further goal of our research will be to reveal the coordinated biochemical mechanism involving sequential and hierarchical multisite phosphorylation of the receptor.

1P-10 CAPS1 stabilizes SVs docking state in hippocampal CA3–CA1 synapses

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AbstractCalcium–dependent activator protein for secretion 1 (CAPS1) is a cytosolic protein, which associates with dense–core vesicle secretion in endocrine cells, however, their neuronal function is still largely unknown because of *Caps1* knock–out (KO) results in prenatal death. Here we show that CAPS1 stabilizes the docking state of synaptic vesicle (SV) to presynaptic active zone using forebrain specific *Caps1* conditional KO (cKO) mice. The synaptic transmission is strongly reduced and paired–pulse facilitation shows significant alteration in *Caps1* cKO so that the impairment of SV release is expected. Morphological analysis shows accumulation of SVs in presynapse without any morphological changing. Interestingly, even though SV accumulation is occurred, the percentage of presynaptic button contained docked vesicle is markedly reduced in *Caps1* cKO. Finally, SV release experiment revealed by time–lapse imaging indicates the decreased SV release in the absence of CAPS1. These data suggest that CAPS1 stabilizes SV docking state to enhance SV release.

1P-11 Conditional knockout and optogenetic study on the involvement of the secretion–related protein CAPS1 in oxytocin–associated social and maternal behavior

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Calcium–dependent activator protein for secretion 1 (CAPS 1) plays a regulatory role in dense–core vesicle (DCV) exocytosis pathway. CAPS1 is widely expressed in the mouse brain including paraventricular nucleus (PVN) and supraoptic nucleus (SON) that contain oxytocin (OXT)–producing neurons. OXT is known as a neuropeptide, which is associated with social and maternal behavior. Thus, we hypothesized that CAPS1 potentially regulates social and maternal behavior through regulating OXT secretion. In this study, we generated conditional knock–out (cKO) mice lacking *Caps 1* in OXT neurons and tried to compare WT and cKO mice by optogenetic control of PVN or SON via microinjection of adeno–associated virus vectors containing channelrhodopsin 2 (ChR2). The effect of CAPS1 deficiency in OXT neurons was analyzed in terms of activity–dependent OXT secretion, social and maternal behavior. We will show the recent progress in testing the implication of CAPS1 in OXT–related brain function and behavior.

1P-12 IRBIT suppresses CaMKII-alpha activity and contributes to catecholamine homeostasis through tyrosine hydroxylase phosphorylation

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Inositol 1,4,5-trisphosphate receptor (IP$_3$R) binding protein released with IP$_3$ (IRBIT) contributes to various physiological events (electrolyte transport, mRNA polyadenylation, and the maintenance of genomic integrity) through its interaction with multiple targets. However, little is known about the physiological role of IRBIT in the brain. In this report, we identified calcium calmodulin-dependent kinase II alpha (CaMKIIα) as an IRBIT-interacting molecule in the central nervous system. IRBIT binds to and suppresses CaMKIIα kinase activity by inhibiting the binding of calmodulin to CaMKIIα. In addition, we show that IRBIT knockout mice show elevated catecholamine levels, increased locomotor activity, and social abnormalities. The level of tyrosine hydroxylase (TH) phosphorylation by CaMKIIα, which affects TH activity, was significantly increased in the ventral tegmental area of IRBIT deficient mice. We concluded that IRBIT suppresses CaMKIIα activity and contributes to catecholamine homeostasis through TH phosphorylation.

1P-13 Generation of GAD65 knockout rats using TALEN-mediated genome editing

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GABA is the major inhibitory neurotransmitter in the adult mammalian CNS. Glutamate decarboxylase (GAD) is the rate-limiting enzyme that catalyzes the production of GABA from glutamate. There are two isoforms of GAD, GAD65 and GAD67 according to their molecular masses, and they are encoded by independent genes. GAD65 knockout mice showed an increase in susceptibility to seizures and changes in emotional behavior such as anxiety and aggression, whereas GAD67 knockout mice were shown to die of cleft palate. However, the size of the mouse is a potential limitation for some types of physiological monitoring, behavioral testing, brain mapping and repeated blood sampling. To overcome the problem, we have generated GAD65 knockout rats using TALEN genome editing. mRNAs encoding TALENs targeted to exon 1 of GAD65 were microinjected into single-cell rat embryos and transferred to pseudopregnant recipients. Three founders with monoallelic or biallelic mutations were backcrossed to wild-type rats, and then heterozygous mutants were obtained. These heterozygous rats possessed both wild-type GAD65 allele and GAD65 mutant allele containing either 8 bp, 314 bp or 490 bp deletions. Homozygous GAD65 mutant rats with 8 bp or 314 bp deletions were generated by crossing their respective heterozygous mutant rats each other. Western blot analysis demonstrated that GAD65 protein was not detected in the homozygous mutant brain. This rat model provides a new experimental tool for investigating the pathophysiology of GABAergic transmission.

1P-14 Functional characterization of oxytocin/vasopressin-like peptide in social insect, ants

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Oxytocin (OXT) and arginine-vasopressin (AVP) are neurohypophysial hormones composed of 9 amino acids and known to function as neuromodulators. OXT/AVP-like peptides are evolutionarily conserved among wide range of animals, from worms to humans. Their function is well studied in highly social mammals to regulate social behaviors. In addition, recent studies also suggest that OXT/AVP-like peptides function to regulate social behaviors such as courtship and mating in birds, fishes and some invertebrates such as molluscs and nematodes. However, the evolutionary origin of their molecular function is still unrevealed and we aim to study the neuronal function of their homologous nonapeptide, inotocin (INT) in social insects, ants. Ants exhibit sophisticated social organization within their colonies which is characterized by the reproductive caste differentiation and the division of labor. Ant colonies have a reproductive caste which consists of queens and males, and a non-reproductive caste, workers. Workers show the division of labor, that is, each worker specializes in one job such as foraging, nurturing and nest construction. They can flexibly change their jobs according to surrounding environment and colony demands. From this, we hypothesize that INT signaling would be involved in the regulation of the unique social behaviors in ants. We firstly established the *in vitro* assay system to measure the INT receptor activity by utilizing cultured cells, and confirmed that the putative INT receptor is activated by INT peptide. We also found that OXT peptide can activate the INT receptor at high dose, which suggests the similarity of INT and OXT signaling pathways. We are now testing the effect of OXT/AVP signaling agonists/antagonists in INT signaling, for the further *in vivo* study to manipulate INT signaling pathway and examine whether it is involved in the regulation of social behaviors in ants.

1P-15 Dual imaging of SVs and DCVs exocytosis

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Exocytosis of synaptic vesicles（SVs）mediates the release neurotransmitters into synaptic cleft through the sequential process of vesicle docking, priming and fusion, when the action potential arrives at the presynaptic terminals. In neuronal cells, there is another secretory vesicle, called dense-core vesicles（DCVs）that play a major role in the release of neuropeptides and peptide hormones. Very little, however, is known about the regulatory process of DCV exocytosis. To clarify the distinction between SV and DCV exocytosis regarding the underlying mechanisms including subcellular release patterns and stimulus-dependent release kinetics, simultaneous cell imaging of both exocytosis events must be informative. We constructed a pH-sensitive red fluorescent protein mOrange2 fused with the DCV luminal protein chromogranin A（ChgA）,"ChgA-mOrange2" as a fluorescent probe for DCV exocytosis. We also used a pH-sensitive green fluorescent protein pHluorin（pH）fused with the SV membrane protein synaptophysin（SYP）,"SYP-pH" as a fluorescent probe for SV exocytosis. Two probe constructs were co-transfected into rat primary cultured cortical neurons and were subjected to live-cell time-lapse imaging. At present, we successfully detected exocytosis events indicated by ChgA-mOrange2 and SYP-pH, most of which likely showed different subcellular sites and different mode. We will further repeat dual imaging and will present the data on their comparative exocytosis mechanisms.

1P-16 Neuropsin dependent synaptic tagging in vivo

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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 tag setting 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 tag setting in vivo.

1P-17 The role of metabotropic glutamate receptor on structural plasticity of dendritic spines in cultured hippocampal neurons

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The morphology of dendritic spines is closely related to higher brain functions. Various external signals including glutamate regulate the spine morphology. The group I metabotropic glutamate receptor（mGluR）is one of regulators of spine morphology, but details of the underlying mechanism still remains unclear. We have demonstrated that drebrin a major F-actin-binding protein in dendritic spines is important for spine morphogenesis and plasticity. Drebrin in its sequence has two binding motifs for Homer that is scaffolding protein of mGluR. We then propose that group I mGluR activity regulates spine morphology through drebrin-Homer interaction in dendritic spines. To elucidate this working hypothesis, using cultured hippocampal neurons, we examine the relationship between mGluR5 activity and localization of mGluR5, drebrin and Homer1 in dendritic spines.
Mouse hippocampal neurons were cultured with Banker method. At 21 days *in vitro*, neurons were incubated with 1 mM CHPG, a selective mGluR5 agonist for 15min, then processed for immunocytochemistry for mGluR5, drebrin and Homer. Neurons were also stained with fluorescence-conjugated phalloidin for F-actin staining.
Quantitative analyses of confocal microscopic images revealed that the CHPG treatment significantly increased the number of spine in which drebrin and Homer were co-localized. The same treatment affected spine morphology. These findings suggest that mGluR5 activity regulates spine morphology through drebrin-Homer interaction.
mGluR has recently been shown to participate in several neuropsychiatric diseases. The elucidation of functional roles of mGluR in spine morphogenesis helps us to gain better understanding of the mechanism of higher brain functions.

1P-18 Deletion of drebrin A impairs hippocampal synaptic plasticity and hippocampus-dependent fear learning in adulthood

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Structural plasticity of dendritic spines, that underlies higher brain function including learning and memory, is dynamically regulated by actin cytoskeleton and its associated proteins. Drebrin A（DA）is an actin binding protein preferentially expressed in the brain and localized highly in dendritic spines of mature neurons. The isoform conversion from drebrin E（DE）to DA and its accumulation into dendritic spines occur during synaptic maturation. We have demonstrated that DA has a pivotal role in spine morphogenesis and plasticity. However, it is not determined which process is required, the accumulation of drebrin（either DE or DA）within spines or the isoform conversion of drebrin. To answer this question we further analyzed mutant mice（named DAKO mice）in which the isoform conversion from DE to DA was disrupted by a deletion of the DA-specific exon. In adult DAKO mouse brain DE continued to be expressed instead of DA. Electrophysiological study using hippocampal slices revealed that LTP induced by high frequency stimulation of CA1 synapses was impaired in older（than 30 week old）DAKO mice, but not in younger（than 8-9 week old）ones. In contrast, LTD was abnormally induced by low frequency stimulation of CA 1 synapses in older（than 30 week old）DAKO mice, but not in younger（than 8-9 week old）ones. Unlike the LTD in juvenile mice, this form of LTD does not depend on the NMDA receptor activity, rather depends on metabotropic glutamate receptor 5（mGluR5）activity, suggesting that mGluR5-signaling is altered in old DAKO mice. In parallel with the electrophysiological phenotype these mice exhibit impaired hippocampus-dependent fear memory in an age-dependent manner. The impairment was evident in mice older than 30 week old, but not in mice younger than 10 week old. Thus, our data indicate that the isoform conversion of drebrin is critical and DA is indispensable for normal synaptic plasticity and hippocampus-dependent types of fear memory.

1P-19 EphB Extracellular Phosphorylation Controls Pathological Pain and Synaptic Function of NMDA Receptors

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N-methyl-d-aspartate receptors (NMDARs) are localized to synapses to drive adaptive and maladaptive changes in response to sensory experience. Synaptic organizing proteins control recruitment and retention of NMDARs by inducible intracellular and extracellular interactions. However the mechanisms enabling extracellular interactions are largely unknown. Here we show that synaptic accumulation of GluN 2B-containing NMDARs and pathological pain are controlled by ephrin-B-induced extracellular phosphorylation of a tyrosine in the fibronectin type III (FN3) domain of EphB2. Ligand-dependent extracellular tyrosine phosphorylation drives the EphB-NMDAR interaction and surface retention of EphB2 and NMDARs. In contrast, in the absence of NMDAR, phosphorylation of this tyrosine residue regulates endocytosis and degradation of EphB2. Viral transduction of EphB2 and drug administration demonstrate that extracellular phosphorylation mediates EphB and injury-induced pathological pain behavior. FN3 domains of other synaptic proteins contain a homologous residue suggesting that extracellular phosphorylation is novel candidate mechanism. Together these data identify tyrosine residue in FN3 domain of EphB and extracellular phosphorylation as possible therapeutic targets.

1P-20 NMDA receptors are involved in X-irradiation-induced decrease in drebrin clusters within dendritic spines of cultured hippocampal neurons

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Therapeutic X-irradiation of the brain possibly causes cognitive impairment, which is associated with synaptic dysfunction. We have reported that cranial 10 Gy X-irradiation has acute effects on fear memory in adult mice. This impairment of the memory was associated with the decrease in immunostaining intensity of an actin-binding protein, drebrin in molecular layer of dentate gyrus *in vivo*. Drebrin is usually concentrated in dendritic spines, postsynaptic sites of excitatory glutamatergic synapses and correlates well with the severity of cognitive impairment. We have shown that glutamate-induced decrease in drebrin clusters within dendritic spines of cultured neurons is mediated by NMDA receptor activity. However the mechanism regulating this X-irradiation-induced decrease in drebrin immunostaining intensity is unknown. In order to examine whether NMDA receptors is involved in X-irradiation-induced decrease in drebrin in postsynaptic sites, we used primary hippocampal neuronal culture and analyzed the acute effect of X-irradiation on drebrin accumulation within dendritic spines *in vitro*. The neurons were treated with 50 μ M Amino-5-phosphonovaleric acid (APV ; an NMDA receptor antagonist) 1 hour before 10 Gy of X-irradiation at 21 days *in vitro*. The neurons were fixed 8 hours after X-irradiation. Immunocytochemical analysis showed that drebrin cluster density along dendrites significantly decreased 8 hours after X-irradiation. This decrease was blocked by pretreatment with APV. In addition, we also analyzed the cluster density of GluN1 subunit of NMDA receptors. The GluN1 cluster density was significantly increased 8 hours after X-irradiation. Our results suggest that X-irradiation induces decrease in drebrin clusters within dendritic spines by inducing accumulation and activation of NMDA receptors. Antagonists of NMDA receptor may provide a new avenue toward therapeutic tools to mitigate X-irradiation-induced synaptic dysfunction.

1P-21 Effect of histone deacetylase inhibitor on synaptic dysfunction elicited by X-irradiation

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Treatments for cancers have been developing surprisingly due to progresses of radiotherapy techniques. It has been known, however, that cranial irradiation causes cognitive deficits, although the underlie mechanism of such cognitive deficits is still remain unknown. We have been studying the effect of X-irradiation on neurons in vitro, and have reported that the density of dendritic spines was decreased or the changes in spine morphology by X-irradiation. Furthermore, using drebrin, an actin binding protein, as a marker for synaptic function, we found the number of drebrin clusters decreased transiently by X-irradiation. We have also shown that amyloid beta oligomers-induced change of drebrin accumulation is mediated by histone deacetylase（HDAC）(Ishizuka et al., 2014). And some of HDAC inhibitors are known to protect normal cells from radiation-induced damage. In this study we examined if the accumulation change of drebrin by X-irradiation is also mediated by HDAC, and tested the possibility of HDAC inhibitors usage as therapeutic tools to weaken irradiation effects on synaptic function. We used primary hippocampal cultured neurons and suberoylanilide hydroxamic acid（SAHA）as a HDAC inhibitor. Drebrin was used as a marker of synaptic function and a post synaptic marker and Synapsin I was used as a pre synaptic marker and these proteins were analyzed immunocytochemically. The cultured neurons were pretreated with SAHA 1 hour before irradiation and were fixed at 2, 8, and 24 hours after the irradiation. We evaluated the effect of SAHA by counting drebrin and Synapsin I clusters. Although the data was not significantly different due to the small sample number, it suggested that low dose of SAHA blocks the X-irradiation induced transient decrease of drebrin accumulation.

1P-22 The effect of carbon ion irradiation on cell motility in human glioblastoma cell lines

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This study aimed to investigate the effect of carbon ion（C-ion）irradiation on cell motility in glioblastoma cells. Cell motility was assessed by a wound-healing assay, and the cell survival was evaluated by clonogenic assay.

1P-23 The molecular mechanisms of cell motility by X-ray irradiation in human glioblastoma cell lines

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This study aimed to investigate the molecular mechanisms of cell motility by X-ray irradiation（X-irradiation）in glioblastoma cells. Human glioblastoma cell lines was used U251 and T98G. Cell motility was assessed by a wound-healing assay, and the cell survival was evaluated by clonogenic assay.

1P-24 Maternal separated mice show the anxiety- and fear-related behavior and change neurogenesis in the limbic system

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Early life stress is known to induce long-term alterations in emotional and anxiety-related behaviors. Rodent models of neonatal maternal separation（MS）stress have been used to explore the effects of early stress on changes in affective and cognitive behaviors. MS are associated with structural changes in brain regions linked to cognition and mood regulation. Here, we studied the effects of MS on the alteration of neurogenesis in the limbic system and anxiety-related behavior on C57Bl/6 mice. The MS was performed daily for 3 hr from P1 to P14 and behavioral test was started at 10 weeks of age. We used a battery of stress and anxiety-related behavioral tests in C57Bl/6 mice.（1）The open field test, which measures the basal anxiety level, showed that MS mice tended to spend shorter time in the center area, although total moving distance did not differ.（2）The acoustic startle response induced by the sudden loud tone stimulus was significantly elevated in MS mice.（3）The contextual and cued fear conditioning test provides a measure of memory by assessing a memory for the association between an aversive stimulus and a tone stimulus. MS mice showed decreased fear conditioning to the context and the tone compared to the control. While startle response was elevated in MS mice, freezing time during tone stimulus was significantly attenuated, suggesting that the fear memory formation or maintenance was impaired in MS mice.（4）Neurogenesis in the limbic system was increase in MS mice. These results suggest that neonatal MS treatment enhances the neurogenesis and alters the anxiety- and fear-related behavior. We are investigating whether or not MS treatment alters the differentiation of neural progenitor cells into excitatory and inhibitory neurons or even glial precursor cells, such as NG-2 positive oligodendrocyte precursor cells.

1P-25 Proteomic characterization during differentiation from human embryonic stem cells into early and late neural stem cells by neural stem sphere method

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Understanding neurogenesis is valuable for the treatment of nervous system disorders. However, there is currently limited information about the molecular events associated with the transition from human ES cells to neural stem cells. We therefore investigated the differentially expressed genes during differentiation of highly homogeneous human embryonic stem cells to early and late neural stem cells by neural stem sphere method, using SDS-PAGE and liquid chromatography-tandem mass spectrometry. We identified 1145 differentially expressed proteins involved in these three differentiation stages. Together with the results of classification of protein functions and search of metabolic pathways related to differentially regulated proteins using DAVID bioinformatics tools and KEGG, respectively, suggested that ES cells differentiated to early neural stem cells via extracellular matrix-receptor interactions followed by their signal transduction, and that early neural stem cells differentiated to late neural stem cells via reorganization of cytoskeleton followed by extension of the cells with increase of differentiated neural stem cells.

1P-26 Role of Kruppel-like factor 5 in neural precursor cells during brain development

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Kruppel-like factor (Klf) 5 is a member of Klf family proteins, which are members of DNA-binding transcriptional factors with highly conserved sequences and redundant functions in the regulation of cell cycle, cell differentiation and tissue organization. Among Klf family, Klf4 is one of the defined factors which reprogram somatic cells to induced pluripotent stem cells (iPS cells). Klf4 is a key regulator of pluripotency in embryonic stem cells (ESCs), inner-cell mass (ICM) and neural precursor cells (NPCs). Klf5 possesses overlapping function with Klf4 in the induction of iPS cells and ESCs self-renewal. Klf5 is shown to be essential for the blastocyst implantation, the three germ layers development, and the formation of cardiovascular system and optic vesicle. Although these preceding reports suggest that Klf5 has broad roles in the organogenesis, its function in the central nervous system has not been investigated despite of its expression in the developing brain. In this study, we have investigated roles of Klf5 in the proliferation and maintenance of NPCs in the developing cortex. When knockdown or overexpression of Klf5 was performed by in utero electroporation, aberrant differentiation and migration of NPCs were observed. We also found that BrdU incorporation of NPCs was altered after the knockdown/overexpression of Klf5. In addition, we performed a colony-forming neurosphere assay using NPC-specific Klf5 conditional knockout mice, which revealed the impairment of self-renewal of neural stem cells. To examine overlapping function of Klf4 and Klf5, we have also analyzed NPC-specific Klf4 and Klf5 double deficient embryos and observed accumulating effects of gene deletion in the brain development. Our data suggests that Klf5, as well as Klf4, plays an important role in the proliferation, differentiation and migration of NPCs.

1P-27 Role of Protease-activated receptor-1 in proliferation of neural stem/progenitor cells derived from the adult mouse hippocampal dentate gyrus

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It is now clear that there is a continual turnover of the mammalian hippocampal dentate gyrus neurons throughout life even in adult. Various neurological injuries are widely recognized as promoting endogenous neurogenesis in hippocampal dentate gyrus. Thrombin-activated/protease-activated receptor-1 (PAR-1) is known to regulate proliferation of neural cells following brain injury including intracellular hemorrhage. To elucidate involvement of PAR-1 in neurogenesis occurred in the adult hippocampus, we evaluated the effect of thrombin and PAR-related peptides on proliferation of the neural stem/progenitor cells (NPCs) derived from the hippocampal dentate gyrus of adult mouse. Immunostaining revealed that PAR-1 was co-localized with nestin, which is a marker for NPCs. Reverse transcription-PCR analysis showed the expression of mRNA encoding all subtypes of PAR in the NPCs. Exposure of the cells to thrombin significantly attenuated the cell proliferation without morphological change and cell damage. However, the cell proliferation was not affected by the PAR-1 negative control peptide, RLLFT-NH2, which is an inactive peptide for PAR-1. Thrombin had no effect on lactate dehydrogenase release during the culture condition. In addition to thrombin, the PAR-1 agonist peptide, SFLLR-NH2, also attenuated the cell proliferation in a concentration-dependent manner. Moreover, the attenuation induced by thrombin was completely abolished by RWJ56110, which is a PAR-1 antagonist. These data suggest that PAR-1 negatively regulates proliferation of the NPCs in the adult hippocampus.

1P-28 The dorsoventral boundary of the germinal zone is a specialized niche for the generation of cortical oligodendrocytes

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Oligodendrocyte precursor cells (OPCs) appear in the late embryonic brain, mature to become oligodendrocytes (OLs) and form myelin in the postnatal brain. Recently, it has been proposed that early-born OPCs derived from the ventral forebrain are eradicated postnatally and that late-born OLs predominate in the cortex of the adult mouse brain. However, intrinsic and extrinsic factors that specify the ability of self-renewing multipotent neural stem cells in the embryonic brain to generate cortical OL-lineage cells remain largely unknown. Using an inducible Cre-loxP system to permanently label Nestin- and Olig2-lineage cells and using an in utero electroporation technique, we determined when and where cortical OL-lineage cells differentiate from neural stem cells in the developing mouse brain. We show that neural precursor cells in the dorsal VZ/SVZ are inhibited by Wnt signaling from contributing to cortical OLs in the adult brain. By contrast, neural precursor cells present in the dorsoventral boundary VZ/SVZ produce a significant amount of OLs in the adult cortex. Our results suggest that neural stem cells at this boundary are uniquely specialized to produce myelin-forming OLs in the cortex.

1P-29 Expression profiling of ubiquitin ligases with transmembrane domain in the brain

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Studies on endoplasmic reticulum (ER)-associated degradation (ERAD), in which unfolded proteins accumulated in the ER are selectively transported to the cytosol for degradation by the ubiquitin-proteasome system, have been focused on molecular mechanisms in yeast. In human, disruption of the ER quality control system causes various diseases, such as neurodegenerative disease, lifestyle disease, and cancer. Furthermore, ER stress has become more important because it is also involved in cellular differentiation and tissue development. We have identified human 44 ubiquitin ligases (E3) with transmembrane domain, which are potentially involved in ERAD. As reason for so many genes in mammals compared with the yeast 3 ubiquitin ligases, they are assumed to have tissue-specific and/or developmental stage-specific roles. Here, we investigate the tissue distribution and cellular expression the ubiquitin ligases. First, we specialized high expression organization of each ubiquitin ligases in the human and the mouse tissues by quantitative PCR. Several kinds of tissue-specific and embryonic-specific ubiquitin ligases genes have been found, whereas most of ubiquitin ligases genes were expressed in nervous tissues. Furthermore some ubiquitin ligases were upregulated in the mouse brain at late embryonic stage. In addition, the expression levels of ubiquitin ligases were upregulated during retinoic acid-induced neural differentiation of mouse embryonal carcinoma P19 cells. Therefore, ubiquitin ligases with membrane may play roles in the regulation of neuronal differentiation and function in the brain development.

1P-30 Evidence for involvement of apoptosis in establishing proper cerebrospinal fluid hydrodynamics

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Apoptosis is a major form of cell death to remove unnecessary cells during development and adult tissue in many organisms. In vertebrate, a large number of cells undergo apoptosis during neural tube closure（NTC）in apoptosome-dependent manner. Mice lacking apoptosome activation often exhibit defects in NTC, which has hampered physiological roles of apoptosome-dependent caspase activation in brain development after NTC. We generated a transgenic mice in which broad spectrum of caspases can be suppressed in spatio-temporal pattern by the expression of p35, a pan-caspase inhibitor protein obtained from baculovirus. Mice expressing p35 by nervous-system specific drivers（NCre；p35V mice）were given birth at expected mendelian ratio, but most of them died by 1 month after birth. They showed severe postnatal growth retardation and hydrocephalus. Flow of cerebrospinal fluid（CSF）between 3rd and 4th ventricle was disturbed, while neither stenosis nor abnormality in ciliary morphology and motility was observed in the path of CSF flow including the aqueduct. The hydrocephalus and growth retardation of NCre；p35V mice was not rescued by simultaneous deletion of RIP3, an essential factor inducing necroptosis in the absence of capase-8 activation. The CSF of NCre；p35V mice contained a larger amount of secreted proteins than that of controls. These data suggest that establishment of proper CSF dynamics requires caspase activity during brain development after neural tube closure.

1P-31 Age-related cell death of *Drosophila* Or42 b neurons is induced by activation of innate immune response

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During normal aging or in neurodegenerative diseases, our brain functions such as cognition and memory get decline. However, the mechanisms of age-related impairments in brain function during normal aging are not well known. Here we show that age-related caspase activation and cell death of specific neuron is caused by activation of innate immune response.Recently, we found that caspase, the executor protease of apoptosis is activated in a subset of olfactory receptor neurons（ORNs）, especially in Or42b neurons during normal aging（PLOS Genetics 10, e1004437, 2014）. ORN is the first order neuron of *Drosophila* olfactory system, and Or42b neuron is known to be necessary and sufficient for innate attractive behavior to food-like odors. Thus, aging can affect the defined animal behavior by affecting the death of specific neurons, such as Or42b neurons. In this report, we investigate the molecular mechanism underlying the age-related caspase activation in Or42b neurons. To investigate the impact of aging on ORNs including Or42b neurons, we first performed gene expression profiling of young or aged antenna with microarray analysis. We found that expression of antimicrobial peptide（AMP）genes were significantly up-regulated in aged antenna, suggesting that innate immune response is induced in aged antenna. Consistent with this, age-related caspase activation was suppressed in mutants for innate immune response. These results indicate that caspase activation is the consequence of activated innate immune response. Our results suggest the possible link between innate immune response and age-related decline of brain functions during normal aging.

1P-32 ER and Golgi stresses upregulate ER-Golgi SNARE Syntaxin5 and suppress Aβ peptide secretion in primary hippocampal neurons

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Endoplasmic reticulum（ER）stress has been implicated in neurodegenerative diseases such as Alzheimer's disease（AD）. We have been focusing on the neuronal function of ER-Golgi soluble N-ethylmaleimide-sensitive factor-attachment protein receptors（ER-Golgi SNAREs）. We previously demonstrated that manipulation of Syntaxin5（Syx5）protein causes changes in the Golgi morphology and processing of AD-related proteins such as β-amyloid precursor protein（βAPP）. We also showed that ER stress upregulates *de novo* synthesis of ER-Golgi SNAREs Syx5 and Bet1 in Neuroblastoma-Glioma hybrid cell line NG108-15（Suga K. *et al., Exp. Cell Res.*, 2015）. In addition, while ER stress caused the reduction of β-amyloid peptide（Aβ peptide）secretion during the adaptive stage of the response, knockdown of Syx5 proteins enhanced the secretion of Aβ. Furthermore, reduction in Aβ secretion by ER stress was significantly suppressed by Syx5 knock down. However, it is neither clear how such stress signal propagates from the ER through the Golgi apparatus, nor how it affects the transport and the processing of AD-related proteins in neurons. In this study, to clarify the role of Syx5 proteins in neuronal βAPP processing and viability, we examined the effects of ER and Golgi stress on the expression of ER-Golgi SNAREs, βAPP processing, and cell viability in hippocampal culture neurons. We found that whereas ER stress and Golgi stress caused upregulation of Syx5 proteins, apoptosis induction using Staurosporine caused down regulation of Syx5 proteins due to the degradation by activated Caspase-3. Knockdown of Syx5 protein under ER stress enhanced vulnerability of neurons. In addition, Golgi stress decreases the secretion of Aβ peptides from neurons as in ER stress. These findings suggest that ER-Golgi SNARE Syx5 serve as a new responder to Golgi stress and regulates Aβ peptide secretion and affects neuronal survival during organelle stress.

1P-33 Reactive oxygen species-generating activity in lysosomes contributes to an iron-dependent form of cell death

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Oxidative stress-induced cell death has been implicated in acute injury such as ischemia. Here we assessed a novel type of oxidative cell death, ferroptosis, which requires intracellular iron. We found that ferroptotic compounds-induced cell death could be prevented by inhibitors of autophagic/lysosomal activity. Analyses with a fluorescent reactive oxygen species (ROS) sensor revealed constitutive formation of ROS in endo-lysosomes, and treatment with lysosome inhibitors decreased both lysosomal ROS and a cell death-associated ROS burst. These inhibitors partially prevented intracellular iron usage by attenuating intracellular transport of transferrin or autophagic degradation of ferritin. Furthermore, fluorescent analyses with a membrane peroxidation sensor represented formation of lipid peroxidation in these compartments. Thus, lysosomal activity may be involved in ferroptosis by modulating iron equilibria and ROS formation. Our spatiotemporal analysis with effective probes will contribute to understand the mechanisms of neural cell death during cerebral ischemia.

1P-34 Effect of Arginine methylation via PRMT1 on organella

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Cumulative of reports have shown the importance of ER stress in pathology of neurodegenerative diseases, such as Alzheimer's disease, Parkinson disease, etc. These studies indicate that the cellular events in response to ER stress should relate to the pathology of neurodegenerative diseases. To elucidate the pathogenesis of neurodegenerative diseases from the point of ER stress, we investigated the altered genes in SK-N-SH cells in the condition of tunicamycin-induced ER stress by gene fishing method. As the result, we found that Protein arginine N-methyltransferase 1, PRMT1, is up-regulated in SK-N-SH cells under ER stress. Based on this result, we examined the importance of PRMT1 in the ER stress pathway and cell, PRMT1 permanently knock down cells were constructed and the cells showed the abnormal golgi formation and increased UPR, unforded protein resonse. To elucidate the mechanism of such alterations, we screened the methylated proteins under ER stress condition by IP-MS and we got several candidates. In this poster, we showed the effect of methylation on the physiological functions of them.

1P-35 Characterization of zinc uptake by mouse primary cultured astrocytes and microglia

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Under severe pathophysiological conditions, zinc is released excessively from glutamatergic synaptic boutons, and induces brain injury. Neuronal death is exacerbated by excessive activation of microglia induced by zinc released from astrocytes in addition to that from the neurons. Therefore, regulation of the extracellular zinc is important for maintenance of brain homeostasis, and the extracellular zinc has to be cleared by specific systems. To elucidate the regulatory mechanism for extracellular zinc in the CNS, we examined the zinc uptake characteristics in mouse astrocytes and microglia. Zinc was taken up into mouse astrocytes and microglia time-dependently, and the cell-to-medium concentration (C/M) ratio in the initial uptake phase in astrocytes was significantly smaller than that in microglia, while in the steady state phase, there was no difference in their C/M ratios. In both astrocytes and microglia, the zinc uptake was mediated, at least in part, by high- and low-affinity systems. There were no differences for both in the Km values for zinc uptake between astrocytes and microglia, and those for the low-affinity system in both cell types were the same as that for mouse ZIP1 reported previously. On the other hand, the Vmax values for both systems were greater in microglia than in astrocytes. Among ZIP isoforms, expression of ZIP1 was high in astrocytes and microglia. Nickel, a competitive inhibitor of ZIP1, and ZIP1 knock-down decreased zinc uptake by both types of cells. Overall, it is demonstrated that astrocytes and microglia had a similar uptake system for zinc including ZIP1, and the differences found in their uptake profiles imply that they play different roles in the regulation of extracellular zinc to maintain brain homeostasis.

1P-36 Oxidative stress enhances zinc clearance via upregulation of ZIP1 expression at the plasma membrane in astrocytes

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Zinc plays roles as neuro- and glio-transmitters in maintenance of brain homeostasis. Under pathological conditions, zinc is released from glutamatergic boutons and astrocytes, and excessive zinc in extracellular space kills neurons and also induces extensive activation of microglia, resulting in exacerbation of brain injury. Therefore, extracellular zinc levels have to be strictly regulated in narrow physiological ranges. Recently, we demonstrated that astrocytic uptake of zinc has a primary role in zinc clearance, and a zinc transporter ZIP1 expressed by astrocytes is one of the responsible molecules for the uptake. On the other hand, under pathological conditions, it is reported that astrocytic clearance for transmitters such as glutamate and GABA is upregulated, but that for zinc is unknown yet. Here, we examined whether functional expression of zinc clearance system is altered under oxidative stress-loaded cultured astrocytes. Treatment of astrocytes with hydrogen peroxide at 0.4 mM for 24 h treatment caused apparent activation of them with increased expression of GFAP and 4-hydroxynonenal without cell toxicity. The activated astrocytes exhibited increased zinc uptake activity, and the Vmax value for the uptake was significantly greater than that in control group, but there was no change in the Km value, which is comparable wih that of ZIP1. Expression of ZIP1 in the activated astrocytes was increased in whole cell lysates and plasma membrane fraction. Taken together, it is indicated that under oxidative stress-loaded conditions, astrocytes increase the zinc clearance activity and this is due, at least in part, to the increase of ZIP1 expression at their plasma membrane.

1P-37 Involvement of Mlc1 in white matter development and maintenance

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For many decades, astrocytes have been considered as supporting cells for neuronal and brain functions. However, this notion has been strongly modified by studies on neuron–astrocytes and blood vessel–astrocytes interactions that should modulate synaptic transductions and blood flow. These new findings have been proposed during last decade, while little is known about relationship between astrocytes and white matter development and/or maintenance. Recent studies have suggested that astrocytes are involved in the white matter development and maintenance. Deficiency in several genes that are expressed in astrocyte specific manner results in leukoencephalopathy, and the damaged astrocytes could contribute to the pathological onset of the leukoencephalopathy. To expand the knowledge on the relationship between astrocytes and the white matter development, we focused on astrocyte-specific gene, Mlc1. Mlc1 is a mouse homologue of the human MLC1 that is responsible for a human leukoencephalopathy, Megalencephalic leukoencephalopathy with subcortical cysts（MLC：# 604004 at Online Mendelian Inheritance in Man（OMIM））. MLC is a rare autosomal recessive neurological disorder with an infantile-onset leukoencephalopathy, which is characterized by a chronic white matter edema, macrocephaly, a slowly progressing deterioration of motor function, cerebellar ataxia, spasticity, and mental decline. Although human MLC1 and murine Mlc1 encode an eight transmembrane protein, its precise function has remained unclear. Here, we generated Mlc1 null mouse and Mlc1 over-expressing mouse, and examined their loss-of- and gain-of-functional effects against astrocytes and brain white matter.

1P-38 Activation of P2X7 receptor/HIF-1α signal pathway in astrocytes induces ischemic tolerance

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A mild ischemic episode（preconditioning；PC）induces resistance to a subsequent severe ischemic injury. This phenomenon, known as ischemic tolerance, is an endogenous process that provides robust neuroprotection. We previously showed that PC-induced activation of astrocytes and subsequent upregulation of P2X7 receptor are essential for ischemic tolerance（astrocyte-mediated ischemic tolerance）. However, the downstream signals of P2X7 receptors responsible for the ischemic tolerance remain unknown. Here we show that hypoxia inducible factor-1α（HIF-1α）, a master molecule that induces various neuroprotective factors in astrocytes has an indispensable role for this event. Using *in vivo* middle cerebral artery occlusion（MCAO）model in mice, we found that PC（15 min-MCAO）increased HIF-1α in both neurons and astrocytes. It is well-known that decrease in the oxygen supply is a main mechanism that promotes increase in HIF-1α, and actually, the neuronal HIF-1α increase was dependent on hypoxia/ischemia. In contrast, as for astrocytes, activation of P2X7 receptors, rather than decrease in the oxygen supply, was important. We also confirmed these mechanisms using primary cultures of neurons or astrocytes. Furthermore, PC-induced increase in HIF-1α in neurons was transient, whereas that in astrocytes lasted much longer. Such characteristic features of HIF-1α in astrocytes, but not in neurons, were well correlated with that of ischemic tolerance. Thus, it is strongly suggested that P2X7 receptor/HIF-1α signal pathway plays an indispensable role in astrocyte-mediated ischemic tolerance.

1P-39 Hedgehog signaling modulates the release of gliotransmitters from cultured cerebellar astrocytes.

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The hedgehog（Hh）signaling pathway is conserved in diverse species from Drosophila to human and plays a key role in regulating organogenesis. Sonic hedgehog（Shh）, a member of the Hh family, is an essential factor in the development of the central nervous system. Recent studies have implied that the Hh signaling pathway also functions in mature astrocytes under physiological conditions. The present study focused on the functions of the Hh signaling pathway in the adult mouse brain. We first examined the expression of Hh signaling molecules in the adult mouse brain by in situ hybridization and immunohistochemistry. Patched homolog 1（ptch1）, a receptor for Hh family members, was expressed in S100beta positive astrocytes and Shh mRNA was expressed in HuC/D-positive neurons in the adult mouse cerebellum. These results suggested that the Hh signaling pathway is involved in neuro-glial interactions. To test this hypothesis, we next examined the effects of recombinant Shh N-terminal（rShh-N）on the functions of cultured cerebellar astrocytes. While glutamate uptake was not affected by activation or inhibition of Hh signaling, activation of Hh signaling by rShh-N influenced gliotransmitter release such as glutamate, ATP and D-serine from cultured astrocytes. However, cyclopamine pretreatment interfered with the release of glutamate and ATP, but not of D-serine. These results suggest that non-canonical Hh signaling pathways such as the MAPK and AKT pathway are evidently important in the release of D-serine from astrocytes. We conclude from these results that the Hh signaling pathway modulates the release of gliotransmitters and participates in neuro-glial interactions in the adult mouse brain.

1P-40 The mechanism of Denosomin in astrocytes leading to release of axonal growth factors

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We previously found that a novel compound Denosomin improved hindlimb motor dysfunction of spinal cord injury（SCI）mice, induced axonal growth and increased astrocytes in the injured site. Denosomin enhanced secretion of the intermediate filament protein, vimentin, from cultured astrocytes. The direct treatment of vimentin to cortical neurons increased axonal density. In addition, ratios of astrocytes expressing vimentin and 5-HT-positive axons co-localizing with vimentin were increased inside of the glial scar in SCI mice administered Denosomin. These results suggest that the functional change to astrocyte secreting vimentin as an axonal growth factor is induced by Denosomin treatment, which may contribute to recovery from motor dysfunction. Generally, astrocytes secreting chondroitin sulfate proteoglycan are considered to inhibit axonal regeneration in SCI. Therefore Denosomin-induced conversion of astrocytic properties into beneficial ones of secreting vimentin would be valuable for therapy of SCI. However, the mechanism of Denosomin in astrocytes has not been elucidated. In this study, we aimed to clarify a direct target protein of Denosomin to know its signal pathway in astrocytes. DARTS analysis was peformed using cultured astrocytes（ddY mice, E14）. As a result, one candidate protein was supposed as a vulnerability changed protein against proteolysis with Denosomin coexistence. Confirming that the candidate is a direct binding protein of Denosomin is ongoing.

1P-41 Acetate attenuates LPS-induced nitric oxide production in cultured astrocytes

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The biomolecule acetate can be utilized for energy production, lipid synthesis, and several metabolic processes. Recently, acetate supplementation reduced neuroglial activation in the model of neuroinflammation induced by intraventricular injection of lipopolysaccharide（LPS）. To understand the mechanisms underlying the anti-inflammatory effect of acetate on glial cells, we examined the effect of acetate on nitric oxide（NO）production in cultured astrocytes, which is experimentally stimulated by LPS. Increasing acetate concentration attenuated the LPS（1 μg/ml）-induced NO production in a dose-dependent manner, significantly more than 5 mM, although cell viability was not affected. The LPS-induced expression of inducible NO synthase protein was significantly decreased by acetate（10 mM）. Acetate also reduced the LPS-induced phosphorylation of p38 MAPK at 24 hr, whereas ERK was not affected. LPS-induced intracellular reactive oxygen species（ROS）productions were decreased at 4-24 hr by the addition of acetate. Furthermore, the addition of acetate significantly reduced hydrogen peroxide（H_2O_2）-induced cytotoxicity by increasing cell viability through the attenuation of intracellular ROS level. These findings suggest that attenuation of NO production by acetate may alleviate glial cell damage during neuroinflammation. Acetate may offer its glioprotection by reducing the oxidative stress.

1P-42 Potent induction of glycogen metabolism by pituitary adenylate cyclase-activating polypeptide on cultured astrocytes

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Glycogen is stored in astrocyte located in regions of high synaptic density, and is important for a variety of brain functions including learning and memory. However, the mechanism how the astrocytic glycogen metabolism is regulated has not been completely elucidated yet. Previously, it was reported that glycogen metabolism was activated by vasoactive intestinal polypeptide（VIP）, which shares common receptors with pituitary adenylate cyclase-activating polypeptide（PACAP）. In addition, gene ontology analysis revealed that carbohydrate metabolism was the closest network induced by PACAP in astrocytes. Therefore, we investigated the effect of PACAP on glycogen metabolism using cultured astrocytes. PACAP or VIP induced glycogenolysis dose-dependently 1 hr after exposure, and these EC_{50} values were 0.0084 nM or 0.43 nM, respectively. Interestingly, EC_{50} value of PACAP was at least 50 times less than these of neurotransmitters previously reported to induce glycogenolysis such as VIP, noradrenaline or serotonin. Although PACAP decreased glycogen content 1 hr after exposure, it was over-compensated about 3 times more than control level 7 hr after exposure. This glycogenesis by PACAP or VIP was induced dose-dependently, and these EC_{50} values were 0.086 nM or 185 nM, respectively. Ratio between EC_{50} values of glycogenolysis and glycogenesis by PACAP was just 10 times, but that by VIP was about 500 times. In addition, co-application of maxadilan, PAC1 receptor selective agonist, with VIP further improved the VIP mediated glycogenesis by that of PACAP. These results suggested that PACAP and its receptor PAC1 strongly activates glycogen metabolism including glycogenolysis and glycogenesis in cultured astrocytes.

1P-43 Characterization of Olig2-positive astrocytes in the normal adult forebrain

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Expression of Olig2, a basic helix-loop-helix（bHLH）transcription factor, persists from embryonic to adult stages in the central nervous system. In the adult stage, most of the Olig2-positive cells co-express NG2 proteoglycan, and constitute a population of oligodendrocyte precursors（OPCs）. "Adult OPCs" have abilities of self-renewal and differentiation. We previously reported that genetically labeled Olig2-positive cells in the adult brain generate NG2 glia, oligodendrocytes and astrocytes（Tatsumi et al., 2008；Islam et al., 2009；Okuda et al., 2009）. We recently found a distinct population of Olig2-positive cells in the gray matter of the adult forebrain. In contrast to the OPCs, these cells are postmitotic and positive for s100β, a marker of mature astrocyte. They are relatively abundant in basal ganglionic nuclei such as the globus pallidus and substantia nigra pars reticulata. Since these nuclei receive inhibitory GABAergic inputs from the striatum and globus pallidus, respectively. Olig2-positive astrocytes are preferentially localized in the vicinity of the inhibitory synapses. Assuming the tripartite synapses theory, Olig2-positive astrocytes may contribute to inhibitory transmission in the adult forebrain.

1P-44 The role of CD38, an Autism Spectrum Disorder（ASD）-associated molecule, in the development of glial cells

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CD38, a type II transmembrane protein with ADP-ribosyl cyclase activity, is involved in Ca2＋ -induced Ca2＋ -release in different types of cells, and plays an important role for oxytocin（OXT）secretion in the hypothalamus. Deletion of CD 38 gene caused reduction of the central OXT secretion, and caused impaired social behaviors associated with Autism Spectrum Disorder（ASD）. In this study, we investigated the expression and possible role of CD38 in the postnatal development of neurons and glial cells in mice. qPCR and western blot analysis revealed enhanced expression of CD38 from P14 to P28 in wild-type（WT）mice brain. Analysis with WT and CD38 knockout（KO）mice revealed that the expressions of MBP, MAG and CNP, markers of oligodendrocytes, were significantly decreased at mRNA level in the cerebral cortices of CD38 KO mice from P7 to P14. The reduced levels of expression of the MBP and CNP proteins were also observed in CD38 KO mice from P7 to P21 by western blotting and immunohistochemistry. Further analysis using qPCR, western blotting and immunohistochemistry revealed that the expression of GFAP, a marker of astrocytes, was also reduced, and the processes of astrocytes were shorter in CD38 KO mice from P1 to P7. Taken together, CD38 may have some roles not only in the oxytocin neurons but also in the development of glial cells, such as oligodendrocytes and astrocytes.

1P-45 Functional analysis of a Down syndrome-associated gene.

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Trisomy of chromosome 21 is the major genetic cause of intellectual disability, collectively known as Down syndrome. The neuropathology of Down syndrome suggests that the gross brain pathology is associates with the specific profile of working memory and/or verbal short-term memory. These pathophysiological changes of Down syndrome also include the changes in size of specific brain regions and their connectivity and alternations in the number and/or the morphology of a certain population of neurons. Recently, the candidate genes and their interactions have been explored, however, the whole picture of pathological process of Down syndrome has not been revealed.Previously, we reported that a down syndrome-associated gene regulates the neuronal migration and eventual distribution in the midbrain at embryonic stage. In this study, we find that a down syndrome-associated gene is expressed in the cerebellum at postnatal stage. Moreover, we show the new function of this gene in both neurons and glial cells in the cerebellum.Several studies in Down syndrome fetal brain and in the trisomy mouse, which express this protein at higher level, have reported the reduction in the brain volume and cell number in the hippocampus and cerebellum. Consistently, we found that the number of neurons in mutant mice, which express this protein at lower level, was increased. We will discuss the functional role of this gene in the pathogenesis of Down syndrome.

1P-46 Involvement of Ndrg2 in blood-brain barrier disruption after stroke

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Disruption of blood-brain barrier（BBB）is known to be occurred in various pathological conditions including ischemic stroke. However, its regulating mechanism remains elusive. We previously showed that Ndrg2, a gene responding to various stresses in astrocytes, is involved in the regulation of reactive astrogliosis and the protection from infarct damage in a mouse experimental stroke model. This study was aimed to investigate the functional role of Ndrg2 in BBB dysfunction after brain ischemia using a mice middle cerebral artery occlusion（MCAO）model. Vessel permeability, determined by tracer leakage and the extravasation of internal serum proteins, was enhanced in Ndrg2-knockout（KO）mice compared to wild-type（WT）mice after MCAO. Moreover, flow cytometry analysis showed increased infiltration of immune cells in ipsilateral brain hemispheres from Ndrg2-KO mice compared to that from WT mice. Further study revealed that Ndrg2 deficiency results in enhanced expression level of matrix metalloproteases in ipsilateral cortex after MCAO. Similar results were observed in cultured astrocytes isolated from Ndrg2 KO mice. These results suggest that Ndrg2 expressed in astrocytes may play a critical role in the regulation of BBB permeability and immune cell infiltration after ischemic brain stroke.

1P-47 **Treatment of hyperbaric oxygenation combined with radiotherapy improves radioresponsiveness of Glioblastoma.**

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Glioblastoma multiforme（GBM）is the most aggressive malignancies among primary malignant brain tumors. Certain areas of the tumor tissues are coursed to hypoxic condition because of insufficient blood vessel supply. Such hypoxic condition area is also considered to induce radioresistance. Molecular oxygen has been recognized an enhancer of radiatiosensitivity. Hyperbaric oxygenation（HBO）improves the oxygen supply to hypoxic tumor cells. We examined the effect of radiotherapy just after HBO breathing in experimental tumors using a tumor growth assay. U87-MG cells were transplanted into balb/c nu/nu mice leg. After the subcutaneous xenograft reached approximately 200mm^3, mice were started by radiotherapy, 2Gy/day for 10days, with or without HBO, 2.5 atmosphere with 100% oxygen for 40min. A significant growth delay was observed in the xenograft with radiotherapy after HBO, and the tumor size increased 7.0 fold in no-treatment, 4.2 fold only radiation treatment, and 2.4 fold in radiation after HBO treatment, respectively. Next we analyzed the changes of gene expression of tumor cells using mRNA differential display method. Gene expressions induced by HIF-1, a hypoxic response transcription factor, were reduced by HBO. The result indicates that HBO treatment induced oxygenation of hypoxic tumor cells and activated sensitivity of radiation.

1P-48 Development of a novel method for assessing motivation in male mice

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Anhedonia is frequently observed in patients with psychiatric disorders. Sucrose preference is widely used as an index of motivation in rodents. However, previous studies show a strain difference in sucrose preference in mice. Here, we demonstrate a novel method for assessing motivation based on sexual orientation in mice. Six strains of mice, CD-1, ddY, BALB/c C57BL/6J, DBA/2J and C3H/HeJ mice, were purchased and used at 3 to 40 weeks of age. The test apparatus consists of three open chambers. Each male subject was habituated in the apparatus, and then two encountered mice, one female and one male, were introduced into wire-mesh boxes in the left and right chambers, respectively. The time the subjects spent in these two areas was measured for 10 min. All strains of mice showed a significant preference for female encounter regardless of its estrus cycle. The significant preference was observed repeatedly from 7 to 30 weeks old. The preference disappeared in castrated test mice. In addition, the preference was not observed in mouse models of depression such as isolation-reared, lipopolysaccharide (LPS)-treated and corticosterone-treated mice. Fluvoxamine improved the impaired preference in isolation-reared and LPS-treated mice. The metabotropic glutamate 2/3 receptor antagonist LY341495 had an antidepressant-like activity and improved the impaired preference in corticosterone-treated mice. The encounter to a female, but not male, mouse caused an increase in c-Fos expression in the nucleus accumbens shell of test male mice. In addition, dopamine D1 and D2 receptor antagonists blocked both the preference and increased c-Fos expression. These findings indicate that the novel method female encounter test can measure easily motivation in adult male mice.

1P-49 Antidepressant-like effect of resolvin E2 against lipopolysaccharide-induced depression-like behavior in mice

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Epidemiological studies suggest that dietary deficiency of n-3 polyunsaturated fatty acids (n-3 PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid, is correlated to the prevalence of depression and that a chronic supplementation with n-3 PUFA exerts antidepressant-like effects. However, little is known about the underlying mechanisms. In this study, we examined whether resolvin E2 (RvE2), one of the mediators generated from EPA, exerts antidepressant-like effect against lipopolysaccharide (LPS)-induced depression-like behavior. A tail suspension test (TST) was carried out 24 h after intraperitoneal administration of LPS (0.80 mg/kg) in male BALB/c mice. LPS challenge increased in immobility in the TST, an index of behavioral despair, which was dose-dependently reversed by intracerebroventricular (i.c.v.) injection of RvE2 (1 or 10 ng) 2 h before the TST. As RvE2 is reported to act on chemerin receptor 23 (ChemR23) as a partial agonist and leukotriene B4 receptor BLT1 as an antagonist, we next examined which receptors are involved in antidepressant-like effect of RvE2. LPS-induced increase in immobility was dose-dependently reversed by i.c.v. injection of ChemR23 agonist peptide chemerin (50 or 500 ng), but not by the BLT1 antagonist U75302 (10 or 50 ng). Last, we examined whether LPS challenge itself or combination with i.c.v. injection of RvE2, chemerin or U75302 alter locomotor activity. LPS and these treatments did not alter locomotor activity. Taken together, the present findings indicate that RvE2 exerts antidepressant-like effect via ChemR23, but not BLT1.

1P-50 Antidepressants via nitric oxide system – A pilot study in acute depressive model with arginine.

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Nitric oxide（NO）may be one of neurotransmitters related to Major Depressive Disorder（MDD）because a selective neuronal NO synthase（NOS）inhibitor, 7-nitroindazole, induces dose-dependent antidepressant-like effects. However, its role in MDD is not known yet. The purpose of the study is whether antidepressants improve depression via NO pathway using an acute depressive rat model with L-arginine（AR）. We used three different types of antidepressants：fluoxetine（FLX, 10 mg/kg）, milnacipran（MIL, 30 mg/kg）, and mirtazapine（MIR, 10 mg/kg）, in the depressive model using AR（750 mg/kg）pretreatment. We analyzed the mRNA expression levels of three NOS with real-time PCR method and serum NO levels. There are significant increases in the mRNA expression levels of the iNOS gene in brain regions after AR treatment although those of the eNOS gene tended to decrease with AR injection. After antidepressant treatments, there were no mRNA expressional changes in either nNOS or iNOS. However, the eNOS mRNA expression were significantly increased with FLX（cerebellum：$P=0.011$, hippocampus：$P=0.011$, midbrain：$P=0.011$, pons：$P=0.013$, striatum：$P=0.011$, thalamus：$P<0.001$）. There was a statistically significant increase of serum NO levels with MIL（$P=0.011$）. We conclude that changes of both eNOS mRNA level in the brain with FLX and the amount of serum NO with MIL may be related to their antidepressive effects of both agents but further experiments will be needed to ensure the involvement of NO system in MDD.

1P-51 Search for the blood-based biomarkers of late-onset major depressive disorder from the patients and the model mice

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The current diagnosis of major depressive disorder（MDD）is based on the evaluation of symptoms and relies on clinical interview. Use of MDD biomarkers will aid the accurate diagnosis, disease classification, and outcome evaluation of MDD treatment. To date, however, the biomarker which is accepted and available worldwide is nothing, and the discovery is strongly desired. Here we identified the state-dependent biomarkers in late-onset MDD（LOD）patients from the gene expression patterns of blood cells by cross-matching with the gene expression patterns in the blood cells of the model mice of postmenopausal depression, ovariectomized（OVX）mice with exposure to the chronic ultra-mild stress（CUMS）. This study was performed in accordance with the Helsinki Declaration, as revised in 1989, and was approved by the Institutional Review Board of the Gunma University Hospital. All the participants received complete information on this study and signed an informed consent document. The mRNA levels of cell death-inducing DFFA-like effector c（*CIDEC*）, ribonuclease 1（*RNASE1*）, solute carrier family 36 member-1（*SLC36A1*）, and serine/threonine/tyrosine interacting-like 1（*STYXL1*）differentiated depressed from non-depressed states in patients with LOD. The expression levels of these genes were significantly correlated with the severity of depression measured by the Structured Interview Guide for the Hamilton Depression rating scale. Of these, the mRNA level of *Slc36a1* in the blood cells was also an objective index for identifying the depressive state-like blood condition in OVX＋CUMS mice. These blood biomarkers will be helpful for properly diagnosing LOD and bridging the gap between animal studies and human clinical trials.

1P-52 MicroRNA normalizes glucocorticoid receptor levels in neuron and oligodendrocytes after stress exposure

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Major depressive disorder is one of the leading causes of disturbances in emotional, cognitive, autonomic, and endocrine functions, affecting nearly 7% of the population in Japan. According to the large amount of information on depressive diseases that has been accumulated during recent years, patients with major depressive disorder show an enhanced biologic stress-response mechanism, especially a hyperactive hypothalamic-pituitary-adrenal（HPA）axis and high levels of circulating cortisol. Although dysregulation of the HPA axis by chronic stress is indicative of major depressive disorder, the molecular mechanisms and functional changes in the brain underlying depression are largely unknown. It is well known that glucocorticoid receptor（GR）signaling regulates the hypothalamic-pituitary-adrenal（HPA）axis and GR expression level is associated with HPA axis activity. MicroRNAs（miRs）are noncoding RNAs that inhibit the translation and/or decrease the stability of their target mRNAs, ultimately decreasing their proteins expression. A previous report suggested that GR protein levels might be regulated by microRNA（miR）-18 and/or -124a in the brain. Furthermore, the Kampo medicine Yokukansan can affect psychological symptoms such as depression and anxiety that are associated with stress responses.Recently, we reported that stressed mice with elevated plasma levels of corticosterone exhibit morphological changes in the oligodendrocytes of nerve fiber bundles, such as those in the corpus callosum. However, little is known about the molecular mechanism of GR expression regulation in the oligodendrocytes after stress exposure.In this study, by using water-immersion and restraint stress as a stressor for mice, we attempted to elucidate the GR regulation mechanism in the paraventricular nucleus（PVN）of the hypothalamus and in the oligodendrocytes of corpus callosum, and evaluate the effects of Yokukansan on GR protein level regulation.

1P-53 Influence of aminergic modulation and stress on kainic acid-induced neuronal oscillations in anterior cingulated cortex

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Neuronal oscillation is a prominent form of rhythmic activity occurring in the brain. Fast neuronal oscillations（30-100 Hz）are frequently observed in the thalamo-cortical structures when an animal is awake or attentive state. Abnormalities in these oscillations in the anterior cingulated cortex（ACC）, a medial part of the prefrontal cortex, might underlie neuropsychiatric illnesses such as schizophrenia. Furthermore adjustment of these oscillatory activities by amine systems, such as VTA and basal forebrain, also plays an important role in higher brain function of the prefrontal cortex including ACC. We developed an *in vitro* model of neuronal oscillation in coronal slice preparation from young adult mice. In the current study, to elucidate physiological significance of ACC oscillation and its correlation with behavior, we first investigated the effects of aminergic modulation, and then examined influence of the stress on animal behaviors and the neuronal oscillation in the ACC.We performed extracellular field recording from layer 2/3 in cg1 regions in bilateral ACC and evoked neuronal oscillation by perfusing 1 or 3 μM kainic acid（KA）for 1 minute. This manipulation induced oscillatory activity with various frequency bands：θ（5-10 Hz）；α（10-15 Hz）；β（15-30 Hz）；low-γ（30-50 Hz）and high-γ（50-80 Hz）. These activities were evaluated by power-spectral density analysis. Dopamine（DA, 10μM）and noradrenaline（NA, 10μM）were administered by perfusion for 10 min before KA activation. These amines increased oscillation power evoked by kainic acid depending on the frequency ranges. We will report the correlation of aminergic action on the oscillation power and stress-induced behavioral changes.

1P-54 Contrasting feature of ERK1/2 activation and synapsin I phosphorylation at the ERK 1/2-dependent site in the rat brain during epileptic seizure activity in vivo

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Extracellular signal-regulated kinase 1/2 (ERK1/2) that belongs to a subfamily of mitogen-activated protein kinases (MAPKs) plays diverse roles in the central nervous system. There have been a number of studies showing activation of ERK1/2 in various types of seizure activity in vivo and in vitro, but few studies have been conducted to examine the relationship between ERK1/2 activation and its substrate phosphorylation in seizure models. We have been studying the phosphorylation state of a presynaptic protein, synapsin I at ERK1/2-dependent and -independent sites in various types of seizure models, i.e., a cortical slice model of seizure activity and electroconvulsive treatment-induced seizure activity in rats in vivo. Here in this study, we examined the effects of prolonged seizure activity on ERK1/2 activity and synapsin I phosphorylation by using status epilepticus induced by kainic acid (KA-SE) in rats in vivo. In KA-SE, robust ERK1/2 activation was observed in the hippocampus, a representative limbic structure, and lesser activation in the parietal portion of the cerebral cortex, a representative non-limbic structure. On the other hand, the phosphorylation level of synapsin I at ERK1/2-dependent phospho-site 4/5 was profoundly decreased, the extent of which was much larger in the hippocampus than in the parietal cortex. Based on the present and previous results, we will discuss the relationship between neuronal excitation, ERK1/2 and phosphatase activities, and phosphorylation state of synapsin I in vivo.

1P-55 BRINP expressions in pentylenetetrazol-kindled mice

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BRINP (BMP/RA-inducible neural-specific protein)-1, 2, 3 are family genes that are specifically expressed in the nervous system. Among the three family members, BRINP1 is most highly expressed in various brain regions including hippocampus, and its expression is further up-regulated in dentate gyrus (DG) by intraperitoneal (i.p.) administration of kainic acid. Each BRINP possesses an ability to suppress cell cycle progression in cultured neural stem cells, and disruption of BRINP1 by homologous recombination led to the increase in the neurogenesis in subgranular zone of adult mice (BRINP1-KO mice). Furthermore, BRINP1-KO mice exhibited abnormal behaviors with an increase in locomotor activity, reduced anxiety-like behavior, poor social interaction and slight impairment of working memory, which are relevant to symptoms of human psychiatric disorders such as schizophrenia and ADHD. In this study, we examined BRINP expressions in pentylenetetrazol (PTZ)-kindled mice as an epileptic model. Kindling was induced by i.p. injection of 35 mg/kg PTZ into C57BL/6J male mice every 48 hrs. PTZ-kindled mice exhibited no impaired working memory in Y-maze test, but they showed hyperactivity as indicated by the increases of the total number of arm entries and the number of rearing actions. Expression levels of BRINP-mRNAs in hippocampus of the PTZ-kindled mice at steady state are the same as those in control mice. On the other hand, BRINP1-mRNA expression as well as that of BDNF-mRNA was increased in hippocampus of the kindled mice 3hrs after reinjection of PTZ. We also examined induction of neuronal death by the PTZ-exposure to organotypic hippocampal slice culture. Increased fluorescence of propidium iodide (PI) incorporated into dead neurons was detected in dentate granular cells and CA3 pyramidal neurons from 1 day after PTZ exposure and in CA1 pyramidal neurons at later periods.These results suggest that BRINP1 expression is regulated in an activity dependent manner in PTZ-kindled mice. DG and CA3 neurons are more sensitive to PTZ-induced excitotoxicity than CA1 pyramidal neurons.

1P-56 The basic mechanisms underlying ketogenic diet：a neuronal autocrine regulation through adenosine receptor

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A ketogenic diet (a low-carbohydrate high-fat protocol) was designed in the 1920s to mimic fasting and it has been used successfully to treat pediatric and medically-refractory epilepsy. Despite decades of clinical use, the neural mechanisms underlying the anticonvulsant effects of a ketogenic diet are not well understood. To elucidate this, we fed rats a ketogenic or control diet for 2-3 weeks, prepared acute hippocampal slices, and performed electrophysiology and pharmacology in the seizure-prone CA3 region. In slices prepared from ketogenic diet-fed animals we found reduced excitability and seizure propensity. Similar to clinical observations, reduced excitation depended on maintaining reduced glucose；changes reversed rapidly when glucose was increased. We found decreased excitability depended on increased pannexin-1 channel, adenosine A_1 receptor and K_{ATP} channel activation, thus identifying specific mechanisms influencing neuronal activity. These results suggest that the reduction of neuronal activity through activation of adenosine A_1 receptor via purinergic autocrine regulation is one of the key mechanisms underlying anticonvulsant effects of ketogenic diet.

2P-1 Subcellular localization of the SRF coactivators, MKL1 and MKL2, in the brain：possible involvement in dendritic spine morphology.

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Morphological changes of neurons and gene expression play important roles in the formation of proper neuronal circuit and plasticity. Megakaryoblastic leukemia（MKL）family members, MKL1 and MKL2, the serum response factor（SRF）coactivators, have actin-binding motifs suggesting that actin-mediated morphological change affects MKL-mediated gene expression.

Our previous studies have demonstrated that MKL1 and MKL2 are highly expressed in the brain and play an important role in activating SRF-mediated gene expression and regulating dendritic morphology in rat cortical neurons. Nuclear and cytoplasmic extraction from cultured cortical neurons and western blotting revealed that they are localized in both cytoplasm and nucleus. However, little is known about the subcellular localization of MKL.

In this study, we have analyzed the subcellular distribution of MKL1 and MKL2 in the rat brain and found that they are relatively concentrated in the postsynaptic density（PSD）fraction which was insoluble in 1% triton X-100. Conversely, they were not detectable in the crude synaptic vesicles（CSV）. Furthermore, RNAi-mediated knock down of MKL1 and MKL2 was performed in long-cultured cortical neurons with synapses. As a result, the knock-down caused a decrease of spines with mushroom-shape, indicating that they play important roles in maturation of spines.

Brain-derived neurotrophic factor（BDNF）influences dendritic morphology. We have found that MKL1 and MKL2 are phosphorylated in neurons stimulated with BDNF. Therefore, it is thought that BDNF signaling pathway may mediate MKL phosphorylation and regulate dendritic spine morphology. To address this, we investigate the relationship between phosphorylation of MKL and alteration of dendritic spine morphology.

2P-2 Studies on possible PSD-core structure of type I excitatory synapse from rat forebrain

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Postsynaptic density（PSD）is a specialized cytoskeleton, localizing immediately underneath the postsynaptic membrane and play important roles in signal processing upon receiving neurotransmitter and in generation of synaptic plasticity. Model for architecture of PSD of type I excitatory synapse comprises of several scaffolding proteins including shank, PSD-95, GKAP and homer, to which various molecules involved in postsynaptic signaling are associated. On the contrary, PSD-lattice structure has long been known to be an architectural base for type I PSD, of which major constituents are not clearly known. Lattice-like structure of type I PSD can be seen after solubilization of PSD with deoxycholate. We identified such lattice-like structures under other conditions, such as purification of PSDs from forebrains of immature rats and purification of PSDs from brains rapidly frozen after decapitation. The latter condition produces lean PSD（Suzuki et al., 1994, 63：1529-1537）. To know the structural organization of synapses at molecular level, we investigated systematically purification process of PSD and postsynaptic membrane rafts（PSRs）from synaptic plasma membrane（SPM）of rat forebrain after treatment with three different detergents, Triton X-100, n-octyl β-D-glucoside and 3-（[3-Cholamidopropyl] dimethylammonio）-2-hydroxy-1-propanesulfonate（CHAPSO）at varied concentrations（Zhao et al., J. Neurochemistry, 2014, 131：147-162）. We found clear difference in the separation of subsynaptic structures among these detergents. In particular, we identified several novel subsynaptic fractions, and one of them contained mainly mesh-like structures, which resembled previously reported PSD-lattice structure. This preparation may be of use to resolve the molecular architecture of type I excitatory PSDs of mammalian brain.

2P-3 SUMO1 Affects Synaptic Function, Spine Density and Memory

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Small ubiquitin-like modifier-1（SUMO1）plays a number of roles in cellular events and recentevidence has given momentum for its contributions to neuronal development and function. Here, wehave generated a SUMO1 transgenic mouse model with exclusive overexpression in neurons in aneffort to identify in vivo conjugation targets and the functional consequences of their SUMOylation.A high-expressing line was examined which displayed elevated levels of mono-SUMO1 and increasedhigh molecular weight conjugates in all brain regions. Immunoprecipitation of SUMOylated proteinsfrom total brain extract and proteomic analysis revealed ～95 candidate proteins from a variety offunctional classes, including a number of synaptic and cytoskeletal proteins. SUMO1 modification ofsynaptotagmin-1 was found to be elevated as compared to non-transgenic mice. This observationwas associated with an age-dependent reduction in basal synaptic transmission and impairedpresynaptic function as shown by altered paired pulse facilitation, as well as a decrease in spinedensity. The changes in neuronal function and morphology were also associated with a specificimpairment in learning and memory while other behavioral features remained unchanged. Thesefindings point to a significant contribution of SUMO1 modification on neuronal function which mayhave implications for mechanisms involved in mental retardation and neurodegeneration.

2P-4 Analysis of Arhgef2 phosphorylation at Ser 885.

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In neuron, Rho family small GTPases regulate axon elongation or dendritic spine formation through F-actin cytoskeleton. The small G proteins function as molecular switches, cycling between inactive GDP-bound state and active GTP-bound state. Arhgef2（Lfc or GEF-H1）is a major Rho guanine exchange factor（RhoGEF）, that activates RhoA by exchanging GDP for GTP. Depending on PKA, phosphorylation at Ser 885 of Arhgef2 inactivates its GEF activity. In vitro investigation of the Ser885 phosphorylation has been conducted, however, the effect of such phosphorylation in vivo yet remains unknown. Assuming that phosphorylation of Arhgef2 at Ser 885 alters neuronal morphology, we tried to produce Ala885 mutant Arhgef2 mouse with CRISPR/Cas9 system targeting Arhgef2 Ser885. We checked F0 mice and have detected knock-in allele in some mice. We will analyze RhoA activity, neuronal morphology and behavior in Arhgef2 mutant mice.

2P-5 5-HT_{2A} receptor antagonist, Ketanserin induces change in the localization of an actin-binding protein drebrin in hippocampal neurons.

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Serotonergic transmission plays important modulatory roles in higher brain function and is deeply involved in the pathogenesis of psychiatric disorders. Patients with depression have decreased level of serotonin in their brain. We have reported that an actin-binding protein, drebrin at postsynaptic sites plays a pivotal role in the effect of a serotonin-norepinephrine reuptake inhibitors imipramine (Kojima et al., 2010). Our previous study also demonstrated that activation of 5-HT_{2A} receptor reduces clusters of drebrin in dendritic spines of cultured hippocampal neurons (Roppongi et al., 2013) through the activation of NMDA receptors. To examine the role of endogenous 5-HT_{2A} receptor activity on the distribution of drebrin-binding actin cytoskeleton in neurons, we examined the effect of 5-HT_{2A} receptor antagonist, Ketanserin on the localization of drebrin in cultured hippocampal neurons. Primary hippocampal neurons prepared from hippocampi of mouse embryo are treated with Ketanserin for 15 min at 21 days in vitro. Immunocytochemical analysis using anti-drebrin antibody showed that Ketanserin induces accumulation of drebrin in cell body with cluster-like distribution. These results suggest that endogenous 5-HT_{2A} receptor activity regulates the distribution of drebrin in mature neurons.

2P-6 Allopregnanolone induces increase of excitatory but not inhibitory synapses via protein kinase A activation

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Allopregnanolone（APα；5α-pregnan-3α-ol-20-one）is a steroid synthesized in both the periphery and central nervous system. Because APα was suggested to improve the symptoms of depression and Alzheimer´s disease, which involve synaptic dysfunction and loss, we hypothesized that APα increases excitatory synapses. Drebrin, an actin binding protein, facilitates the accumulation of other postsynaptic proteins in dendritic spines. Additionally, drebrin accumulation in dendritic spines is inhibited by soluble amyloid β oligomers, which are involved in the pathogenesis of cognitive decline. These suggest that the accumulation of drebrin within the dendritic spine is a good marker of mature synaptic function. In this study, we investigated whether APα increases mature excitatory synapse density by the analyses of dendritic spine morphology and drebrin accumulation. We prepared primary cultures of hippocampal neurons by Banker´s method. After the cells were incubated for 20 days in vitro, they were treated with various dosages of APα（0.1, 0.3 and 1 μM）for 24 hours, and then analyzed morphologically and immunocytochemically. 0.3 and 1 μM APα significantly increased dendritic spine density. The length and width of these dendritic spines were not altered by APα treatment. In addition, drebrin cluster density was increased by 0.3 and 1 μM APα treatment. Moreover, APα increased VGLUT1 cluster density but not VGAT. These data suggest that APα increases mature excitatory synapses. Interestingly, the protein kinase A inhibitor H-89 pretreatment inhibited the APα-induced increase in drebrin cluster density, while sole treatment of H-89 did not alter the density of drebrin clusters in control neurons. These results demonstrate that APα increases mature excitatory synapses via activation of protein kinase A.

2P-7 A role for BMP4 signaling pathway in mouse neural stem cell survival

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We recently reported that bone morphogenetic protein 4 (BMP4) does not promote differentiation of CD44-positive astrocyte precursor cells into mature astrocytes but greatly promotes their survival (1). Although only few studies have examined the survival-promoting effects of BMPs in the nervous system, it has been shown that BMPs acts as important survival factors in various kinds of cells including those constituting primordial follicles (2). While the anti-apoptotic effects of BMPs have been described in these reports, the molecular mechanism by which they inhibit apoptotic death remains unclear.
We isolated neural stem/progenitor cells (NSCs) from ganglionic eminence of postnatal day 0 mouse brain, and examined the effects of BMP4 on their proliferation and survival. BMP4 did not promote their proliferation but promoted their survival, just as in the case of CD44-positive APCs. Microarray analysis suggested us some candidate molecules in the signaling pathway downstream of BMP4. We will present the data and discuss the mechanism by which BMP4 promotes survival of NSCs.

1). Cai N, Kurachi M, Shibasaki K, Okano-Uchida T, Ishizaki Y. CD44-positive cells are candidates for astrocyte precursor cells in developing mouse cerebellum. Cerebellum. 2012；11：181-193.
2). Nilsson EE, Skinner MK. Bone morphogenetic protein-4 acts as an ovarian follicle survival factor and promotes primordial follicle development. Biol. Reprod. 2003；69：1265-1272.

2P-8 PACAP induces Bdnf expression through selective activation of NMDA receptor/calcineurin pathway in neurons.

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It has been reported that the activation of GPCR (G protein-coupled receptor), which is a major receptor for modulatory neurotransmitters such as monoamines and neuropeptides, modulates the function of NMDA (N-methyl-D-aspartate), one of ionotropic glutamate receptors. We here focused on the regulation of gene expression under the activation of GPCR in cultured cortical cells. However, intracellular signaling pathways evoked by the activation of these two receptors are still unclear. We found that the activation of PAC1, a Gs/q-coupled GPCR, with PACAP (Pituitary adenylate cyclase-activating polypeptide) induced the expression of a group of genes including Bdnf (brain-derived neurotrophic factor) through the NMDA receptor and calcineurin pathway. Interestingly, co-activation of NMDA receptor and PAC1 selectively enhanced the NMDA receptor/calcineurin pathway, resulting in the synergic induction of Bdnf expression. We demonstrated that the activation of the NMDA receptor/calcineurin pathway induced CREB (cAMP response element-binding protein)-dependent transcription mediated by nuclear localization of CRTC1 (CREB-regulated transcriptional coactivator 1). Taken together, it is suggested that glutamatergic and modulatory neurotransmissions activate CRTC1/CREB-dependent gene expression via the selective activation of the NMDA receptor/calcineurin pathway in neurons. To further elucidate the molecular mechanisms underlying the selective activation of this pathway, we are now focusing on intracellular anchoring molecules like AKAP (A kinase anchoring protein), which can coordinate multiple kinases and phosphatases such as PKA (protein kinase A), PKC (protein kinase C), and calcineurin, and modulate the function of NMDA receptor.

2P-9 Dopamine phosphorylates GEF-H1 through PKA to regulate GEF-H1 activity in striatum

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In the brain, dopamine functions as a neurotransmitter and associates with emotion regulation. The functions of dopamine are mediated by Protein kinase A（PKA）-dependent signaling cascades in the neurons. Phosphorylation of PKA substrates regulates the structural and functional plasticity of neurons.

However, many important PKA substrates are still unknown, so further investigation is required to determine the substrate for PKA and its function.

Here, we treated mouse striatum with D1 agonist（SKF81297）or cyclic AMP（cAMP）inducer（forskolin）and then concentrated phosphoprotein with GST-14-3-3z pull-down assay followed by LC-MS/MS analysis. We identified GEF-H1, a Rho guanine nucleotide exchange factor, as a novel PKA substrate. Although present throughout the brain, GEF-H1 is enriched in striatum and is a major RhoGEF in striatum. GEF-H1 is phosphorylated by PKA at Ser-885 in response to activation of D1 dopamine receptors. Phosphorylation of GEF-H1 is associated with inhibition of GEF activity as demonstrated by GST-RhoA-G17A pull down assay.

These results suggest that dopamine can decrease RhoA activity through phosphorylation of GEF-H1 in striatum so as to exert its control over behavior.

2P-10 Transduction from the protein kinase C pathway to the tyrosine kinase pathway in cultured hypothalamic neurons

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The receptor for gonadotropin-releasing hormone（GnRH）belongs to the G-protein-coupled receptors, and its stimulation activates extracellular signal-regulated protein kinase（ERK）. We found that the transactivation of ErbB4 was involved in GnRH-induced ERK activation in immortalized hypothalamic GnRH neurons（GT1-7 cells）[1)]. In the present study, we examined signal transduction comprising the activation of ERK after GnRH treatment. Experiments with two types of PKC inhibitors, Go 6976 and bisindolylmaleimide I, indicated that novel PKC isoforms were involved in ERK activation. Our inhibitor experiments indicated that the novel PKC isoforms activated protein kinase D（PKD）after its translocation by treatment with GnRH. Knockdown experiments suggested that PKD stimulated the phosphorylation of Pyk2 by constitutively activated Src and Fyn. We found that Src, Fyn and PYK2 were involved in ERK activation. Taken together, it is highly possible that PKD plays a critical role in signal transduction from the PKC pathway to the tyrosine kinase pathway for ERK activation.

1）J. Cell. Physiol., 2012, 2492-2501.

2P-11 Behavioral phenotypes of neuron-specific Shp2 conditional knockout mice

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A protein tyrosine phosphatase, Shp2 (Src homology 2-containg protein tyrosine phosphatase 2), acts as a positive regulator of Ras-MAPK cascade downstream of growth factor receptors. Forebrain neuron-specific genetic ablation of Shp2 resulted in the reduction of synaptic transmission and of post-tetanic potentiation, a form of short-term synaptic plasticity, in hippocampal slices. To further address the functional roles of Shp2 in the brain, forebrain neuron-specific Shp2 conditional KO (cKO) mice were subjected to a battery of behavioral tests. The mutant mice showed normal behavior in the rotarod test or the thermal preference test, suggesting normal motor coordination, thermosensation, and nociception in the Shp2 cKO mice. In contrast, the mutant animals exhibited reduced stay time in the dark place, as well as increased number of transition and distance traveled, in the light-dark transition test. The mutant mice also exhibited reduced immobility in the forced swim test and an increased acoustic startle response, whereas prepulse inhibition was normal. In addition, Shp2 cKO mice exhibited reduced freezing behavior during re-presentation to the context in the fear conditioning test, while the fear response to an auditory conditioned stimulus (CS) was not affected. The mutant mice also showed temporary impaired memory formation in Morris water maze. Some of these abnormal behaviors are likely due to the hyperactivity in Shp2 cKO mice, because the mutant mice exhibited markedly increased locomotor activity in the open field test and in the home cage as shown in our previous report. In contrast, other abnormal behaviors, such as reduced stay time in the dark place or impaired memory formation, may be independent from the hyperactive phenotype of the mutant mice.

2P-12 Two Gq-coupled octopamine receptors function non-redundantly to mediate food deprivation signaling of *C. elegans*

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Amine neurotransmitters act primarily through G protein-coupled receptors (GPCRs). In many cases, there are multiple receptors that bind to the same neurotransmitter and activate the same intracellular signaling cascades. In a model animal Caenorhabditis elegans, there are two Gq-coupled receptors for octopamine, the biological equivalent of norepinephrine in invertebrate. It has been previously shown that octopamine induces activation of CREB (cAMP response element-binding protein) in the cholinergic SIA neurons during food deprivation and that this activation is mediated through activation of the Gq-coupled octopamine receptor SER-3 that is expressed in these neurons. We also analyzed the other Gq-coupled octopamine receptor, SER-6, which is highly homologous to SER-3. As seen for ser-3 deletion mutants, CREB activation induced by exogenous octopamine and food deprivation was decreased in ser-6 deletion mutants compared to wild-type animals, suggesting that SER-6 is also required for this signal transduction. Expression of SER-6 in the SIA neurons was sufficient to restore CREB activation in the ser-6 mutants, indicating that SER-6 functions in these neurons as does SER-3. Furthermore, overexpression of one receptor subtype did not fully restore CREB activation in the absence of the other receptor. These results demonstrate that two types of similar GPCRs, SER-3 and SER-6, are required for normal signaling and function in the same cells in a non-redundant manner.

2P-13 The Strip–Hippo pathway regulates synaptic terminal formation by modulating actin organization at the *Drosophila* neuromuscular junction

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The Hippo（Hpo）pathway is well known for its role in growth control in both flies and mammals. Although postmitotic roles of Hpo have also been uncovered such as regulation of dendrite tiling and photoreceptor specification, there has been no report of Hpo function in synapse development. Here, we show the role of Hpo and its negative regulator Strip in synapse formation of *Drosophila* larval neuromuscular junction（NMJ）. Strip, an evolutionarily conserved protein functions as a platform for endosome maturation that is required for axon elongation（*Nat Commun* 5, 5180, 2014）. In addition, we found that endogenous Strip is predominantly localized at presynaptic sites of NMJ. *strip* knockdown in motor neurons resulted in the significant increase in the number of small synaptic bouton called satellite bouton. Furthermore, *strip* knockdown larvae exhibited the defects in synapse transmission. As Strip was also reported as a component of STRIPAK（PP2A）complex, a negative regulator of Hpo in growth control, we examined the relationship between Strip and Hpo. First, we found that *strip* knockdown in S2 cells significantly increased the phosphorylation level of Hpo. Consistent with this, the satellite bouton phenotype by *strip* knockdown was significantly suppressed in *hpo* heterozygous background, suggesting that Strip negatively regulates Hpo activity in synapse development. We also observed overexpression of Hpo caused the satellite bouton phenotype, suggesting that Hpo positively regulates satellite bouton formation. Here we would like to present the molecular mechanism how Hpo and Strip regulate synapse development.

2P-14 Investigation of novel CREB interacting proteins using DNA affinity beads.

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Dopamine is a neurotransmitter that encodes emotional information such as reward, pleasure, attention and anxiety. It acts as a modulator of synaptic plasticity by altering the resting potential of the post-synaptic membrane and changing gene expression. In brain, most of the dopaminergic neuron project to striatum. Dopamine receptors comprise 7-transmembrane domain receptors and are associated with guanosine triphosphate-binding proteins（or G proteins）that mediate their effects. One major effect of D1 receptors is to raise cAMP levels and thereby activate a cyclic AMP dependent protein kinase（PKA）. PKA phosphorylates various substrates including cyclic AMP response element binding protein（CREB）and regulate their functions. CREB is a transcription factor that binds to cyclic AMP response element（CRE）sequence and activate transcription phosphorylation-dependent manner. Although we know that CREB activity is regulated by phosphorylation at Ser133 and interaction with cofactors such as CBP/p300, the precise mechanism of transcription induced by CREB is yet to be revealed. Therefore, we tried to obtain comprehensive understanding of protein complex that is responsible for CREB-induced transcription. We prepared the DNA affinity beads coupled to oligonucleotides containing CRE sequence in promoter region of *c-fos* gene. And the ability of the beads to enrich CREB and the specificity of the beads were confirmed both *in vitro* and *in vivo*. By LC/MS/MS we identified several proteins that bound to the beads with sequence specificity. Using DNA affinity beads enables us to elucidate the regulatory mechanism of transcriptional activation and to obtain comprehensive understanding of protein complex that acts in that process.

2P-15 Physiological analysis of lipid raft molecules on mouse brain slices

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The brain consists of numerous neural cells which give rise to the voluntary action potential resulting in the transmission of complex and intermittent signals to the succeeding cells. The constitution of such neural network is quite essential to maintain higher brain function in vertebrate. Also, the signal transduction system simultaneously contributes to not only neural cell functions but also higher brain function. It has been known that lipid raft domain serve as cell surface platforms of signal transduction especially plays the crucial roles of neural cell functions, such as the release and internalization of neurotransmitter in presynaptic nerve terminal. Although lipid raft in the brain has been intensely investigated, the neural culture cells or brain lysates that do not retain native neural network were used for experimental materials because of the limitation in biochemical strategies. The physiological living brain tissue（brain slice）is, however, considered to be appropriate to explore the relationship between higher brain functions and actual lipid raft domains. Herein, we focused on lipid raft domains on living brain slices（200μm）derived from several portions of mouse brain processed by using brain slicer. To identify the lipid raft molecules, Enzyme-Mediated Activation of Radical Sources（EMARS）method previously developed（Kotani N. et al. Proc Natl Acad Sci U S A. 105：7405-7409（2008））was applied to label specific lipid raft molecules on living brain slices. The physiological lipid raft molecules in mouse brain were significantly different among brain region, suggesting that there needs to use living brain slice for understanding the contribution of lipid raft domain to neural network and higher brain functions.

2P-16 VAMP7 regulates autophagy to maintain mitochondrial homeostasis and to control second phase insulin secretion in pancreatic β-cells.

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VAMP7 is a member of VAMP family proteins required for membrane trafficking and the fusion, and recently reported to be involved in autophagy. VAMP7 is expressed in pancreatic β-cells but its physiological function have not been elucidated. In this study, we investigated the role of VAMP7 in the β-cell function using the β-cell specific VAMP7 deletion（VAMP7 βKO）mice. In isolated VAMP7 βKO islets, the glucose-induced second phase but not the first phase insulin secretion was decreased. The deletion of VAMP7 did not affect the distribution of insulin granules and the glucose-induced cytosolic Ca2＋ dynamics during the second phase. On the other hand, p62 was slightly accumulated and the starvation-induced LC3-II accumulation was disturbed in VAMP7 βKO islets, indicating a defect in autophagy. Because damaged mitochondria are eliminated by autophagy to maintain mitochondrial homeostasis, we focused on the mitochondrial function in VAMP7 null β-cells. The glucose-induced ATP production was significantly reduced during the second phase in VAMP7 βKO islets. Both the hyperpolarization of mitochondrial membrane and increase in mitochondrial Ca2＋, which was essential for the second phase insulin secretion, was blunted in VAMP7 null β-cells. In addition, morphologically abnormal mitochondria were found in VAMP7 null β-cells. Finally, we found that the expression of VAMP7 in VAMP7 null β-cells restored the glucose-induced secretion and the starvation-induced LC3-II accumulation. These results suggest that VAMP7 plays an important role in the maintenance of mitochondrial homeostasis which is critical for the regulation of the second phase insulin secretion.

2P-17 A novel effect of tributyltin on mitochondrial quality control

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Organotin compounds, such as tributyltin（TBT）, are well-known endocrine disruptors. TBT is known to cause neurodevelopmental defects such as behavioral abnormalities and teratogenicity. Several reports have shown that micromolar TBT levels induce neuronal degeneration via mitochondria-mediated ROS generation in neurons. We have recently reported that nanomolar TBT levels reduce cell growth and ATP content via mitochondrial NAD^+-dependent isocitrate dehydrogenase（NAD-IDH）, which metabolizes isocitrate into α-ketoglutarate, in human embryonic carcinoma cells NT2/D1. However, the molecular mechanisms by which NAD-IDH mediates TBT toxicity remain unclear. In the present study, we evaluated the effects of TBT on mitochondrial dynamics. Staining with MitoTracker revealed that nanomolar TBT levels induced mitochondrial fragmentation. TBT also degraded the mitochondrial fusion proteins, mitofusin 1 and 2. Interestingly, apigenin, an inhibitor of NAD-IDH, mimicked the effects of TBT. Incubation with an α-ketoglutarate analogue partially recovered TBT-induced mitochondrial dysfunction, supporting the involvement of NAD-IDH. Our data suggest that nanomolar TBT levels impair mitochondrial quality control via NAD-IDH in NT2/D1 cells. Thus, mitochondrial function in embryonic cells could be used to assess metal toxicity.

2P-18 Roles of the autism susceptibility candidate gene Auts2 for neuronal migration and neuritogenesis in the developing brain

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Previous studies have demonstrated that mutations in Autism susceptibility candidate 2 gene were associated with multiple psychiatric illnesses including autism spectrum disorders（ASD）, intellectual disability（ID）. In developing brain, Auts2 mRNA is highly expressed at several brain regions responsible for the cognitive brain functions such as prefrontal cortex, hippocampus and cerebellum. The physiological roles of this gene in CNS, however, remain largely unknown. Here we reveal a novel function of the protein for AUTS2 in the cytoplasm, regulating cytoskeleton and neural development. Immunohistochemical analysis reveals that this protein is exclusively localized at nuclei of postmitotic neurons at cerebral cortex in an early embryonic stage. In later stage, however, it also appears at the neurites including axons. Immunocytochemistry shows, in neuronal cells, it localizes not only in nuclei but also in cytoplasm including growth cones. AUTS2 activates Rac1 via interaction with several Rac1-GEFs including P-Rex1 and Elmo2/Dock180 complex to induce lamellipodia in neuroblastoma cells and promote the neurite-outgrowth in primary hippocampal neurons. Our loss-of-function experiments revealed that AUTS2 participates in cortical neuronal migration and neuritogenesis by activating Rac1 signaling in the developing cerebral cortex. Moreover, the KO mice display behavioral abnormalities including anxiety-related emotion and memory formation. Thus, our findings indicate that AUTS2 contributes to cortical development and is critical for the acquisition of neurocognitive function.

2P-19 Acute inflammation induces the proliferation of radial glial cells in the optic tectum in response to traumatic brain injury

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Adult neurogenesis is a phenomenon that neural stem cells（NSCs）produce new neurons, astrocytes or oligodendrocytes in the adult brain. It is conserved among various vertebrates. In the adult mammalian brain, neurogenesis is restricted to the subventircular zone（SVZ）and the subgranular zone（SGZ）. In contrast, zebrafish have 16 NSCs niches and can continue to produce new neurons through life. In the optic tectum where optic nerves project, neuroepithelial-like cells in the dorsomedial margin of the perventricular gray zone（PGZ）have the property of self-renewal and multipotency and continue to supply new neurons, radial glial cells（RGCs）and oligodendrocytes. RGCs in the deeper layer expressing several stem cell markers as Sox2 and msi1 are quiescent, while RGCs in the telencephalon are proliferative and work as NSCs. In this study, we found that acute inflammation induced not only the proliferation of neuroepithelial-like NSCs but also that of RGCs in the PGZ. In addition, RGCs were also activated by stab injury, suggesting that RGCs in the deeper layer have a key role in the regeneration from the tissue damage in the PGZ.

2P-20 The role of natural killer cells in developmental brain

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During brain development, neural stem cells proliferate and generate neurons and glial cells in ventricular zone（VZ）and subventricular zone（SVZ）. As the proliferation and differentiation of neural stem cells determines the brain organization and function, it is important to understand the mechanism of neural stem cells activity for the treatment of psychiatric disorders. Recent study demonstrates that some immune cells such as microglia regulate the activity of neural stem cells. However, it has not been reported whether peripheral immune cells are involved in brain development. In current study, we examined the infiltration of peripheral immune cells to embryonic and postnatal mouse brain by flow cytometry analysis, and found that NK1.1＋ natural killer（NK）cells infiltrate embryonic and postnatal brain. To identify the role of NK cells in developmental brain, we intraventricularly injected NK1.1 neutralizing antibody at E16 to deplete NK cells from embryonic brain, and found that the number of BrdU labeled mitotic cells around SVZ decreased by NK1.1 antibody injection. Moreover, BLBP＋ astrocyte precursors and Tbr2＋ basal progenitors were also reduced by NK1.1 antibody injection. These results suggest that NK cells promote neurogenesis and gliogenesis in postnatal brain by regulating the proliferation of neural stem cells.

2P-21 Role of the Meis1 in the development of cerebellum.

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Meis1, which is a transcription factor, is involved in neural differentiation in neural stem cells, however the function of Meis1 in brain development remains unclear. We determine that Meis1 is expressed in the developing cerebellum, especially in granule cells. To investigate the role of Meis1 in granule cells, Meis1 granule cells–specific conditional knockout（cKO）mice are generated. Meis1 cKO mice exhibit the small cerebellum and abnormalities in cerebellar foliation. In addition, decreased proliferation of granule cells and increased number of immature granule cells are observed in Meis1 cKO mice. We reveal that these abnormal phenomena are regulated by Meis1 via the Pax6/Smad1/BMP signaling cascade. Our results suggest that Meis1 participates in granule cell development, including proliferation and differentiation, and is a key factor for correct cerebellar development.

2P-22 Knockdown of glycoprotein M6a in utero delayed the determination of neuronal polarity

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Determination of the neuronal polarity is essential to the proper neural network organization of the developing brain. However, its molecular mechanisms have not been understood completely. We previously found by proteomics that glycoprotein M6a was one of the most abundant membrane proteins in the growth cone, and our many in vitro experiments indicated that M6a is involved in the determination of the neuronal polarization, thus, we suspected that M6a is responsible for the neuronal polarity in in vivo.

To confirm this hypothesis, we investigated physiological roles of the M6a in the developing cerebral cortex using in utero M6a-knockdown (KD). Since M6a is highly expressed in the developing cortical neurons (E12～), in utero electroporation was performed at E14.5 and the fetus were fixed at E16.5 or P2.5. On two days after electroporation (E16.5), the normal neurons migrated into the intermediate zone with changing their shapes, from the multipolar to the bi- or unipolar. In contrast, M6a-KD impaired the normal morphological transition, resulting in the increased proportion of the multipolar neurons. In addition, the number of neurites increased in M6a-KD neurons than the ones using the negative control shRNA. On seven days after electroporation (P2.5), the axon elongation remained immature and the neurons with shorter axons increased in number. These results are consistent with our in vitro data, indicating that M6a participates in the physiological steps of neuronal polarization.

2P-23 Contactin associated protein (Caspr) 4/LNX2 signaling pathway modulates neuronal differentiation of mouse neural progenitor cells

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[INTRODUCTION] The contactin-associated protein (Caspr) family includes 5 members：Caspr1, Caspr2, Caspr3, Caspr4 and Caspr5. Two of them, Caspr1 and Caspr2, have been well characterized as key molecules for central and peripheral myelination. However, the roles of Caspr4 have not been well characterized yet. Caspr4 is type I trans-membrane protein that have relatively large extracellular domain and short intracellular domain. It has been reported that Caspr4 is mainly expressed in specific neuronal subpopulations (Spiegel I et al, 2001). Recently, it has also been reported that Caspr4 is a susceptibility gene of autism spectrum disorders (ASDs) (Karayannis T et al, 2014). However, the molecular function of Caspr4 in the brain has yet to be identified. To understand the function of Caspr4, we have identified Ligand of Numb X2 (LNX2) as a binding partner of Caspr4 intracellular domain using yeast-two hybrid analysis. LNX2 consists PDZ domains, the PDZ domains of LNX2 is a specific binding for PDZ binding motif of Caspr4. In this study, we focused on analyses of functional interaction of Caspr4 and LNX2, especially, the distribution in the developmental brain. Furthermore, we have investigated whether the interaction is related to neuronal differentiation. [RESULTS AND DISCUSSION] We have shown that both Caspr4 and LNX2 expressed in the ventricular zone (VZ) and neural progenitor cells (NPCs) isolated in embryonic 14 days from mice.In vitro differentiation assay, neuronal differentiation was significantly reduced when shRNAs were applied to decrease the expression of either Caspr4 or LNX2 and increased when either Caspr4 or LNX2 was overexpressed in NPCs. In utero electroporation, neuronal differentiation was also increased when either Caspr4 or LNX2 was overexpressed. These results describe positive modulation of neuronal differentiation by Caspr4/LNX2 signaling.

2P-24 Analysis of PKC-dependent phosphorylation and cell adhesion property of myelin P 0 readthrough isoform (L-MPZ)

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Homophilic interaction of PNS myelin protein zero (MPZ/P0) between the extracellular Ig domain leads to tight adhesion between each layers in myelin. This adhesion is affected by the PKC-dependent phosphorylation site in the cytoplasmic region of P0. A novel readthrough isoform of P0, large myelin protein zero (L-MPZ), has the same PKC-dependent phosphorylation site as well as an additional putative PKC phosphorylation site in the extra L-MPZ specific domain. Since L-MPZ is localized in the PNS compact myelin and cell-cell adhesion sites in the L-MPZ transfected cells, L-MPZ may be potentially involved in cell adhesion and myelination. However, adhesion activity of L-MPZ and role of PKC-mediated phosphorylation are still unknown. To elucidate PKC phosphorylation of L-MPZ, we performed Western blot analysis of rat sciatic nerve homogenate using phospho-(Ser) PKC substrate antibody. PKC phosphorylation of L-MPZ was detected in the unique two-dimensional electrophoresis system using cationic detergent. The increase of phosphorylated L-MPZ was observed during early postnatal development of sciatic nerve. Additionally, two states of phosphorylation in L-MPZ were demonstrated by Western blotting using Phos-tag. Next, to clarify adhesion activity of L-MPZ, we performed the adhesion assay using HeLa cells which semipermanently expressed P0, L-MPZ, or phosphorylation site mutants. L-MPZ exhibited a cell adhesion activity which was clearly weaker than P0. This binding activity was affected by mutation of phosphorylation sites. Further, heterophilic binding of L-MPZ to P0 was demonstrated by fluorescence-labeled cells. These results suggest that content of L-MPZ in P 0-rich myelin membrane may be related to flexibility of myelin structure which affects myelin function.

2P-25 Mechanical stress disrupts neuron-glia interactions at nodes of Ranvier

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Peripheral nerves are often exposed to mechanical stress leading to compression neuropathies such as carpal tunnel syndrome. The mechanisms of how mechanical forces cause peripheral nerve dysfunction still remain largely unknown. In myelinated nerve fibers, action potential conduction depends on high densities of voltage-gated sodium channels at the nodes of Ranvier. At paranodes flanking nodes, myelinating Schwann cells interact with axons and form junctions that restrict the mobility of sodium channel complex at nodes. We hypothesized that peripheral nerve compression alters the molecular composition of nodes and paranodes causing nerve conduction failure. To test this hypothesis, we utilized a chronic nerve compression model in which a silastic tube is placed around the mouse sciatic nerves. Two weeks after compression, motor nerve conduction velocity was significantly decreased across the compression site. Immunohistochemistry showed dispersed and reduced clusters of paranodal proteins that form axon-glial junctions. Clusters of nodal proteins were occasionally elongated in association with severely disrupted paranodal junctions. Quantitative PCR showed that cysteine protease calpain2 was up-regulated in the compressed nerves. Cytoskeletal proteins alpha II and beta II spectrins expressed in Schwann cells were down-regulated in the compressed nerves, whereas these spectrins were presumably proteolyzed by activated calpain 2. These results suggest that the disruption of nodes and paranodes contributes to nerve conduction failure in compression neuropathies. Calpain-mediated proteolysis together with Schwann cell gene modulation may be involved in the disruption and/or rearrangement of paranodal structures during peripheral nerve compression.

2P-26 Phosphoglycerate mutase 1 is concentrated in the paranodal loops of myelinating Schwann cells

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Phosphoglycerate mutase 1 (PGAM1) catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate during glycolysis.Recently, autoantibodies against PGAM1 have been found in sera from patients with multiple sclerosis by proteomics-based analysis. However, why the PGAM1 autoantibodies are produced in these patients is uncertain. In the present study, we examined distributions of PGAM1 and glycolysis related enzymes in rodent CNS and PNS to understand why the PGAM1 autoantibodies are produced and contribute to demyelinating disease.Western blot analysis showed that PGAM1 is abundantly detected in the PNS as well as the CNS. Immunohistological analysis showed that PGAM1 was present in GFAP-positive cell bodies and their processes including perivascular end feet and myelinated tracts in corpus callosum, white matter of spinal cord and cerebellum.In white matter, PGAM1 was co-localized with MBP-positive signals, indicating that this enzyme is enriched in myelin sheath. In contrast, PGAM1 was mainly observed in paranodal loops of the PNS myelin. No prominent staining was found in the cell bodies of non-myelinating Schwann cells, perinuclear cytoplasm of myelinating Schwann cells, nor Schmidt-Lanterman incisures where Schwann cell cytoplasm was present.Staining intensity was significantly reduced in the mice with disruption of paranodal axo-glial junction.Thus, present results suggest that PGAM1 is present in astrocytes and myelin and may contribute to glycolysis and energy metabolism in the CNS and PNS.Abundance of this enzyme in myelin membrane may be related to production of autoantibodies against PGAM1 in demyelination.

2P-27 DBZ, a CNS-specific DISC1 binding protein, positively regulates oligodendrocyte differentiation

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Schizophrenia (SZ) is a serious and disabling mental disorder with a lifetime prevalence of about 1% of the population worldwide, and commonly has a chronic course. The underlying pathological mechanisms are still largely unknown, but a growing body of evidence suggests that it is a multifactorial disorder influenced by genetic, neurodevelopmental and social factors. Disrupted-in-schizophrenia 1 (DISC1) is a gene disrupted by a (1；11)(q42.1；q14.3) translocation that segregates with major psychiatric disorders including schizophrenia, recurrent major depression and bipolar affective disorder in a Scottish family. Here we report that DBZ (DISC1 Binding Zinc-finger protein), a brain-specific member of DISC1 interactome, positively regulates oligodendrocyte differentiation. In an in vitro oligodendrocyte primary culture, the expression of DBZ was increased after induction of differentiation of oligodendrocyte by deprivation of PDGF and siRNA knockdown of DBZ decreased the expression level of myelin related markers such as MBP, MAG and CNPase. In mouse corpus callosum, DBZ mRNA expression in oligodendrocyte was intense at P7, the period of myelination, and it was hardly detectable in adult by in situ hybridization. Furthermore, a delay of oligodendrocyte maturation in DBZ knockout mice was revealed by the electron microscope analysis. These results indicate that DBZ is involved in oligodendrocyte differentiation. As multiple lines of evidence obtained by brain imaging, studies in postmortem brains and genetic association studies have implicated oligodendrocytes and myelin dysfunction in SZ, these results may provide important clue about theunderlying etiology of SZ.

2P-28 Differential expression and distribution of myosin superfamily in oligodendrocyte

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Motor proteins are required for myelin formation and maintenance. One of motor proteins, unconventional myosin Va (Myo5a) has been reported to control morphology of oligodendrocytes (OLs) and CNS myelination. In the transcriptome database of OLs, another unconventional myosin VI (Myo6) mRNA has been found in O4-positive differentiated OLs. Recently, we revealed that the other unconventional myosin Id (Myo1d) is expressed in mature OLs (Yamazaki, R., et al., 2014) and is required for myelin-like-membrane formation in vitro. However, the expression and the localization of these three unconventional myosins in OLs are still unclear. To examine the expression of myosin superfamily in OLs in vivo, we performed immunofluorescence staining using cuprizone induced demyelination model mice. We revealed that all myosins are present in OLs in vivo. Their expressions in OLs are reduced in parallel with demyelination and then recovered in remyelination. To clarify the timing of expression during differentiation process and intracellular distribution, we performed double immunostaining with stage-specific OL makers and each myosin antibody using cultured OLs. Myo5a signals were detected in all stage of differentiation from A2B5-positive early progenitor to mature OL. Myo6 signals were present in most of O4-positive OLs but not in early progenitors. Myo1d were found only in mature OLs. In cultured mature OLs, Myo5a signals were distributed in main thicker processes, Myo6-positive signals were detected in MBP-positive myelin-like membrane sheets, whereas Myo1d was enriched in the leading edge of these membrane sheets. Therefore, these myosins may have different roles in developing OLs. Thus, these myosins may be involved in myelin formation and remyelination by the different ways.

2P-29 Kallikrein 6-mediated CNS myelin pathology in experimental autoimmune encephalomyelitis.

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Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the CNS. Demyelination and axonal damage are responsible for neurological deficits in MS. However, the mechanism of demyelination has not been fully understood. We have reported that Kallikrein 6 (KLK6), a serine protease secreted by mainly oligodendrocytes in the CNS, plays the crucial role in the EAE pathogenesis. Here, we examined KLK6-mediated morphological changes of myelin in MOG35-55-induced experimental autoimmune encephalomyelitis (MOG-EAE). Osmium-maceration scanning electron microscopic (SEM) analysis displayed the ultrastructural abnormalities of myelin in the white matter of the EAE spinal cord. In the acute phase of EAE, myelin detachment from the axon was observed in wild-type mice at day 3 after MOG immunization. At this point, infiltrating immune cells into the CNS were not observed in the spinal cord of wild-type mice. On the other hand, KLK6 knock out mice inhibited myelin detachment from the axon even at day 7 after MOG immunization. These observations suggest that KLK6 plays a crucial role for demyelination at the acute phase of MOG-EAE.

2P-30 Brain microvascular endothelial cells promote survival of oligodendrocyte precursor cells

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Background and Purpose We previously showed that transplantation of brain microvascular endothelial cells（MVECs）stimulated remyelination in the white matter lesion induced by endothelin-1（ET-1）injection and improved the behavioral outcome. In the present study, we examined the effect of MVEC transplantation on the behavior of oligodendrocyte lineage cells（OLCs）in vivo. We also examined the effect of conditioned medium（CM）from MVEC cultures on survival of oligodendrocyte precursor cells（OPCs）in vitro. Additionally, we confirmed the therapeutic effect of MVEC transplantation on the white matter lesion using magnetic resonance（MR）imaging. Methods MVECs prepared from rat cerebral cortex were transplanted into ET-1-induced demyelinating lesion in the internal capsule（IC）of rat brains. Cell densities of OPCs, OLCs, immature oligodendrocytes（OLs）and mature OLs, apoptotic deaths of OPCs, and proliferative state of OLCs in and around the ET-1-induced lesions in IC of MVEC-transplanted animals were analyzed. The effect of CM from MVEC cultures on OPCs was analysed by counting pyknotic nuclei in OPC cultures. MR imaging was used to examine the changes in ischemic white matter lesions. Results MVEC transplantation reduced apoptotic death of OPCs and increased the number of OPCs and immature OLs in and around the ischemic lesion in the IC without increasing their proliferation. All these effects were independent of increased angiogenesis or blood flow. Conclusions Presence of MVECs per se has a beneficial effect on ischemic white matter damage in vivo. Further study of the molecular mechanisms by which MVECs inhibit apoptotic death of OPCs may lead to the establishment of a therapeutic strategy against ischemic demyelinating diseases.

2P-31 Effect of exosomes derived from vascular endothelial cells on OPC survival, proliferation and motility

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We examined the effect of brain microvascular endothelial cell（MVEC）transplantation on rat white matter infarction, and found that MVEC transplantation promoted remyelination of demyelinated axons in the infarct region. To help clarify the molecular mechanism of this phenomenon, we examined in vitro the effect of exosomes derived from MVECs and other cells on oligodendrocyte precursor cells（OPCs）. We isolated OPCs from postnatal day 0-2 rat cerebral cortices by the immunopanning method, and cultured them in serum-free medium containing PDGF as a mitogen for several days. MVECs were prepared from adult rat cerebral cortices, and cultured in endothelial cell growth medium. Human umbilical vein（HUVECs）and aortic（HAECs）endothelial cells, immortalized mouse cerebral endothelial cell line（BEND3）and rat fibroblast-like cell line（Rat-1）cells were also used. We prepared exosomes from conditioned medium of each culture, using exosome precipitation solution. After 2 days in culture, there were significantly less number of pyknotic OPCs in the presence of exosomes derived from MVECs, HUVECs and HAECs when compared to control. A larger number of BrdU-positive OPCs were seen in the presence of exosomes derived from endothelial cells（MVECs, HUVECs, HAECs and BEND3）when compared to control. We also examined the effect of exosomes on motility of OPCs. OPCs migrated longer in the presence of exosomes derived from endothelial cells when compared to control. These results suggest that exosomes derived from endothelial cells promote survival, proliferation and migration of OPCs. Identification of molecules contained in the exosomes derived from endothelial cells may be useful for establishment of the therapeutic strategy against demyelinating diseases.

2P-32 Microglia-dependent neurodegeneration in demyelinating mouse model

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Oligodendrocytes are glial cells that myelinate neuronal axons in the central nervous system. Neural network is disrupted when demyelinating diseases occur, such as multiple sclerosis. In the Experimental Autoimmune Encephalomyelitis（EAE）demyelinating mouse model, microglia respond to EAE-induced inflammation and produce various cytokines including IL-1β. Since expression of non-canonical Wnt signaling components in the EAE spinal neurons was observed beside cytotoxic M1 microglia, we postulate the interplay of the activated microglia and non-canonical Wnt pathway in the surrounding EAE spinal neurons. Application of recombinant IL-1β to cultured spinal neurons or co-culture of microglia up-regulated non-canonical Wnt signaling components. While neuronal degeneration was observed in the spinal cord of demyelinating EAE mice, IL-1β or activated microglia induced neuronal cell death via Ror2-c-Jun N-terminal kinase（JNK）pathway. The expression of non-canonical Wnt signaling components was significantly increased in the spinal cord of EAE mice. In vivo analysis of Wnt5a transgenic mice mating with a chronic demyelinating mouse model indicates that non-canonical Wnt signaling aggravates the demyelinating pathology through neurodegeneration. Activated microglia and Wnt-Ror2-JNK axis may provide a possible candidate target for therapeutic approaches to demyelinating disorders.

2P-33 Mitochondrial fission and elongation in microglia induced by activation with LPS

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Macrophage/microglia activation is important for pathophysiology of central nervous system disorders including demyelinating diseases. Although mitochondrial biogenesis and metabolism modulate behavior of activated monocytes and peripheral macrophages, mitochondrial changes in activated microglia are largely unknown. In this study, we investigated mitochondrial morphology in lipopolysaccharide（LPS）-induced activation of primary microglia culture. Mixed glial cultures were prepared from neonatal C57BL/6 mice, and microglia were purified following 7-10 days of maintenance. The cultured microglia were activated by 1 μg/mL LPS with or without treatment with 1 mM N-acetyl-L-cysteine（NAC）, an antioxidant, and morphology of microglia and their mitochondria as well as phosphorylation state of mitochondrial fission protein, Drp1, were examined after fixation and immunostaining. Immunostaining for a microglial marker, Iba1, revealed that the culture contained ＞95% of microglia, and LPS stimulation induced typical changes of microglial morphology during the first few hours. Immunofluorescence of multiple mitochondria markers, including TOM 20, COX I, COX Va and VDAC1, showed that microglial mitochondria after 1-3 hours of stimulation were significantly shorter compared with those under vehicle treatment and also those after 6-12 hours of stimulation. Consistently, the measurements of immunofluorescence intensity showed that phosphorylation of Drp1 at Ser 616, which induces Drp1 activation, was increased during the first few hours after stimulation. The decrease of mitochondrial length during the first few hours of stimulation was partially inhibited by treatment with NAC. These results indicate that mitochondrial fission and subsequent elongation are induced by stimulation with LPS, and at least partially mediated by phosphorylation of Drp1 at Ser616 and production of reactive oxygen species.

2P-34 Cell–cell interactions via CD47–SIRPα signal regulate microglial activation

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Signal regulatory protein α（SIRPα）, an immunoglobulin superfamily（IgSF）membrane protein, mediates cell–cell communication signal by interacting with CD47, another IgSF membrane protein. SIRPα is predominantly expressed in dendritic cells（DCs）or macrophages in the immune system, while both SIRPα and CD47 are predominantly expressed in neurons of the brain. In the central nervous system（CNS）, SIRPα is also expressed in microglia, while the functional significance of the CD47–SIRPα signal in regulation of microglial cell functions remains unclear. We found that whole–body knockout（KO）of SIRPα resulted in an increase in the number of cells that expressed CD11c, a cell marker of DCs, in the brain and spinal cord. These CD11c–positive cells were thought to be a subpopulation of brain–resident microglia, because they expressed microglial marker proteins, CD11b and Iba–1. These CD11c–positive microglia were predominantly found in white matter regions, such as corpus callosum, anterior commissure and fimbria. The number of the CD11c–positive microglia was also increased in the brain of CD47 KO mice, suggesting that the increase of CD11c–positive microglia was due to the lack of cell–cell interactions between CD 47 and SIRPα. Subsequently, to evaluate inflammatory responses to lipopolysaccharide（LPS）in SIRPα KO mice, gene expression changes of proinflammatory cytokines were measured after 3hr and 24hr after LPS treatment in the brain and spinal cord. In SIRPα KO mice, LPS treatment induced elevated gene expression of proinflammatory cytokines, such as IL–1β and TNFα, compared with WT mice. These data demonstrate that deletion of CD47–SIRPα signal turns off the inhibitory control of microglial activity, leading to phenotypes such as an appearance of CD11c–positive microglia and hyper–responses to LPS in the CNS.

2P-35 Activation of mitochondrial transient receptor potential vanilloid 1 channel contributes to microglial migration.

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Microglia, the resident immune cells in the brain, survey the environment of the healthy brain. Microglial migration is essential for many physiological and pathophysiological processes. Although microglia express some members of the transient receptor potential（TRP）channel family, there is little knowledge regarding the physiological roles of TRP channels in microglia. Here, we explored the role of TRP vanilloid 1（TRPV1）, a channel opened by capsaicin, heat, protons, and endovanilloids, in microglia. We found that application of capsaicin induced concentration–dependent migration in microglia derived from wild–type mice but not in those derived from TRPV1 knock–out（TRPV1–KO）mice. Capsaicin–induced microglial migration was significantly inhibited by co–application of the TRPV1 blocker SB366791 and the Ca^{2+} chelator BAPTA–AM. Using RT–PCR and immunocytochemistry, we validated that TRPV1 was expressed in microglia. Electrophysiological recording, intracellular Ca^{2+} imaging and immunocytochemistry indicated that TRPV1 was localized primarily in intracellular organelles. Treatment with capsaicin induced an increase in intramitochondrial Ca^{2+} concentrations and mitochondrial depolarization. Furthermore, microglia derived from TRPV1–KO mice showed delayed Ca^{2+} efflux compared with microglia derived from wild–type mice. Capsaicin–induced microglial migration was inhibited by membrane–permeable antioxidants and MAPK inhibitors, suggesting that mitochondrial TRPV1 activation induced Ca^{2+}–dependent production of ROS followed by MAPK activation, which correlated with an augmented migration of microglia. Moreover, a mixture of three endovanilloids augmented microglial migration via TRPV1 activation. Together, these results indicate that mitochondrial TRPV1 plays an important role in inducing microglial migration.

2P-36 Neuroprotective function of microglia

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Microglia are the resident macrophages in the central nervous system（CNS）. It is known that microglia are involved in the surveillance of the CNS under the physiological conditions, and are required for the construction of neural circuitry during development. We previously reported the neuroprotective behavior of microglia in the developing brain. We found that microglia accumulated along subcerebral projecting axon, and their levels peaked at postnatal day 3 to 7. Inactivation or ablation of microglia increased apoptosis in layer V subcerebral and callosal projection neurons. Further, CX3CR1 is required for the survival effect of microglia. We tested candidate factors derived from microglia, and identified that microglia-derived insulin-like growth factor 1（IGF 1）supports the neuronal survival. Thus, we demonstrated the mechanism of a trophic role of microglia in the developing brain. However the mechanism of neuron-microglia interaction during a specific period remains unclear. Although it is well established that Fractalkine（CX3CL1）-CX3CR1 signaling is involved in neuron-microglia interaction, the number of microglia did not decrease in the brain of Cx3cr1-deficient mice. This suggests that Fractalkine-CX3CR1 signaling is not required for the migration of microglia. We examined whether microglia distribute along postnatal axons depending on the axon-derived factor, and what factor attracts microglia to the axon.

2P-37 Functional analysis of protein arginine N-methyltransferase 8（PRMT8）in activated microglia that are induced by spinal cord injury.

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Dendritic transport of αCaMKII mRNA is mediated by recognition of the cis-acting element located on its 3′-untranslated region（3′-UTR）by putative trans-acting factor（s）. To identify the factor（s）that directly bind to αCaMKII 3′-UTR and enable the dendritic translocation of αCaMKII mRNA, we affinity-purified proteins that bound to the 15 segments of αCaMKII 3′-UTR immobilized on streptomycin Sepharose through a Strepto-tag RNA aptamer. A list of proteins, including RNA-binding proteins（RBPs）, were identified with MALDI-TOF mass analyses. Among the detected proteins, hnRNP K, a multifunctional RBP, was rich in quantity in the purified fractions from the restricted fragments of the 3′-UTR, suggesting that it binds to the 3′-UTR in a sequence-specific manner. As expected, hnRNP K knockdown of rat primary culture neurons exhibited a significant reduction of localized αCaMKII mRNA signals in the dendrites, demonstrating an involvement of hnRNP K in the mRNA transport. In this study, we further analyzed the selective activities on the mRNA transport by two hnRNP K variants with different last exons, Ka and Kb, because merely anti-Kb immunoprecipitated αCaMKII mRNA, but not anti-Ka. Accordingly, impaired dendritic localization of αCaMKII mRNA induced by hnRNP K knockdown was rescued by Kb variant, but not by Ka. To address what makes the differences in binding competence between the variants, we focused on their methylated states on arginine residues. As a result, Kb turned out to be a major methylated target by the enzyme, protein arginine N-methyltransferase（PRMT1）, in the neurons. PRMT1 knockdown also impaired the dendritic distribution of αCaMKII mRNA, just as being observed in the hnRNP K knockdown. Since the mRNA delocalization induced by PRMT1 knockdown was recovered by protein transduction of *in vitro* methylated recombinant Kb protein fused with cell penetrating peptide, it is likely that the Kb-specific methylation is a vital event for transport of αCaMKII mRNA.

2P-38 **Acidic pH inhibits interleukin-1β production by down-regulation of mitogen-activated protein kinase activity through the TDAG8/protein kinase A pathway in mouse microglia**

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Objective：OGR1 family G-protein coupled receptors (GPCRs), including OGR1, GPR4, G2A, and TDAG8, sense extracellular protons, resulting in the stimulation of intracellular signaling pathways (Okajima F. (2013) Cell. Signal., 25, 2263-2271). It is known that proinflammatory cytokines, such as interleukin-1β (IL-1β), are released from activated microglia and involved in neurodegeneration of acute and chronic brain disorders, such as stroke and Alzheimer disease. Extracellular acidification in brain (pH = ～6.5) has been observed in ischemia and neurodegenerative disorders, in which lactate and by-products of glycolysis are accumulated in association with impairment of mitochondrial function. Acidic pH is also shown to regulate microglia functions; however, the mechanism underlying acidic pH-induced actions remains unknown. Here, we examined whether extracellular acidic pH regulates IL-1β production, especially focusing on TDAG8 in mouse microglia. **Results**：(1) Extracellular acidification inhibited lipopolysaccaride (LPS)-induced IL-1β production, which was associated with the inhibition of IL-1β cytoplasmic precursor and mRNA expression. (2) The IL-1β mRNA and protein responses were significantly, though not completely, attenuated in microglia derived from TDAG8-deficient mice compared with those from wild-type mice. (3) The acidic pH also stimulated cellular cAMP accumulation, which was completely inhibited by TDAG8 deficiency. (4) Forskolin and a cAMP derivative, which specifically stimulates protein kinase A (PKA), mimicked the proton actions, and PKA inhibitors reversed the acidic pH-induced IL-1β mRNA expression. (5) The acidic pH-induced inhibitory IL-1β responses were accompanied by the inhibition of extracellular signal-related kinase (ERK) and c-Jun N-terminal kinase (JNK) activities. (6) The inhibitory enzyme activities in response to acidic pH were reversed by the PKA inhibitor and TDAG8 deficiency. **Conclusion**：Extracellular acidic pH inhibits LPS-induced IL-1β production, at least partly, through the TDAG8/cAMP/PKA pathway, by inhibiting ERK and JNK activities, in mouse microglia. TDAG8 may be a potential target of neurodegenerative disorders.

2P-39 SOLOIST, a novel isoform of SRF coactivator MKL2 that is enriched in neurons and negatively regulates dendritic complexity of cortical neurons

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The accurate construction of neural network via neuronal plasticity, the morphological and functional change of neurons, are important for the acquisition of higher brain function such as memory, learning, recognition and emotion. It is thought that the rearrangement of cytoskeleton and the expression of genes related to cytoskeleton are deeply involved in neuronal plasticity.
Recently, MKL（megakaryoblastic leukemia）family members have been paid attention as molecules which are involved in neuronal morphology. MKL1 and MKL2 are SRF（serum response factor）coactivators with G-actin binding domains. Our previous studies revealed that MKL1 and MKL2 are highly expressed in the brain and regulates dendritic morphology and SRF-mediated gene expression.
In this study, we identified and characterized a novel MKL2 isoform named SOLOIST（spliced neuronal long isoform of SRF transcriptional coactivator）. Our findings have shown that SOLOIST is highly expressed in the brain and is enriched in neurons. The expression of SOLOIST increased during brain development. SOLOIST regulated the SRF-mediated transcription. Additionally, overexpression of SOLOIST in cultured cortical neurons decreased dendritic complexity, whereas MKL2 isoform 1 increased. Taken together, the roles of SOLOIST in neurons seems different from other MKL2 isoforms. Interestingly, our preliminary data has shown that the influence of SOLOIST on endogenous SRF-target genes may be different from those of other MKL2 isoforms. It would be interesting if SOLOIST-target gene expression is involved in the opposite roles between SOLOIST and other isoforms in dendritic complexity.

2P-40 MAPK-mediated phosphorylation of NPAS 4 regulates memory formation by modulating its interaction with CBP

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Dopamine（DA）is involved in emotional learning, perception, and memory formation. DA activates cAMP/Protein kinase A（PKA）-signaling pathway acting through D1 receptor and then activates Mitogen-Activated Protein Kinase（MAPK）in striatal medium spiny neurons（MSNs）and plays a pivotal role in regulating gene expression. However, how DA signaling regulates gene expression through the phosphorylation of transcriptional factors（TFs）is not fully understood. The cAMP response element-binding protein（CREB）-binding protein（CBP）is a critical for neuronal plasticity and memory formation and is believed to participate in the activities of hundreds of different TFs. The interactions of CBP with TFs are regulated by phosphorylation. To isolate the transcriptional factor regulated by phosphorylation downstream of DA, we performed proteomic analyses of CBP-interacting proteins and identified Neuronal Per Arnt Sim domain protein 4（NPAS4）, as a novel CBP-interacting protein. NPAS4, a brain-specific basic helix-loop-helix transcription factor, regulates the expression of several genes that are important for synaptic plasticity and plays an important role in synapse formation and memory formation. We found that NPAS4 strongly interacted with the kinase-inducible domain interacting（KIX）domain of CBP, weakly Histone acetyltransferases（HAT）domain. NPAS4 was phosphorylated at Thr-427 by MAPK in vivo and the phosphorylation of NPAS4 increased the interaction of NPAS4 with CBP. Furthermore, the phosphomimic mutant of NPAS4 enhanced the exon I and IV-BDNF promoter activity. These results imply that MAPK phosphorylates NPAS4 at Thr-427 and increases its binding with CBP, thereby regulating BDNF expression and memory formation.

2P-41 Effects of Oxytocin on the respiratory circuit in isolated brainstem–spinal cord preparation from neonatal rat.

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Oxytocin is well known as a hormone affecting uterine contraction and inducing lactation. Moreover, oxytocin plays a key role as a neurotransmitter affecting an autism. Previous report showed that oxytocin–containing neurons of the hypothalamic paraventricular nucleus project to the rostral ventrolateral medulla region (RVL) and phrenic motoneurons innervating the diaphragm. And oxytocin–containing neurons in the PVN is mediating increased respiratory output elicited by PVN stimulation. However, oxytocin in the neonatal breathing were not so well understood. We examined oxytocin regulate respiratory activity using brainstem–spinal cord preparation of the neonatal rats. The brainstem and spinal cord were isolated from postnatal day 0 to 4 (P0–P4) with deep isoflurane anesthesia. The respiratory rate and spinal tonic activity were significant increased by application of 10 μM oxytocin in P0–2. After P3, oxytocin was not significant increased the respiratory rate and spinal tonic activity. The respiratory facilitation and spinal tonic activity were inhibited by oxytocin receptor antagonist. Furthermore, respiratory facilitation and spinal tonic activity induced by oxytocin were abolished by APV (NMDA receptor antagonist). On the other hand, respiratory increase was depressed by treatment of CNQX (non–NMDA receptor antagonist) but CNQX was not inhibited spinal tonic activity. These results suggested that 1) the respiratory facilitation and spinal tonic activity by oxytocin were seen in early neonatal stage ; 2) oxytocin excited respiratory rhythm via non–NMDA receptors and excited spinal tonic activity via NMDA receptor. Oxytocin may play a crucial role in assisting spontaneous breathing after birth.

2P-42 The role of ventrolateral striatal dopamine receptor type 2 expressing medium spiny neurons in motivation

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The ventral striatum is known as a key node mediating motivational processes. However, cell–based further understanding of ventral striatal function was not easy because researchers had to address the effects by cell location (medial vs lateral subregion) and cell type (dopamine receptor type 1 vs type2 (D2) expressing medium spiny neurons (MSNs)), separately. Here, we combined an expansive yet reversible loss–of–function with day–by–day instrumental task in order to search the responsible region of mediating motivation and examine how specific cells at the confined region modulate instrumental motivation. Our main findings are：
1) Bilateral loss–of–function of striatal D2–MSNs caused motivation deficits.
2) Loss–of–function of 17% of D2–MSNs within the ventrolateral striatum (VLS) was sufficient to decrease motivation.
3) Anatomical expansion of loss–of–function manipulation exacerbated motivation deficits.
4) Rescue–of–function ameliorated motiovation deficits.
Our data demonstrate that the D2–MSNs in the VLS are essential for maintaining motivation.

2P-43 How does the ventral hippocampus respond to optogenetic activation of the raphe nucleus?

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Serotonin（5-hydroxytryptamine or 5-HT）is a neurotransmitter involved in a wide range of brain functions such as emotion, cognition, motor control, and autonomic function. 5-HT containing axons distribute throughout the CNS and originate from cells located in the raphe nucleus. The ventral hippocampus（vHP）is one of target regions of 5-HT neurons in the raphe nucleus and expresses 5-HT receptors（Htrs）including Htr1a, 1b, 2a, 2c, 3a, 4, 5a, and 7. The vHP is involved in emotional responses, i.e. anxiety；hippocampal theta activity increases in an anxiety provoking situation, like the open arm of the elevated plus maze. However, it is unclear how 5-HT neurons behave in an anxious situation and how 5-HT neuron activation modulates vHP activities. Here we addressed the latter question by optogenetics in vivo. We previously succeeded in generating transgenic mice that expressed a step-function-type channelrhodopsin-2 variant ChR2（C128S）in 5-HT neurons. To clarify the effect of the optogenetic serotonergic manipulation on vHP activities, we recorded local field potential（LFP）in the vHP under urethane anesthesia. Our preliminary results showed that the optogenetic activation of 5-HT neurons decreased theta power in the vHP.

2P-44 Neonatal isolation augments social dominance by altering actin dynamics in the medial prefrontal cortex

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Social maltreatment early in life can lead to the development of impaired interpersonal relationships and profound social disorders. However, the underlying cellular and molecular mechanisms involved are largely unknown. Here, we found that isolation of neonatal rats induced social dominance over nonisolated control rats from the same litter in juveniles that was glucocorticoid-dependent. Furthermore, neonatal isolation inactivated the actin-depolymerizing factor（ADF）/cofilin in the juvenile medial prefrontal cortex（mPFC）. Isolation-induced inactivation of ADF/cofilin resulted in the decrease of glutamate synaptic AMPA（α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid）receptor（AMPAR）contents by the increase of stable actin fractions at dendritic spines in the juvenile mPFC. The expression of constitutively active ADF/cofilin in the mPFC rescued the effect of isolation on social dominance. Thus, neonatal isolation traumatizes spines in the mPFC by altering actin dynamics, leading to abnormal social behavior later in life.

2P-45 A New Inhibitory Pathway Toward the Mouse La via the mITC Revealed by VSD Imaging

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It is well known that the lateral amygdala nucleus（La）receives and integrates sensory inputs from the cortex and the thalamus to establish emotional memory. Inhibitory GABAergic inputs in the La play very important roles in controlling the strength of sensory inputs and interfering with the acquisition of fear memory in the initial step. Thus, we used voltage sensitive dye（VSD）imaging, and investigated the spatial and temporal patterns of the inhibitory responses in the mouse La. Direct stimulation of the external capsule（EC）induced large and long-lasting hyperpolarizing signals in the La. We focused on these hyperpolarizing signals to identify the source of the inhibitory inputs. We prepared the slice preparation with four patterns of surgical cuts on the possible afferent pathways. The induction of the hyperpolarization were strongly suppressed by isolating the medial branch of EC（ECmed）, but not the lateral branch of EC（EClat）. Interestingly, the hyperpolarization was not suppressed by isolating the ECmed from the caudate putamen, while the surgical cut of the ECmed fiber tract moderately suppressed it. The hyperpolarizing signals could be completely suppressed in the presence of glutamatergic antagonists. Additionally, the early component（51-100ms）and slow component（201-300ms）of the hyperpolarizing signals could be largely suppressed by GABAA and GABAB antagonists, respectively. When directly stimulating the dorsal, middle or ventral part of ECmed fiber tract in the presence of glutamatergic antagonists, only the stimulation in the middle part of the ECmed caused hyperpolarization.These results suggest that the GABAergic neurons in the medial intercalated cluster（mITC）, which receive glutamatergic excitatory inputs via two pathways, one is from the ECmed fiber tract and the other is from the La, send inhibitory afferents to the La. Here we identify a new inhibitory pathway toward the La via the mITC. This new pathway might have inhibitory effects on the acquisition of fear memory.

2P-46 γ-Aminobutyric acid in anterior cingulate cortex predicts intra-cingulum functional connectivity in human：A magnetic resonance imaging study

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γ-Aminobutyric acid（GABA）is the major inhibitory neurotransmitter in the central nervous system and has important roles in synchronous neural activity. Recent studies employing magnetic resonance spectroscopy（MRS）and functional neuroimaging have revealed a relationship between regional GABA concentration and brain activity in human without invasive experiment. Two studies have demonstrated that GABA concentration in posterior cingulate cortex negatively correlated with functional connectivity within default mode network in healthy subjects. On the other hand, a relationship between functional connectivity at rest and GABA concentration in anterior cingulate cortex（ACC）remains unclear. In this study, we performed MRS using MEGA-PRESS sequence to determine GABA concentration in perigenual ACC（pgACC）and resting-state functional magnetic resonance imaging（fMRI）to measure intrinsic neuronal activity for 25 healthy subjects. Data processing and statistical analysis were performed by SPM8, REST and R software. SPM analysis showed that the GABA/creatine（Cr）ratio is positively associated with the strength of functional connectivity between pgACC and posterior midcingulate cortex（pMCC）[$p < 0.001$, small volume correction for whole cingulate cortex, MNI coordinate；$x = -3, y = -6, z = 32$]. We extracted the values of functional connectivity from the significant cluster in pMCC, then carried out post hoc correlation analysis. The analysis confirmed positive correlation between GABA/Cr and pgACC-pMCC connectivity [$r = 0.76, p < 0.001$]. In addition, the GABA/Cr in the pgACC is positively correlated with the amplitude of fMRI signal fluctuation. These findings suggest that GABA has an important role in intra-cingulum connectivity and local neuronal activity at resting state.

2P-47 Translational regulation by the neuronal RNA binding protein Elavl2 in the brain

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Neuronal Elavls (nElavl) are the first defined neuron specific RNA binding protein (RNABP), implicated in the nervous system development, making spatialmemory or RNA regulation in drug induced seizure brain. However, their roles have not fully been elucidated, in part due to their in vivo handful targets and redundancy. Here we focused on a specific member of nElavls, Elavl2, which mRNA is expressed in the earliest stage in developing cortical neuronsamong other nElavls. Our histological analysis by Elavl2 specific antibody revealed that Elavl2 protein showed unique expression patterns in the adult mouse brain, especially inhibitory neuron in hippocampus compared with othernElavls. To understand a comprehensive role of Elavl2, we generated genome-wide Elavl2-RNA binding map on the mouse embryonic brain by using HITS-CLIP,High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation methods. We are now validating Elavl2 specific RNA targets with Elavl2 knock-out mouse and this genome-wide Elavl2-RNA binding map, biological functions of Elavl2 by Gene Ontology and in vivo Elavl 2 binding motif by CIMS (Crosslinking induced mutation site) analysis, detecting the in vivo Elavl2 binding motif in single nucleotide resolution. In addition, we are also analyzing the mechanisms of Elavl2 function in the brain, especially translational control of target mRNAs with Ribosome profiling analysis. Ribosome profile using Elavl2 KO mice revealed specific RNA targets of Elavl2 in the brain.Lastly, we will discuss how Elavl2-RNA targets link to brain complexity usingthese our two layered in vivo foot printing analysis.

2P-48 Overactivation of the VPAC2 receptor during postnatal brain maturation induces changes in synaptic proteins and selective alterations in prepulse inhibition in mice

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Clinical studies have shown that microduplications at 7q36.3, containing *VIPR2*, confer significant risk for schizophrenia. *VIPR2* gene encodes the VPAC2 receptor for VIP (vasoactive intestinal peptide) and PACAP (pituitary adenylate cyclase-activating polypeptide). Lymphocytes from patients with these mutations exhibited higher *VIPR2* gene expression and VIP responsiveness, but mechanisms by which overactive VPAC2 signaling may lead to these psychiatric disorders are unknown. Here we aimed to determine in a C57 BL/6 mouse model if the *VIPR2*-linkage to mental health disorders might be due to overactive VPAC2 receptor signaling during postnatal brain maturation by daily administration of the highly-selective VPAC2 receptor agonist Ro 25-1553 from postnatal day 1 (P1) to P14. Western blot analyses on P21 revealed significant reductions of synaptophysin and postsynaptic density protein 95 in the prefrontal cortex, but not in the hippocampus, in Ro 25-1553-treated mice. The same postnatally-restricted treatment resulted in a disruption in prepulse inhibition of the acoustic startle in adult mice. No effects were observed in locomotor activity, sociability in the three-chamber social interaction test, or fear conditioning or extinction. In addition, Ro 25-1553 and VIP, but not PACAP, caused reductions in total numbers and length of neuronal dendrites and length of axon in mouse primary cortical neurons. These results suggest that overactivation of the VPAC2 receptor in the postnatal mouse results in a reduction in synaptic proteins in the prefrontal cortex and selective alterations in prepulse inhibition. These findings imply that the *VIPR2*-linkage to mental health disorders may be due in part to overactive VPAC2 receptor signaling during a critical time of synaptic maturation.

2P-49 Microglial property changed in a maternal immune activation animal model with relevance to schizophrenia.

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Schizophrenia is a serious psychiatric disorder with a disabled neurodevelopmental basis. Inflammatory and immunological events interfering with brain development are discussed as one of causes of schizophrenia. In our analysis of post-mortem brain, mRNA expression of PBR/TSPO, which is known as microglial activation marker, increased in patients with schizophrenia. Based on this an aberrant neuro-immune system and epidemiological study, maternal immune activation (MIA) as a neurodevelopmental animal model with high validity for schizophrenia has been developed. However microglial property in MIA is unclarified well. The goal of this study is to investigate whether microglia is activated, and then microglial activation is a neurobiological correlate to the altered behavior in the MIA model. In the present study, MIA was induced in pregnant SD rats by injecting intraperitoneally 20 mg/kg poly I：C 2 times per a day at gestational day 13. We examined the number and morphology of microglia in prefrontal cortex（PFC）and hippocampus（HIP）at 4 or 8 weeks old by immunohistochemical stain for Iba1. And we checked mRNA expression of microglial activation markers（IL6, CD68, CD86, IL1b, IL10）in PFC and HIP at 4 or 8 weeks old using real time qPCR. As a result, the cell body of microglia at 8 weeks old in MIA model became bigger and their processes was thicker than control. Although the number of microglia in PFC was not different from control in PFC. On the other hands, the mRNA expression of PBR was upregulated in HIP in MIA at 8 weeks old. And mRNA expression of CD86, which is known as M1 marker, increased in PFC and HIP at 4 and 8 weeks old. These results suggested that microglial property was changed in MIA model and might support the hypothesis that MIA contributes to microglial activation in the offspring. We are examining whether microglial activation relate to abnormal behavior in MIA.

2P-50 Activation of prefrontal dopamine system by attention deficit/hyperactivity disorder drugs improves prenatal valproic acid-induced behavioral abnormalities in mice

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Rodents prenatally exposed to valproic acid（VPA）are used as animal models of autism spectrum disorder（ASD）. We have also shown that male mice prenatally exposed to VPA at embryonic day 12.5 display ASD-like behavioral abnormalities such as social interaction deficits and recognition memory impairment at 8weeks old. More recently, we have demonstrated that the prenatal VPA exposure causes hypofunction of prefrontal dopamine（DA）system in male mice. The finding implies that hypofunction of prefrontal DA system is associated with behavioral abnormalities observed in the prenatal VPA-exposed mice and activation of prefrontal DA system may result in treatment of the abnormal behaviors. We have found that the attention deficit/hyperactivity disorder (ADHD) drugs methylphenidate (MPH) and atomoxetine（ATX）enhance prefrontal noradrenaline（NA）and DA functions in mice. Thus, the present study examined the effects of ADHD drugs on abnormal behaviors in VPA-treated mice. Chronic, but not acute, treatment with MPH or ATX for two weeks improved social interaction deficits and recognition memory impairment. These drugs also improved the decrements in dendritic spine density in the hippocampus, prefrontal and somatosensory cortices. Furthermore, the improvement of behavioral abnormalities by ATX was blocked by the DA-D_1 receptor antagonist SCH39166 or the DA-D_2 receptor antagonist raclopride, but not by the α_2-adrenergic receptor antagonist idazoxan. These results suggest that ADHD drugs improve VPA-induced abnormal behaviors via activation of DA-D_1 or DA-D_2 receptors. Furthermore, the finding supports that hypofunction of the prefrontal DA system is associated with behavioral abnormalities in VPA mice.

2P-51 Analysis for Mechanism of Autism spectrum disorder via serotonin transporter dysfunction

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Autism Spectrum Disorder（ASD）is a pervasive development disorder characterized by（1）severe and sustained impairment of social interaction and communication, and（2）restricted or stereotyped patterns of behavior and interest. Since this disorder is thought to be a risk of secondary psychiatric disorders such as depression, the mechanism of ASD is being researched all over the world.Changes in serotonin transporter（SERT）function and expression have been implicated in autism. Our colleagues recently reported decreased SERT levels throughout the brains of autistic individuals compared with controls by using PET. Then we screened the SERT interacting proteins which may affect the function of SERT by IP-MS methods. To narrow down the candidates, we measured their mRNA expression levels in lymphoblast cells of autistic and control individuals by real-time RT PCR and Factor X showed significant increase in the autistic individuals（$P<0.05$）. These data suggest the involvement of Factor X in autism via the SERT dysfunction. In this presentation, we show the results of Factor X related to SERT function.

2P-52 Effects of prenatal exposure to a sigma-1 receptor antagonist on behavior and neuronal morphology in rat offspring

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Sigma-1 receptors（Sig-1R）have been implicated in the regulation of neuronal differentiation and development. In the present study, we examined the effect of prenatal exposure of Sig-1R antagonist on postnatal neuronal development and behavior in rat offspring. Pregnant Long-Evans rats were administered NE-100, a selective Sig-1R antagonist, once daily at a dose of 1.0 mg/kg（i.p.）from gestation day 14 to 20. Their young-adult（6 weeks old）male offspring were subjected to behavioral tests and morphological analysis using Golgi-Cox staining. Behavioral studies using the Barnes maze and Y-maze showed that prenatal NE-100 exposure was associated with cognitive impairment in the young-adult offspring. Furthermore, anxiety-like behavior, which was assessed using the elevated plus maze, increased in NE-100-exposed rats. In addition, Golgi-Cox staining revealed that NE-100 exposure disrupted the morphologies of dendrites resulting in decreased dendritic length and branching in granular neurons in the hippocampal DG and CA4 regions. These findings suggest that prenatal exposure to a Sig-1R antagonist is associated with impaired cognition and anxiety states through disruption of dendritic morphology in hippocampal neurons.

2P-53 Effects of oxytocin and analog, Lipo-oxytocin 1 on paternal behavior and social memory in CD38-/- mice

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Autism is neurodevelopmental disorder characterized by core deficits in sociability, repetitive behavior and restricted interests. One of the perspective fields of research of autism therapy is nanopeptide oxytocin (OT). We have a lot of proofs about roles of oxytocin in the social interactions and social recognition. Now oxytocin is used intranasaly like an experimental drug for treatment patients with autism. To ensure such roles, an analog with long-lasting and effective blood-brain barrier penetration properties should have a benefit, like a therapeutic drug. To assess this, we synthesized a new oxytocin analog, lipo-oxytocin-1 (LOT-1), which conjugates two palmitoyl groups at the amino group of the cysteine and the phenolic hydroxyl group of the tyrosine in the OT molecule. In previous research, LOT-1 demonstrated long-effect on recovery of sociability in open field test in CD 157-/- mice. Now, were investigated OT and LOT-1 on paternal behavior and social memory in CD38-/- mice. That mice are established model of autism symptoms. In parental behavior test CD38-/- male mice demonstrate low pups retrieving scores and high time for completely retrieve pups in the nest. In the case of treatment by OT or LOT-1, 30 minutes after injection CD38-/- demonstrate decreasing of complete time for retrieving and increase retrieving scores. Also after 24 hours of injection, mice treated by LOT-1 demonstrate increased parental behavior than at 30 minutes, while mice treated by OT demonstrate parameters are constant at 30 minutes and 24 hours.In the social memory test CD38-/- male mice demonstrate low level of social discrimination between familiar and novel mice. The OT and LOT-1 recover social memory in CD38-/- mice and the mice demonstrate similar pattern of behavior with wild-type. Finally, we show LOT-1 in vitro effects for oxytocin receptors. Together, these results suggest that LOT-1 has a functional advantage for recovery of social behavioral impairment.

2P-54 Expression of CD38 and TRPM2 in activated microglia and behavioral impact in mice lacking CD38

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CD38, whose molecular weight is 45 kDa, mainly expresses in immune cells and is involved with Ca^{2+} signaling through synthesis of cyclic ADP-ribose (cADPr). Recently, it was reported CD38 is required for maintaining social behavior by regulating the secretion of oxytocin, a hypothalamus hormone being responsible for trust and generosity, in mice (Jin et al., 2007). Previously we showed that expression of CD38 protein was detected in microglia and more expression of microglial CD38 was observed in the lipopolysaccharide (LPS)-injected mouse brain in vivo (Akimoto et al., 2013). Using primary cultured mouse microglia, we also reported that application of LPS (100 ng/mL), but not ATP (100 μM), for 24 h up-regulated the expression of microglial CD38. In addition, knock-down of TRPM2, which colocalize with CD38, significantly up-regulated the expression of microglial CD38 (Noda et al., 2014). Therefore, the question to be answered is whether the CD38-related system in microglial cells is involved in autism as an immuno-inflammation factor. In the present study, we examined that the expression of microglial CD38 was not up-regulated either at 1, 2, 4, and 6 h after application of ATP (1 mM), suggesting that up-regulation of CD38 is limited to LPS-induced inflammatory condition. We will report the regulation of expression of TRPM2 in CD38-deficient microglia by quantitative RT-PCR. In addition, the behavioral change in wild-type and CD38-knock out mice after application of LPS will be reported.

2P-55 Rheb activation disrupts spine synapse formation through accumulation of syntenin in tuberous sclerosis complex

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Rheb is a small GTP-binding protein and its GTPase activity is activated by the complex of Tsc1 and Tsc2 whose mutations cause tuberous sclerosis complex (TSC). We previously reported that cultured TSC neurons showed impaired spine synapse morphogenesis in an mTORC1-independent manner. Here we show that the PDZ protein syntenin preferentially binds to the GDP-bound form of Rheb. The levels of syntenin are significantly higher in TSC neurons than in wild-type neurons because the Rheb-GDP-syntenin complex is prone to proteasomal degradation. Accumulated syntenin in TSC neurons disrupts spine synapse formation through inhibition of the association between syndecan-2 and calcium/calmodulin-dependent serine protein kinase. Instead, syntenin enhances excitatory shaft synapse formation on dendrites by interacting with ephrinB3. Downregulation of syntenin in TSC neurons restores both spine and shaft synapse densities. These findings suggest that Rheb-syntenin signalling may be a novel therapeutic target for abnormalities in spine and shaft synapses in TSC neurons.

2P-56 Prenatal administration of valproic acid or tributyltin alters developmental transient of hippocampal excitability in juvenile rats

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Valproic acid (VPA) is commonly used as an antiepileptic drug and a mood stabilizer, but is also known as a developmental neurotoxicant because animal models of autism spectrum disorder has been established by prenatal exposure to VPA. In this study we aimed to clarify with our electrophysiological approach whether prenatal VPA exposure affects early postnatal development of neuronal circuitry, before the appearance of neurobehavioral change in adolescent period. VPA was orally administered to the pregnant day15 Wistar rats with the concentrations of 300 mg/kg. On the days from PND 13 to 18, field potentials were recorded from the CA1 area of hippocampal slices obtained from the control and VPA groups to test development of the local circuits. Stimulation/response curves of field excitatory postsynaptic potential and those of population spike (PS) enhanced at PND14 and 15 in the VPA group, suggesting that developmental transient of hippocampal excitability may be hastened. On the other hand, similar approach was applied to juvenile rats which were prenatally exposed to 20 mg/kg of tributyltin (TBT), known to be typical endocrine disrupter, and we found a significant decrease in PS amplitude at PND16 in TBT group, which seems to be a retardation of developmental transient of excitability. These results suggest that our electrophysiological approach using hippocampal slices obtained from juvenile rats may be useful to predict the appearance of developmental neurotoxicity after adolescent period.

3P-01 Anti-phosho-GAP-43 pSer96 antibody as a novel molecular marker for axonal growth and regeneration

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GAP-43 is one of the good molecular markers for axonal regeneration after injury. We performed the phosphoproteomics using the growth cone fraction and identified several novel phosphorylation sites of GAP-43. We established the specific antibodies against these sites (Igarashi et al., in preparation). To evaluate one of them, the anti-phospho-GAP-43 pSer96 antibody, as a marker of the axonal regeneration in vivo, we performed the following experiments. We performed a standard protocol for the sciatic nerve axon injury using adult C57B6N mice (*J Neurosci Methods 227 (2014)*). We evaluated crush nerves (on day 3) and uninjured control nerves, by western blotting and immunohistochemistry using pSer96-GAP-43 antibody. As for the regeneration assay, we adopted the confocal micrographs along the longitudinal nerve section and used Regeneration Index, which is designated by the measured distance, from the crush site (point A) to the site at which pSer96-GAP-43 intensity level is half of that at point A (*Shin J.E. et al. Neuron 74 (2012)*). The western blots showed the high intensity of pSer96-GAP-43 staining in the crushed nerve (day 3) and the very low intensity in the control nerve. Immunohistochemistry revealed the co-localization of pSer96-GAP-43 with TUJ-1 at the injury site and the very low staining of pSer96-GAP-43 in the control nerve. The Regeneration Index with pSer96-GAP-43 was almost equivalent to the result using anti-SCG10 antibody, a marker of sensory axon regeneration at an acute phase. Taken together from these results, we concluded the excellent utility of pSer96-GAP-43 antibody in vivo as a regeneration marker.

3P-02 JNK-mediated phosphorylation of GAP-43 promotes axonal growth

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Growth-associated Protein-43 kDa (GAP-43) is involved in the mechanisms regulating the growth of neuronal processes during development and axon regeneration. However, its role for the molecular signaling is poorly understood. Recently, we had performed a quantity phosphorproteomic analysis of axonal growth cones and determined more than 1,000 phosphorylation sites. We identified some novel phosphorylation sites of GAP-43, which are extensively highly phosphorylated in in vivo. By using specific antibodies of phospho-GAP-43 at these sites, we confirmed that these sites were highly phosphorylated not only in the developing brain but also in the regrowing axons of the spinal cord. In in silico and in vitro examinations, we identified c-Jun N-terminal kinases (JNKs) was a major kinase responsible for these sites. Inactivation of a phosphorylation site by a point mutation delayed axonal growth in vitro. These results suggest that JNK-dependent phosphorylation of GAP-43 is one of the important signaling involved in axonal generation and regeneration in vivo. We are now investigating a role of JNK-dependent GAP-43 phosphorylation in the course of neuronal wiring.

3P-03 Neurite outgrowth and bipolarization in PC 12 cells and cerebral cortical neurons induced by a low concentration of bisphenol A

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Endocrine disrupting chemicals (EDCs) are known to exhibit hormone-like effects and inhibit specific nuclear receptors, such as endogenous hormone receptors that maintain homeostasis in many creatures. Recently, we found that some EDCs induce apoptosis, and these EDCs are referred to as "apoptogens". One of them, bisphenol A (BPA), is widely used in the production of plastics, and it has been reported that BPA is harmful to the central nervous system. In a recent study, we showed that BPA induces neurite outgrowth in PC12 cells at a relatively low concentration. In the current study, we demonstrated that BPA also induces PC12 cells and cerebral cortical neurons to form bipolar neuronal cells.

Specifically, we compared cultured PC12 cells treated with 60 μM BPA and 100 ng/ml NGF. As a result, we found that the neurites induced by BPA exhibited fewer neurite branches compared with those induced by NGF, and that BPA also induced cell body bipolarization. The neurite outgrowth was inhibited by treatment with an estrogen receptor antagonist (ICI182780). Furthermore, we also demonstrated that neurite outgrowth could be induced in cerebral cortical neurons derived from fetal rat brain (E17-18) by a low concentration of BPA. Thus, BPA may seriously affect neuronal differentiation by changing these cells into bipolar neuronal cells. We will further investigate the specific molecules related to estrogen receptor (ER) signaling that are involved in the elongation of short neurites in PC12 cells and cerebral cortical neurons. As this phenomenon may contribute to pathophysiological development, a detailed understanding of the molecular mechanism (s), including ERs, that are affected by BPA, will be essential to prevent neurite outgrowth or bipolarization.

3P-04 Activation of RhoA/Rho-kinase by CaMKI-mediated phosphorylation of GEF-H1 regulates neuronal polarization

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Neurons are highly polarized cells with structurally and functionally different processes, an axon and several dendrites. One of minor neurites begins to extend rapidly and differentiates into the axon. During the axonal outgrowth, minor neurites consistently grow and retract to prevent multiple axon formation, thereby maintaining neuronal polarity. However, the molecular mechanisms that maintain neuronal polarity remain largely unknown. Here, we found that retrograde long-range Ca2+ signaling regulates the maintenance of neuronal polarity by increasing RhoA/Rho-kinase activity thorough GEF-H1/Lfc, a RhoA-specific guanine nucleotide exchange factor (GEF), in a Ca2+/calmodulin-dependent protein kinase I (CaMKI)-dependent manner. The minor neurites were retracted by local application of Ca2+ ionophore to axon terminal, probably through the propagation of Ca2+ wave to soma and/or minor neurite. Local application of Rho-kinase inhibitor to minor neurite induced rapid elongation and subsequent multiple axon formation. Moreover, we found that CaMKI phosphorylated GEF-H1 at Thr103. The phosphorylation of GEF-H1 at Thr103 by CaMKI significantly increased its GEF activity. The phosphomimic mutant of GEF-H1 (T103E) impaired neuronal polarization. Taken together, these results suggest that the long-range Ca2+ signaling from axon terminal activates CaMKI and thereby phosphorylates GEF-H1 at other minor neurites. This phosphorylation leads to increase the GEF-H1 activity and in turn to stimulate the RhoA-Rho kinase activity to prevent the formation of multiple axons.

3P-05 Function and expression of the mouse RasGEF1 family proteins

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The mammalian RasGEF1 is known as one of the protein families containing RasGEF domain which is guanine nucleotide exchange factors for Ras-like small GTPases. RasGEF1 protein family consists of 3 distinct types 1a, 1b, and 1c. It was reported that RasGEF1a and 1b activate a member of the Ras protein family Rap2 which is known to synaptic function, modulate cell adhesion and cell morphology. RasGEF1b was shown to interact with a small GTPase Cdc42 on the midbody during cell division. In terms of gene expression, RasGEF1a is predominantly expressed in the central nervous system in human, and RasGEF1b is expressed in midbrain and hindbrain in zebrafish. However, detailed information on the RasGEF1 family is largely unknown. In this study, we cloned their cDNAs and analyzed expression in mouse brain and effect of their over-expression in culture cells. RT-PCR analysis of mouse tissues showed RasGEF1c was predominantly expressed in brain. Microarray analysis during mouse cerebellar postnatal development showed that RasGEF1a was up-regulated, whereas RasGEF1b and 1c were down-regulated. According to in situ hybridization data of Allen Brain Atlas, mRNA of each RasGEF1 type displays widespread but differential distribution patterns in mouse brain. Our preliminary data also suggested neurite outgrowth and morphological changes of cultured cells exogenously over-expressed RasGEF1 proteins. Together, these data suggest that each member of the RasGEF1 family may play a role in cell signaling during specific developmental stages and in distinct brain regions.

3P-06 Lemur Kinase 1A（LMTK1A）may coordinate membrane and cytoskeletal dynamics in neurite outgrowth.

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Lemur kinase 1A（LMTK1A）, a substrate of CDK5/p35, is a Ser/Thr kinase highly expressed in mammalian brain. LMTK1A consists of an N-terminal kinase domain and long C-terminal tail. It is palmitoylated at three cysteine residues in the N-terminal region that anchors it to recycling endosomes. We have previously reported that LMTK1A inhibits neurite outgrowth via modulation of Rab11A, a small GTPase, which regulates recycling endosome traffic. However, it is unknown yet how the kinase activity is involved in neurite outgrowth. Neurite outgrowth is complicated processes involving both cytoskeletal dynamics and membrane transport, but it is not known how they are coordinated. In this study, we examined the role of kinase activity of LMTK1A in its interaction with the cytoskeletons, especially microtubules（MTs）and actin filaments. We found that wild type（wt）LMTK1A was predominantly localized in pericentrosomal area containing MTOC, while kinase negative（kn）mutant of LMTK1A is distributed evenly throughout the whole cytoplasm. Further, in the neurite tips wtLMTK1A was accumulated at the tip of MTs and did not invade into the cortical actin-rich region. In contrast, knLMTK1A was found in the actin-rich cortical region as well as MT-rich cytoplasm. In addition, the pericentrosomal localization of LMTK1A was abolished when MTs were destabilized with nocodazole, but when nocodazole was washed out and MTs regrew, LMTK1A re-localized to the pericentrosomal area. Although it is not yet clear how LMTK1A affects organization and dynamics of MTs, these results suggest that LMTK1A regulates a critical step of membrane transport from MTs to the cortical actin in neurite tip, which is necessary for neurite outgrowth.

3P-07 Role of *N*-glycans to a function of a trans-membrane protein, seizure-related gene 6 (sez-6)

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In the developing central nerves system, trans-membrane proteins play important roles in various steps for the construction of the neuronal circuit including the cell-migration, differentiation, axon elongation, dendritic formation and synaptogenesis. It is well known that trans-membrane proteins are modified by addition of *N*-glycan. *N*-linked glycan regulates functions of proteins, since it contributes to folding and stability of proteins. Seizure-related gene 6（sez-6）is a trans-membrane protein expressed in cerebral cortex and hippocampus, modulates dendritic branching. Sez-6 contains eleven putative *N*-glycosylation sites. The role of *N*-glycans on sez-6 is still obscure. To understand the function of *N*-glycans on sez-6, we investigated neuro2a cells overexpressing sez-6 mutants. Eleven *N*-glycosylation sites of sez-6 are divided to three clusters which we termed sugar chain（SC）1-3, SC4-7, SC8-11. The mutants we prepared lacked one, two or all *N*-glycosylation clusters. Mutants（sez-6 SC1-3, SC8-11）having one *N*-glycosylation cluster at the position as well as a mutant lacking all clusters（sez-6 Δ1-11）were transported to the cell membrane but were not distributed to fine processes. On the contrary, sez-6 SC4-7 mutant and mutants lacking one *N*-glycosylation cluster（sez-6 ΔSC1-3, ΔSC4-7, ΔSC8-11）were well distributed on the cell membrane like wild type sez-6. Among mutants behaving like wild type sez-6, sez-6 ΔSC1-3 and ΔSC4-7 reduced neurite formation. Interestingly, sez-6 ΔSC4-7 mutant had no effects on the formation of filopodia-like protrusions, which were induced by the overexpression of other mutants and wild type sez-6. Ours results suggest that *N*-glycans on sez-6 modulate cell morphology by maintaining proper distribution of sez-6 protein on the cell membrane.

3P-08 Deletion of FILIP influenced the development of peripheral nerve

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FILIP（filamin A interacting protein）controls neuronal cell migration during the cortical development. We recently reported that FILIP controls neuronal cell morphology via binding to non-muscle myosin IIb. We here found that FILIP was involved in the development of the sensory system. As we observed the expression of FILIP in the neurons of the dorsal root ganglia throughout the development, we investigated the development of the dorsal root ganglia of the FILIP knockout mice. We found that there were mild abnormalities in the neuronal density in the developing dorsal root ganglia of the FILIP knockout mice at the embryonic age. As we suspected developmental delay of the sensory system in the FILIP knockout mice, we studied the innervation of the peripheral nerve to the skin of the FILIP knockout mice using the whole-mount immunohistochemical method. We observed that the delayed innervation of the peripheral nerve to the skin of the FILIP knockout mice. As FILIP controls intracellular distribution of the non-muscle type myosin IIb that plays an important role in the axon elongation, we considered that the deletion of FILIP influenced the elongation of the neurites of somatosensory neurons via myosin IIb.

3P-09 Visualizing single-neuron identity defined by Pcdh-β cluster in mouse brain

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The brain contains a huge number of neurons that have diverse characteristics participating in discrimination between individual neurons. It has been speculated that clustered protocadherins (Pcdhs), which encode cadherin-related transmembrane proteins as gene clusters in vertebrate genome, could provide these kinds of neuronal identity. The murine Pcdhs are further classified into three subfamilies : Pcdh-α (14 genes), Pcdh-β (22 genes), and Pcdh-γ (22 genes). Their loss of function in mice revealed that the Pcdhs play important roles in neuronal survival, axonal projection, synaptic connectivity, and several brain functions including learning and memory. As revealed by histological examinations and single-cell RT-PCR, the Pcdhs show the scattered expression in each cerebellar Purkinje cell. The scattered expressions of the Pcdhs will provide a potential neuronal identity at the single-neuron level. The involvement of the scattered Pcdh expression in neural circuit formation has been inferred on the basis of several genetic analyses including loss of Pcdh-γ and loss of gene regulators of the Pcdhs (CTCF and Dnmt3b). However, several key questions remain unanswered. For example, are the Pcdh expressions scattered in other neuron type? Are the Pcdh expressions dynamically changed in a live neuron? Does the Pcdh expression depend on cell-lineage? In order to answer these questions, we generated knock-in mice that harbor cDNA encoding red fluorescent protein, tdTomato, under the control of endogenous Pcdh-β3 promoter. The mice showed scattered tdTomato fluorescence in various neuron types, including cerebellar Purkinje cells, hippocampal CA1 pyramidal cells, dentate gyrus granule cells, cerebellar molecular layer interneurons, etc. The newly developed antibody against tdTomato enables a high signal-to-noise ratio visualization of single-neuron identity. We are currently addressing the key questions about scattered Pcdh expression.

3P-10 Analysis of protein synthesis in growth cones of rat dorsal root ganglion neurons

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Although the concept of local translation in neurons is widely accepted, there is a debate about whether axonal translation occurs. Herein, we analyzed the presence of ribosomal proteins in the growth cones of rat dorsal root ganglion (DRG) neurons, by immunofluorescence analysis. Actual protein synthesis was monitored by the surface sensing of translation (SUnSET) method. Structural analysis was performed using atomic force microscopy (AFM). DRG neurons were prepared from embryonic rats and dissociated, then resuspended in culture medium and plated onto dishes. They were maintained in DMEM containing CPT-cAMP to facilitate axon elongation and growth cone formation for 48h. Neurons were stimulated with brain-derived neurotrophic factors (BDNF) for 30min to induce translational activation under the presence of puromycin. Low dose puromycin binds to elongated peptide chain, thus newly synthesized proteins coupled with puromycin can be detected by anti-puromycin. After AFM observation, specimens were labeled with Alexa 488 phalloidin for actin filament staining, followed by anti-ribosomal protein P0/P1/P2 antibody. Some specimens were labeled with anti-puromycin antibody and anti-P-eEF2 (Phosphorylated eukaryotic elongation factor 2). Immunofluorescence images revealed that actin filaments were distributed in the peripheral region and in the filopodia. The positive regions of ribosomal protein P0/P1/P2 were closely related to the distribution of actin filaments. AFM images showed that high regions of DRG tended to be rich in actin filaments and ribosomal protein P0/P1/P2, compared with low regions of DRG. BDNF decreased the phosphorylation of eEF2, indicating enhancement of translation in growth cones. Indeed, BDNF increased puromycin signaling, which suggests increased protein synthesis in growth cones. These results are discussed in relation to locally-synthesized proteins and are related to the three-dimensional structure of DRG.

3P-11 Drebrin stabilizes CaMKIIβ in core region but not in postsynaptic density of dendritic spine

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Dendritic spines are actin-rich small protrusions that contain postsynaptic components of excitatory synapse. Many actin-binding proteins have been identified as spine-resident protein, and they regulate actin-cytoskeleton through diverse processes. Drebrin is a major F-actin binding protein in neurons, and is localized in the center of dendritic spines. Drebrin regulates dendritic spine morphogenesis and spine targeting of synaptic proteins such as spikar, PSD-95 and NMDA receptors. Moreover, drebrin is involved in neurological diseases（eg., Alzheimer's disease and schizophrenia）. Although increasing evidences show that drebrin plays pivotal roles in neurons, how drebrin interacts with other proteins in spines is much less known. In this study, we isolated CaMKIIβ as a drebrin-binding protein by yeast two-hybrid screen and investigated the interaction of drebrin-CaMKIIβ in dendritic spines. CaMKIIβ is localized in dendritic spines more than in dendritic shaft. However, drebrin knockdown（KD）caused diffuse localization of CaMKIIβ in dendrites, suggesting that drebrin anchors CaMKIIβ in dendritic spines. To analyze drebrin-dependence of CaMKIIβ stability in dendritic spine, we performed fluorescence recovery after photobleaching（FRAP）experiments on individual dendritic spines. We calculated the stable fraction from the time-series of fluorescence intensity of GFP-CaMKIIβ before and after photobleaching. The stable fraction of GFP-CaMKIIβ in drebrin-KD neurons was greater than that of control neurons. In addition, NMDA receptor stimulation increased the stable fraction of CaMKIIβ in parallel with drebrin-dislocation from dendritic spines. These results suggest that drebin-loss increases the stable fraction of CaMKIIβ in dendritic spines. Therefore, we think that drebrin-independent stable pool became dominant in drebrin-KD neurons and synaptic activity regulates the accumulation of drebrin-independent CaMKIIβ in dendritic spines. Taken together, our study suggests that there are two stable pools of CaMKIIβ in spines, drebrin-dependent and drebrin-independent pools.

3P-12 Structural basis for cargo binding and autoinhibition of retrograde transport adaptor Bicaudal D1

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Bicaudal D1（BICD1）mediates the attachment of specific cargo to cytoplasmic dynein. The attachment regulation toward cytoplasmic dynein by BICD1 plays an essential role in the minus end-directed intracellular retrograde transport along microtubules. Dysfunction of cargo sorting by BICD1 to cytoplasmic dynein causes various diseases such as dominant congenital spinal muscular atrophy（DCSMA）. BICD1 possess three α helical coiled coil（CC）regions：an N terminal CC1, a central CC2, and a C terminal CC3 region. The N terminal region of BICD1, containing CC1 and a portion of CC 2, associates with cytoplasmic dynein, whereas the BICD1 CC3 has an important role in cargo sorting, including intracellular vesicles associating with the small GTPase Rab6 and the nuclear pore complex Ran binding protein2（RanBP2）, and inhibiting the associating with cytoplasmic dynein by binding to the CC1. The cargo binding of CC3 promotes association of cytoplasmic dynein by inducing the release of CC1 from CC3. However, the molecular mechanisms, by which the CC3 binds cargo factors and CC1, are unknown. Here, we report the X-ray crystallographic structural analysis of CC3 and the mutational binding experiments with Rab6, RanBP2, and CC1.
Firstly, we succeed in the crystallization and the structural determination of CC3 by X-ray crystallographic analysis with a resoution of 1.50 Å. The structure revealed that CC3 forms a parallel homodimeric coiled coil with leucine zipper-like heptad repeat sequence. Next, we attempted to determine the binding site for cargo factors on CC3. The mutational binding study based on the CC3 structure indicated that CC3 possesses the binding surface for two distinct cargos, Rab6 and RanBP2, and that the CC1 binding site overlaps with the Rab6-binding site. These findings suggest a molecular basis for cargo recognition and autoinhibition of BICD1 proteins on the dynein-dependent intracellular retrograde transport.

3P-13 The contribution of the di-leucine motif in p35 to determine the distribution difference between neuronal cyclin-dependent kinase 5 (Cdk5) activators p35 and p39

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Cdk5 is a Proline-directed Ser/Thr kinase regulates a variety of neuronal activities. The Cdk5 activator p35 or p39 also determine the distribution of Cdk5. p35 and p39 are isoforms with a high homology in the C-terminal Cdk5-activating domain but low homology at N-terminal except for the N-myristoylation consensus sequence and the Lys cluster. Previously, we have reported that myristoylation and the Lys cluster are important in their cellular distribution. Though both p35 and p39 localizes at the perinuclear region and plasma membrane, p35 distribute more in perinuclear region than plasma membrane and p39 distribute in contrary pattern to p35. Because myristoylated proteins are found in various intracellular membrane-bound compartments, it is unlikely that only myristoylation determines the specific membrane compartments. To answer this question, we interested in the di-leucine motif, which was found as an ER retention motif at first, and has been also considered to contribute the sorting of protein. p35 has this motif, but p39 does not. To search the effect of this motif, we constructed the deletion mutant of p35 di-leucine motif, p35dCT, and chimera mutant of p39 replacing C-terminal with p35 C-terminal that contained the di-leucine motif. The distribution pattern of p35 dCT in N2A cells exhibited less in perinuclear region and more at plasma membrane than that of WT p35, so to say the intermediate pattern between p35 and p39. The distribution of chimera also exhibited the intermediate pattern between p35 and p39. The di-leucine motif participates to determine the specific membrane localization in the cell, but it is not sufficient. Based on these date, we discussed about the mechanism of determination of distribution of p35 and p39.

3P-14 The effects of accumbal BDNF overexpression on aversive memory

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The mesolimbic dopamine pathway, composed of dopaminergic neurons in the ventral tegmental area（VTA）and their projections to the nucleus accumbens（NAc）is affected by aversive stimuli. Abnormalities in this pathway are implicated in neuropsychiatric disorders including depression. It is known that Brain-derived neurotrophic factor（BDNF）in the NAc is increased by aversive stimuli, however, how accumbal BDNF affect aversive memory structure remains unclear.Here we examined the effects of BDNF protein overexpression in the NAc on acquisition, retrieval, and extinction of aversive memory by combining cell type-specific/time controllable BDNF mRNA overexpression technique with passive avoidance test. [Experiment 1] To investigate the effects of BDNF overexpression on aversive memory acquisition and retrieval, animals were first separated in BDNF overexpression and control groups. The animals were then exposed to a light compartment, and when they entered a dark compartment, an aversive foot shock（0.4 mA, 5 sec）was delivered（acquisition training）. The following day, animals were exposed to the same procedure as acquisition training without foot shock（retrieval test）. The latency to enter the dark compartment was not different between groups in acquisition and retrieval phases. [Experiment 2] To investigate the effects of BDNF overexpression on aversive memory extinction, animals first received acquisition training. Then, they were separated into BDNF overexpression and control groups. After 15 days from acquisition training, animals were exposed to the light compartment. Subsequently, animals received extinction training in the dark compartment for 5 min without foot shock. The following day, the animals were placed in the light compartment（extinction test）. The latency to enter the dark compartment decreased in overexpression group in extinction test but not in acquisition or retrieval phases.Taken together, our data suggest that accumbal BDNF overexpression enhances extinction but not acquisition or retrieval of aversive memory.

3P-15 Difference in a translation start site in BDNF exon I and exon IX.

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Brain-derived neurotrophic factor（BDNF）is a member of neurotrophin family and plays a crucial role in expressing various neuronal functions including neuronal survival, differentiation, and synaptic plasticity. Because of the multiple promoters and alternative splicing, multiple BDNF transcripts are produced. Although it is well known that these multiple BDNF transcripts possess a common translation start site in exon IX, another translation start site exists in 3'end of exon I. It is suggested that BDNF precursor protein（preproBDNF）with 8 additional amino acid residues at the N-terminus would be produced from BDNF exon I-IX mRNA. However, it is unclear that the translation start site in BDNF exon I is functional. In this study, therefore, we focused on the difference in a translation start site in BDNF exon I or exon IX. We constructed expression vectors of preproBDNF translated from BDNF exon I-AUG（termed ppBDNF exon I）and that from exon IX-AUG（termed ppBDNF exon IX）. We found that the expression level of ppBDNF exon I was highly than that of ppBDNF exon IX in NIH3T3 cells. This result suggests that the translation start site in BDNF exon I is functional, and the translation efficiency of the translation start site in BDNF exon I is higher than that in exon IX. We are now examining differences in intracellular localization of these BDNF protein in NIH3T3 and neuronal cells.

3P-16 FGF-1 release induced by oxidative stress enhances apoE/HDL generation of rat astrocytes in the autocrine manner

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FGF-1 release from rat astrocytes to enhance apoE/HDL generation under oxidative stress without inducing apoptosis We previously observed that the production and release of fibroblast growth factor (FGF-1) are increased in rat astrocytes during in vitro long-term culture, that FGF-1 enhances the generation of apoE-containing high density lipoproteins (apoE/HDL). In this study, we examined effects of oxidative stress on release of FGF-1 from cultured rat astrocytes. The treatment of rat astrocytes with 100 μM hydrogen peroxide (H2O2) for 10 min enhanced FGF-1 release without inducing apoptosis. The conditioned medium prepared from the cells cultured in a fresh medium after the treatment with H2O2 had the FGF-1-like activities, which enhanced cholesterol synthesis, signalings to phosphorylate Akt and ERK, and apoE secretion. The oxidative stress induced by H2O2 enhanced the release of cytosolic proteins such as HSP70 and HSP90 in addition to FGF-1. The addition of lipoproteins such as low density lipoproteins (LDL), furthermore, canceled H2O2-induced release of FGF-1 and cytosolic proteins. These findings suggest that oxidative stress is one of the candidates which triggers FGF-1 release from astrocytes in the brain accompanied with the release of cytosolic proteins.

3P-17 TNFα and IL-1β are differentially induced in microglia through distinct combination of MAP kinases

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Stimulation of rat microglia with endotoxin in vitro induces production of the inflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin 1beta (IL-1β) along with superoxide anion (O_2^-) and nitric oxide (NO). In this study, we investigated the role of O_2^- and NO in the induction of TNFα and IL-1β in microglia. The lipopolysaccharide (LPS)-inducible TNFα was significantly suppressed by pretreatment with the O_2^- scavenger, but not by the NO scavenger, while the LPS-inducible IL-1β was strongly inhibited by pretreatment with the NO scavenger, but not by the O_2^- scavenger. On the other hand, an O_2^--donor and an NO-donor induced TNFα and IL-1β in microglia, respectively. These results suggested that O_2^- and NO activate each specific signaling cascade, and through which induce TNFα and IL-1β in microglia, respectively. LPS-dependent TNFα induction was significantly suppressed by c-Jun N-terminal kinase (JNK) and p38 inhibitors, whereas the IL-1β induction was significantly suppressed by extracellular signal-regulated kinase(ERK)and JNK inhibitors. These results indicated that TNFα and IL-1β are induced through the action of JNK/p38 and ERK/JNK, respectively. In fact, the O_2^--donor could activate JNK/p38 in microglia, and the NO-donor could activate ERK/JNK. Taken together, these results showed that TNFα and IL-1β are differentially induced through the different combination of mitogen-activated protein kinases (MAPKs) in endotoxin-stimulated microglia.

3P-18 Microglia regulate the cytokine/chemokine dynamics in the brain and enhance the functional maturation of blood-brain barrier.

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The blood-brain barrier (BBB) permeability is regulated by various cells comprised of neurovascular unit (NVU). Microglia are already present in the brain prior to the brain vascular development at the embryonic stage and once the brain capillaries are formed, abundant microglia exist around the capillaries. However, microglial role on the functional maturation of the BBB is still unclear. In this study, we investigated the roles of microlia in the BBB maturation. We used in vitro BBB model comprised of endotherial cells, pericytes, and astrocytes(Pharmaco cell co). When we added microglia on the astrocytes of the in vitro BBB model (brain side) during the maturation period (1-4 DIV), significant increase in the transendothelial electrical resistance (TEER) and the expression levels of tight junction proteins (Claudine-5) were detected. On the other hand, when we added LPS-activated microglia, significant decrease in the TEER and the expression levels of tight junction proteins (Occludin, Claudine-5) were detected. We measured the amounts of cytokines/chemokines in the brain sides of these two situations comprehensively and quantitatively using MAGPIX system (millipore). The dynamics of the cytokines/chemokines are totally different between two situations. We have detected two factors which exhibited the opposite dynamics in these two situations, suggesting that these factors are related to the microglia-induced maturation of BBB function. Currently we are examining the direct effects of these factors on the BBB functional maturation.

3P-19 Function of activated microglia following hypoglossal nerve axotomy.

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Microglia, generally considered to be immune cells of the central nervous system (CNS), are involved in many types of inflammatory processes in the brain. They are critical in developmental processes and are essential for the maintenance of neuronal homeostasis. Experimental axotomy such as hypoglossal nerve transection causes neurodegeneration and glial reactions. After nerve injury, microglia near the injured motor neurons are stimulated, migrate toward the injured neurons, and wrap up motor neuron cell bodies. This model appear to be suitable for studying microglia in the brain because there is no disruption of the blood brain barrier, and macrophage infiltration does not occur. It is thought that the perineuronal microglia protect axotomized motor meurons, whereas non-perineuronal microglia leads to gradual cell death of the injured motor neurons. Recent studies have demonstrated that under specific polarization conditions microglia develop into different phenotypes, termed M1 and M 2. We analyzed the functional relevance of microglia in motor neurons, we examined the M1, M2 phenotype marker and growth factor expressions in hypoglossal nerve transection model. The M1 markers, neurotrophic factors and phagocytosis-related factors were induced after hypoglossal nerve axotomy, however, M2 markers were not changed.

3P-20 Possible involvement of the secretion-related protein CAPS2 in regulation of dynorphin, one of the endogenous opioids, secretion

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Endogenous opioid dynorphin (Dyn) is known to be produced as a precursor protein pro-Dyn and packed into large dense-core vesicle (LDCV), which is widely expressed in the brain including hippocampal dentate gyrus granule cells, and activity dependently secreted to CA3 region via mossy fiber axon terminals. It is relatively well known that their target receptor κ-opioid receptor and their physiological functions. However, the release machinery of Dyn and its related proteins have not been well understood. Calcium-dependent protein for secretion 2 (CAPS2) is identified initially as a cytosolic protein associated with LDCV in endocrine cells and thought to be involved in LDCV secretion. We previously reported that CAPS2 is highly associated with secretion of neurotrophin BDNF and act as facilitator of their secretion. In the present study, we show that the possible involvement of CAPS2 in regulation of Dyn secretion, such as the localization of Dyn and CAPS2 at hippocampus, the alteration of Dyn distribution in CAPS2 KO mice and the alteration of Dyn release probability in the presence and absence of CAPS2.

3P-21 Effect of repetitive and transcranial near infrared irradiation on inflammatory processes in the brain of rats.

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Immune activation processes including microglial activation and increased cytokine/chemokine expression in the brain have been implicated in the pathology of neurodevelopmental disorders such as schizophrenia and autism. Low-level laser therapy (LLLT) has been used in the treatment of inflammatory pathologies, reducing both pain and acute inflammatory process. In this study we examined possible therapeutic effects of LLLT on immune activations in the brain of rat offspring of dams exposed to gestational polyriboinosinic-polyribocytidilic acid (poly I：C). Pregnant rat dams were given intraperitoneally either with poly I：C or saline at gestational day 13. At 8 weeks after birth, offspring of dams treated by poly I：C or by saline were subjected to LLLT. All the animals were photo-irradiated on the frontal cortex under pentobarbital anesthesia, once daily for 3 days. Energy densities were either 0 (sham), 45, 90, 180, 360, or 720 J/cm2. The wave length was 600-1600 nm and its output was 1800 mW. Twenty-four hours after the last irradiation, animals were sacrificed and their brains were subjected to measurement of mRNA expression of immunological markers by real time PCR. The body temperature of rats tended to elevated after photo-irradiation of the frontal cortex. Expression levels of several cytokine (IL-1beta, IL-6, IL-10) were shown to decrease in the prefrontal cortex of photo-irradiated animals in dose-dependent manner. The result suggests LLLT may affect brain function through modulation of expression of cytokines. Further investigations with regard to behavioral changes after LLLT are ongoing.

3P-22 Setpoint of core body temperature is remodeled prior to hibernation in a obligatory hibernator, Mesocricetus auratus.

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Hibernation is a strategy with profound suppression of metabolic rate, motility, and body temperature in order to avoid energy wastes and survive severe winter or harsh environment with a little or no food. It has been suggested that mammalian hibernators remodel their body to develop tolerance against many types of stresses including severe hypothermia, starvation, ischemia–reperfusion injury, and obesity, in the pre–hibernation period, whereas physiological and molecular mechanisms of such adaptive remodeling remain largely unclear. To identify when and how the adaptive remodeling starts during the pre–hibernation period, we utilize syrian golden hamster（Mesocricetus auratus）, which initiates hibernation after prolonged exposure to short day and cold acclimation condition（about 4～12 weeks）. We found that a core body temperature（Tb）was decreased after 8 weeks of exposure, which preceded entrance into hibernation, suggesting that a setpoint of Tb started to be lowered during pre–hibernation period and minimized in hibernation period. The remodeling of Tb setpoint and the efficiency of hibernation induction were affected by animals'body weight. These observations suggest that lowering Tb setpoint is one of crucial aspects of the adaptive remodeling that precedes hibernation induction.

3P-23 Analyses of metabolic changes of neurons using cultured hippocampal slices

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Metabolic changes of the brain have been analyzed as indices for neuronal activity. For examples, the reduced glucose（Glc）consumption and the increased lactic acid（LA）production in the patient of Alzheimer disease are regarded indices of lowered neuronal activity and of disordered oxygen supply, respectively. However, those interpretations may include prejudice. To know the cellular bases of those metabolic changes, we monitored here those changes by HPLC using the hippocampal slice culture that preserves neuronal circuit but allows pharmacological interventions. Induction of LTP with forskolin did not produce significant changes in either Glc consumption or LA production. However, 3 repeated inductions of LTP, known to produce a long–lasting synaptic enhancement coupled with synaptogenesis, brought about an increased Glc consumption leaving LA production unaltered. An application of bicuculline, known to produce epileptic excitation, brought about increases in both Glc consumption and LA production. Those results indicate that the increase in Glc consumption indicates physiologically elevated neuronal activity, while that in LA production indicates pathologically elevated activity. In the cultured slice, the activation of metabotropic glutamate receptor induces LTD and the repeated induction of LTD leads to a long–lasting synaptic suppression coupled with synapse elimination. Neither single LTD nor repeated LTD evoked the changes in Glc consumption and LA production, suggesting that the repetitive–LTD–operated synapse suppression is a physiological process.

3P-24 Shati/Nat8l induces axon outgrowth via energy metabolism in the primary cultured neurons of mice

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We have identified a novel molecule, Shati/Nat8l in the nucleus accumbens (NAc) of mice repeatedly treated with methamphetamine (METH). Shati/Nat8l produces N-acetylaspatate (NAA) from aspartate and acetyl-CoA. Previously we reported that overexpression of Shati/Nat8l in NAc attenuates the response to METH via N-acetylaspartylglutamate (NAAG ; which is derived from NAA)-mGluR3 signaling in the mice brain. In the present study, to clarify the type of cells that produce Shati/Nat8l, we carried out in situ hybridization for the detection of Shati/Nat8l mRNA accompanied by immunohistochemical studies using serial sections of mice brain. Shati/Nat8l mRNA was detected in neuronal cells, but not in astrocytes or microglia cells. Next, we investigated the function of Shati/Nat8l in the neuronal cells in mice brain ; then, we used adeno-associated virus vector containing Shati/Nat8l for transfection and overexpression of Shati/Nat8l protein into the primary hippocortical neurons to investigate the contribution to neuronal activity of Shati/Nat8l. Overexpression of Shati/Nat8l in the mice primary hippocortical neurons induced axonal growth but not dendrite elongation at day 1.5 (DIV). This finding indicated that Shati/Nat8l contributes to neuronal development. LY341495, a selective group II mGluRs antagonist, did not abolish this axonal growth, and NAAG itself did not abolish axon outgrowth in the same cultured system. The cultured neurons overexpressing Shati/Nat8l contained high ATP, suggesting that axon outgrowth is dependent on energy metabolism. This study shows that Shati/Nat8l in the neuron may induce axon outgrowth via ATP synthesis and not through mGluR3 signaling.

3P-25 LAMP2C, a receptor for novel lysosomal RNA/DNA degradation systems, possesses an arginine-rich motif that mediates RNA/DNA-binding

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Degradation of macromolecules by lysosomes is a fundamental event for biological homeostasis in cells and tissues including neurons and brain. Neurons are cells rich in RNA, and aberrant function and accumulation of RNAs in neurons are reported to cause various neurodegenerative diseases. Degradation of cellular RNA can be an important issue for the better understanding of homeostasis of neuron and pathogenesis of such diseases. We previously discovered novel lysosomal degradation systems in which RNA and DNA are directly imported into lysosomal lumen and degraded. These systems, which we termed RNautophagy/DNautophagy (hereafter abbreviated as RDA), are ATP-dependent and a lysosomal membrane protein, LAMP2C was identified as at least one of receptors for both RNA and DNA. In this study, we examined the mechanisms underlying recognition of nucleic acids by LAMP2C. We found that the cytosolic sequence of LAMP2C possesses features of the arginine-rich motif, a well-known RNA-recognition motif found in a wide range of RNA-binding proteins. Substitution of arginine residues in the cytosolic sequence of LAMP2C completely abolished its binding ability to both RNA and DNA. A scrambled form of the sequence showed affinity to both nucleic acids equivalent to that of the wild-type sequence, as is the case for other arginine-rich motif. In addition to these results, we also found that cytosolic sequences of other LAMP family proteins, LAMP1 and CD68/LAMP4, also possess multiple arginine residues, and show affinity for nucleic acids. Together with the fact that RDA activity is not completely abolished in lysosomes derived from LAMP2 deficient mice, these results suggests the existence of other receptors in RDA. Our results provide further insight into the mechanisms underlying RDA, and may contribute to a better understanding of lysosome function. We would also like to discuss physiological roles of RDA and their possible involvement in diseases.

3P-26 The protective effects of high dose adenosine deaminase during oxygen glucose deprivation on rat corticostriatal slices.

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Adenosine increase in response to ischemic brain insults and adenosine receptors are one of the main therapeutic targets. However, some controversy remain regarding to adenosine effects in striatum, where abundant A2aR receptors exist and A2aR antagonists have been shown to be either protect or increase striatal damage. In the present study, we demonstrate that striatal neuroprotection induced by adenosine deaminase（ADA）(EC 3.5.4.4), the enzyme catabolizes deamination of adenosine to inosine and ammonia and decrease adenosine concentration. We used the oxygen/glucose deprivation（OGD）for 10 minutes as model of ischemia in corticostriatal brain slices. In electrophysiological assessment, we used adult Wistar Thy−1.2 promoter channelrhodopsin−2 Venus transgenic rats of both sexes to enable optgenetical evaluation. We recorded time course of corticostriatal extracellular field potential（FP）evoked via a bipolar stimulating electrode placed in the corpus callosum as well as striatal field potential evoked by optogenetic stimulation to striatum（fOPT）. In control group, 30 minutes after OGD, FP and fOPT were decreased. Application of the ADA in artificial cerebrospinal fluid during OGD significantly suppresses the OGD induced reduction. In histological evaluation, dead cell counts with propidium iodide also support this protective effect. These results show that ADA plays a neuroprotective role in corticostriatal pathway.

3P-27 QUANTITATIVE ANALYSIS OF GSK3β ACTIVITY IN CELLS AND BRAINS

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Tauopathy neurodegeneration including Alzhimers disease (AD) is characterized by the intracellular accumulation of hyperphosphorylated tau. More than 40 phosphorylation sites are reported in AD tau. A characteristic feature is Ser-Pro or Thr-Pro Phosphorylation, which are catalysed mainly by GSK3β and CDK5. Together with their co-localization with tau aggregates in brains of the patients, CDK5 and GSK 3β have been considered as prime candidates for AD pathogenesis. Both GSK3β and CDK5 are Proline directed protein kinases, but their phosphorylation site preference is somewhat different. Phosphorylation of tau by GSK3β is accelerated by prime phosphorylation by CDK5. However, it is not known well how these protein kinases cooperate in generation of AD abnormal phosphorylation epitopes. GSK3β activity is regulated by phosphorylation at Ser9, and its activity is usually estimated by Ser9 phosphorylation using phospho-specific antibody. The use of phospho-Ser9 antibody enabled us to measure relative changes in the GSK3β activity but did not provide the absolute kinase activity. Considering GSK3β as a primary pathological kinase, it is important to understand the activity of GSK3β in pathological brains. In this study, we measured the absolute activation of GSK3β in various cultured cells, neurons and mouse brains using Phos-tag SDS-PAGE. GSK3β has two major phosphorylation sites ; Ser9 and Tyr216. Phosphorylation at Ser9 inactivates GSK3β whereas that at Y216 is proposed to activate the kinase. We first analysed GSK3β phosphorylation in CHO-K1 cells using Phos-tag SDS-PAGE, in which phosphorylated GSK3β was retarded extraordinarily. GSK3β was separated into three bands ; non-phosphorylated, Tyr216 phosphorylated, and Tyr216 and Ser9 double phosphorylated GSK3β. GSK3β expressed in CHO-K1 cells were mostly active with Tyr216 phosphorylation. Insulin treatment increased Ser9 inhibitory phosphorylation but most part of GSK3β still remained in an active phosphorylation state. We would like to report GSK3β activation in different types of cells and different regions of brain.

3P-28 An autophagy-inducing herbal extract alleviates the pathology of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disease and is the most common form of dementia in the elderly. AD patients suffer from memory impairment and cognitive deficits. The lack of disease-modifying therapeutics for AD has imposed a huge social burden. It thus becomes a pressing issue to develop effective therapeutic and prevention strategies against AD. The pathological hallmarks of AD include formation of amyloid-β (Aβ) plague in extracellular space and aggregation of Tau protein within neurons. It is widely believed that accumulation of Aβ and Tau is a causative event in the AD pathogenesis. Both Aβ- and Tau-elicited neurotoxicity could significantly contribute to the onset and progression of AD. We have now identified a herbal extract (HE238) that exhibit potent biological efficacy in suppressing the neurotoxicity elicited by amyloidopathy and tauopathy. Our data show that treatments with HE238 can effectively induce autophagy and neprilysin to promote the clearance of Aβ and Tau in cultured cells. Oral administration of HE238 for 2 month also significantly improves the cognitive function in an Aβ42-injection mouse model. Together, the dual modalities existing in the active ingredients of HE 238 obviously present an enormous resource for AD-alleviating agents.

3P-29 The abundance of nonphosphorylated tau among heterogeneously phosphorylated tau species in vivo in mouse and human tauopathy brains

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Tauopathy is neurodegenerative diseases characterized by aggregates of hyperphosphorylated tau. Previous intensive studies have identified many disease-related phosphorylation sites on tau. However, it is not understood how tau is hyperphosphorylated and how hyperphosphorylated tau forms aggregates. It is neither clear yet what extent these sites are phosphorylated in disease brains but also normal brains. Most previous studies have used phospho-specific antibodies in analysis of tau phosphorylation. They were useful but did not provide information of nonphosphorylated tau. Here, we applied the method of Phos-tag SDS-PAGE, in which phosphorylated proteins are retarded extraordinary, making it possible to analyze in vivo phosphorylated, as well as nonphosphorylated, tau more quantitatively. Tau in adult mouse brains was heterogeneously phosphorylated with nonphosphorylated 0N4R isoform strongest. Perinatal tau and tau in cold water-stressed tau showed the similar extent of high phosphorylation. Tau in normal aged human brain was separated into more than 8 discrete bands. Among them, nonphosphorylated 0N3R and 0N4R tau were strongest. A slightly higher phosphorylation of tau, which may represent the initial step of hyperphosphorylation, was detected in Alzheimer's disease (AD) patients at Braak stage V. This phosphorylation state of tau was pelleted by centrifugation and Sarkosyl-soluble tau in either AD or corticobasal degeneration (CBD) brains showed a similar phosphorylation profiles to tau in normal human brains, suggesting that hyperphosphorylation occurs in aggregated tau. These results indicate that tau is present in multiple phosphorylation states in mouse and human brains and nonphosphorylated forms are highly expressed among them.

3P-30 Sustained rise in body temperature exacerbates the pathologies of Alzheimer disease in mice.

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Climate change is affecting our health and economy in diverse ways and global warming is one of the most serious public health threats facing people in the world. Although in industrialized countries, many people live in a comfortable thermal conditions, environmental temperature keeps increasing annually. Effect of temperature on mammals have not been understood well. To explore the effect of high ambient temperature on memory functions in the elderly, we evaluated pathophysiology of Alzheimer disease (AD) under the different thermal conditions using APP-Tg mice. Surprisingly, compared to the room temperature at 23℃, the body temperature was significantly higher (about 0.5～0.7℃) in the Tg mice reared at 30℃. The APP-Tg mice reared at 30℃ showed impaired spatial memory function compared with those reared at 23℃. The levels of Aβ deposits in the cerebral cortex and hippocampus of APP-Tg mice (17-month-old) reared at 30℃ were greater than those of mice at 23℃. In the hippocampus, Aβ peptides level was 1.85-fold higher in the mice reared at 30℃ than those at 23℃. The levels of all the HSPs (HSP90, 70, 60 and 27) examined were increased in the mice reared at 30℃ compared with those at 23℃. In addition, the levels of hyper-phosphorylated tau was significantly increased in the brains of mice reared at 30℃. The phosphorylation levels of JNK, ERK and p38MAPK were also increased in the cortex of mice reared at 30℃ compared with those at 23℃. When the cultured neuronal cells were incubated at 35, 37, and 39℃, the Aβ levels synthesized increased in a temperature-dependent manner, and γ-secretase activity also increased in a temperature-dependent manner. Although, effect of temperature on the phosphorylation state of tau in vitro remains to be addressed, these lines of evidence suggest that even in mammals, thermal conditions affects body temperature and higher body temperature may enhance AD pathophysiology including Aβ synthesis/deposition and tau phosphorylation.

3P-31 Intracellular Aβ Oligomers Cause Tau-Independent Spine Alteration and Defect of Axonal and Dendritic Transport

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Objectives：
Mounting evidence indicates that extracellular amyloid β(Aβ) oligomers cause synaptic dysfunction and that this toxicity requires tau in the dendrites. Meanwhile, it has been suggested that intraneuronal accumulation of Aβ proceeds extracellular Aβ, and is an early event in Alzheimer's disease. It remains unclear whether intraneuronal Aβ also contributes to synaptic alteration, and if so, whether the toxicity requires tau.
Methods：
To address these questions, mouse/rat primary neurons were transfected with human APP with or without the Osaka (E693Δ) mutation which induces intracellular accumulation of Aβ oligomers. The morphology of dendritic spines, and axonal or dendritic transport of BDNF, mitochondria, and transferrin receptor（a marker of dendritic recycling endosomes）were evaluated. For comparison, the effect of extracelluar Aβ on dendritic spines was examined by adding Aβ into untransfected neurons at concentrations comparable to those in culture media of wild-type APP-transfectants. To study the necessity of tau, primary neurons from tau-deficient mice were also analyzed following to APP transfection.
Results：
Neurons expressing APP Osaka, but not wild-type APP, accumulated Aβ oligomers within cells. APP Osaka-transfectants showed reduced numbers of total and mushroom-type spines, but wild-type APP-transfectants and Aβ-added untransfectants did not. The flux values of BDNF, mitochondria, and the transferrin receptor transport in axons and dendrites were reduced only in APP Osaka-transfectants. Intracellular Aβ-induced aberrant spine morphology was observed even in tau-deficient neurons.
Conclusions：
Intraneuronal Aβ oligomers disrupted synaptic integrity independently of tau, and this toxicity was accompanied by an impairment of axonal and dendritic trafficking.

3P-32 Differential effects of angiotensin II receptor blockers on Aβ generation

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Angiotensin II receptor blockers（ARBs）are widely prescribed for the medication of systemic hypertension and congestive heart failure. It has been reported that ARBs can reduce the risk for the onset of Alzheimer's disease（AD）and have beneficial effects on dementia. Neurotoxic amyloid β-protein（Aβ）is believed to play a causative role in the development of AD. However, whether ARBs regulate Aβ generation remains largely unknown. Here, we studied the effect of ARBs on Aβ generation and found that telmisartan significantly increased Aβ40 and Aβ42 generation, but decreased the Aβ42/Aβ40 ratio. However, losartan, valsartan and candesartan did not increase Aβ generation, while olmesartan significantly increased Aβ42 generation. We also found that telmisartan increased the Aβ generation through angiotensin type 1a receptor（AT1a）and the receptor-related phosphotidylinositide 3-kinases（PI3K）pathway. Our findings revealed the different effects of ARBs on Aβ generation and provide new evidence for the relationship between antihypertensive treatment and AD pathogenesis.

3P-33 The role of Rap1A in Cas/HEF1 assocated signal transducer-induced neuronal death

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Aβ plays an important role in the early pathogenesis of AD. However, the molecular mechanisms of neuronal death by Aβ remain to be fully elucidated. We analyzed the gene expression profile of neurons exposed to Aβ for 15 hours comprehensively by microarray. One of induced genes was Cas/HEF1 assocated signal transducer (Chat, also reported as NSP3 or SHEP1). Chat binds to Eph receptor in N-terminus, and binds to Cas in C-terminus. Binding to Cas leads to activation of Rap1. Chat palys a role in cell movement, cell attachment, and axonal guidance. Northern blot analysis showed Chat expression of Aβ treated neurons was 2.8 times higher than those of control neurons, and the expression of Chat in the cortices of AD model mouse, Tg2576 was significantly higher than that in wild mouse. Overexpression of Chat in rat cultured cortical neurons accelerated cell death. C-terminal region of Chat was reported to interact with Cas family proteins, but the co-expression of Chat and p130Cas or NEDD9 interfered Chat-induced neuronal death.The Chat C-terminal region has a guanine nucleotide exchange factor (GEF) like region and is reported to interact with Rap2 and R-Ras and Rap1A, but its GEF activity is obscure. To exclude Chat's Cas-dependent small G protein activation, Chat Y635E which does not interact with Cas was used. As small G proteins, R-Ras, Rap1A, their constitutively active, and dominant negative mutants were used. Chat Y635E induced accelerated neuronal death was reduced when dominant negative form of Rap1A was co-transfected. Reversely, constitutively active Rap1A induced neuronal death acceleration even without Chat transfection. This result indicates that Rap1A activation was working downstream of Chat in Chat induced accelerate neuronal death pathway.

3P-34 Leptin inhibits expression of neprilysin in cultured astrocytes

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Epidemiological studies have suggested an inverse relationship between the adipocytokine leptin levels and the onset of Alzheimer's disease (AD). Pathogenesis of AD is characterized by accumulation of extracellular deposits of amyloid β-protein (Aβ) in the brain. The balance between production and degradation of Ab proteins is critical to amyloid accumulation and resulting disease. The major Aβ-degrading enzymes in the brain are neprilysin (NEP) and insulin-degrading enzyme (IDE), which may promote Aβ deposition in patients with sporadic late-onset AD. However, the mechanisms underlying the relationship remain uncertain. We investigated whether leptin induces Aβ degradation by inducing NEP and IDE expression of astrocytes. Leptin significantly decreased the expression of NEP but not IDE in a concentration- and time-dependent manner through the activation of extracellular signal-regulated kinase (ERK) in primary cultured rat astrocytes. Furthermore, leptin inhibited the degradation of exogenous Aβ in astrocyte-cultured medium. These results suggest that leptin decreases Aβ degradation by NEP through activation of ERK.

3P-35 Coffee reduces BACE1 expression in human neuroblastoma SH-SY5Y cells.

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Objectives Coffee is one of the most world-widely consumed beverage on a daily bases. Recent epidemiological studies have reported that 3-5 cups of coffee per day can reduce the risk of Alzheimer's disease（AD）by 65%. However, the precise molecular mechanisms of the effects of coffee are yet uncertain. Beta secretase（BACE1）is the enzyme that produces amyloid beta by cleaving amyloid precursor protein（APP）at N-terminal and is a potential therapeutic target for AD. Therefore, we investigated the effects of coffee on BACE1 expression in human neuroblastoma SH-SY5Y cells.Methods SH-SY5Y cells were cultured in Ham's F-12/DMEM（1：1）medium supplemented with 15% FBS. The cells were exposed to coffee, decaffeinated coffee, or coffee extracts up to 2.0%（v/v）. After 15 hours, the whole cell lysates were isolated and subjected to immunoblotting for BACE1. The amount of Aβ40 and Aβ42 in the culture medium was measured with ELISA. Results Coffee reduced BACE1 expression in a dose-dependent manner. Mixture of decaffeinated coffee and caffeine（100 μM）showed significant suppression in BACE1 expression, whereas decaffeinated coffee or caffeine（100 μM）itself showed little effect. The active constituents of coffee were produced by roasting process of coffee beans. Discussion Coffee reduced BACE1 expression and, Aβ40 and Aβ42 production in the human neuroblastoma SH-SY5Y cells. Our data suggest that coffee might reduce BACE1 expression by interaction between caffeine and constituents of roasted coffee. This activity may contribute to the preventive effects of coffee on AD. Further studies to identify active components and to elucidate the mechanism of the effects are needed to clarify the molecular basis of prevention of the disease associated with daily coffee consumption.

3P-36 Effect of Aβ on exosome release from astrocytes in culture

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In the central nervous system, exosomes and apoE-HDL are known to be secreted form glia cells and play important roles in the clearance of amyloid β-proiten（Aβ）. Exosomes are small extracellular vesicles（30-100 nm）derived from the endosomal system and secreted by variety of cell types such as neurons, astrocytes and oligodendrocytes. Exosomes are suggested to play important roles in Aβ deposition and clearance. Aβ is well known to induce neuronal cell death, whereas little is known about its effect on astrocytes. The limited information of the effect of Aβ on astrocytes led us to perform experiments to study the effect of Aβ on release of exosome from astrocytes. We characterized and analyzed release of exosomes and apoE, both of which are known to remove/clear Aβ from the brain, in the culture medium of rat astrocytes in culture. Exosome release was determined by western blot analysis using exosome specific marker proteins, flotillin and HSP90. We found that exosome and apoE-HDL were successfully separated by density gradient ultracentrifugation. Their release was confirmed by distribution of their specific markers and lipids, and electron microscopic analysis. Exosome release was significantly reduced by Aβ1-42 treatment in cultured astrocytes accompanied by an increased JNK phosphorylation. Whereas, apoE-HDL release remained unchanged. A JNK inhibitor recovered the decreased levels of exosome induced by Aβ treatment to levels similar to those of control, suggesting that Aβ1-42 inhibits exosome release via stimulation of JNK signal pathway. Because, exosome is shown to remove Aβ in the brain, our findings suggest that increased Aβ levels in the brain may impair the exosome-mediated Aβ clearance pathway.

3P-37 Diosgenin decreases the expression of HSC70 and improves memory function in Alzheimer's disease model mice.

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We previously found that diosgenin, a constituent of Dioscorea Rhizoma, restored axonal degeneration and improved memory function in Alzheimer's disease model mice (5XFAD). In this study, we aimed to investigate diosgenin-elicited expression change of intracellular molecules, to gain insight about diosgenin mechanism leading to axonal regrowth and memory improvement. Vehicle solution or diosgenin (0.1 μmol/kg/day, p.o.) was treated to wild-type or 5XFAD (male, 24-27 weeks old) for 15 days. Object recognition memory of diosgenin-treated 5XFAD was significantly improved. After the behavioral test, cortical lysates were compared on 2D-PAGE. We focused several proteins that showed drastic changes in the expression level and analyzed those by MALDI-TOF/MS. Heat shock cognate 70 (HSC 70) was identified as the protein decreased by diosgenin treatment in 5XFAD. Next, diosgenin (0.1, 1 μM) or the inhibitor of HSC70, VER-155008 (50, 500 and 5000 nM) was treated for 4 days to cultured cortical neuron (ddY, E14). Diosgenin as well as VER-155008 decreased the expression level of HSC70 and increased axonal density. VER-155008 also decreased in HSC70 expression and increased in axonal density although VER-155008 should be just an activity inhibitor of HSC70. Diosgenin (0.1 μmol/kg/day, p.o.) or VER-155008 (10 μmol/kg/day, i.p.) was treated to ddY (male and female, 6-9 weeks old) for 4 days, or wild-type and 5XFAD (female, 32-38 weeks old) for 17 days. Diosgenin and VER-155008 treatment enhanced object recognition memories in normal mice and improved the memories in 5XFAD. These results suggest that a decrease in HSC70 may relate to memory improvement. We are now investigating specific functions of HSC70 in the 5XFAD brain.

3P-38 Cytosolic Asparatate Aminotransferase Relates to Axonal Growth Control under Aβ Treatment

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Alzheimer's disease (AD) is the most common cause of dementia. We are investigating anti-AD drugs from traditional Japanese Kampo medicine, especially kamikihito (KKT). In this study, we aimed to identify the direct binding proteins of KKT. The drug affinity responsive target stability (DARTS) method was used to identify the direct binding proteins of KKT, and cytosolic aspartate aminotransferase (cAST) was identified. Primary culture cortical neurons were treated with amyloid beta (Aβ)(25-35), and the cAST expression and activity were evaluated by Western blotting and an AST activity assay, respectively. To investigate the effect of the inhibition and knockdown of cAST on the KKT activity, cortical neurons were treated with *O*-(carboxymethyl) hydroxylamine hemihydrochloride (OCHH ; an AST inhibitor) or transfected with siRNA for cAST, and the degree of axonal atrophy was evaluated under those conditions. DARTS analysis showed that a 42 kDa protein was protected by proteolysis via KKT coexistence. MALDI-TOF/TOF analysis indicated that the protein was cAST. cAST in Aβ (25-35)-treated neurons showed no change in the expression level but low activity. In contrast, treatment with KKT reversed the cAST activity to control level. Treatment of cortical neurons with Aβ (25-35) significantly decreased the axonal density. KKT treatment restored the axonal density, whereas the KKT-induced increase in axonal density was diminished by OCHH treatment or knockdown of cAST. In normal condition, the down regulation of cAST was not related to axonal damage. Aβ (25-35)-triggered cAST inactivation may relate to axonal atrophy. KKT up-regulates the cAST activity probably via direct binding to cAST, resulting in axonal growth. Functional roles of cAST in AD pathology are under investigation.

3P-39

Tau phosphorylation via microtubule-affinity-regulating-kinase (MARK)/PAR-1 as an initial step in the pathological cascade leading to neurodegeneration

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Tau is a microtubule-binding protein localized to the neuronal axons, where it regulates microtubule stability. However, tau is hyper-phosphorylated and accumulated in the cytosol in the brain neurons under pathological conditions including Alzheimer's disease (AD). Tau phosphorylation at Ser262 has been suggested to occur in the early stages in AD pathogenesis and to play critical roles in tau toxicity. It is not clear how elevated levels of tau phosphorylated at Ser262 leads to neurodegeneration. By using Drosophila as a model system, we found that the events causally related to disease pathogenesis such as expression of β-amyloid peptide or depletion mitochondria from the presynaptic terminals increases the levels of tau phosphorylated at Ser262 via microtubule-affinity-regulating-kinase (MARK)/PAR-1. Tau phosphorylation at Ser262 and Ser356 causes accumulation of tau with a prominent effect on tau species that are not phosphorylated at proline-directed kinase-target sites (SP/TP sites). Tau phosphorylated at Ser262 and Ser356 were subjected to further phosphorylation at disease-associated SP/TP sites. These results suggest that tau phosphorylation at Ser262 and Ser356 via MARK/PAR-1 is a critical step initiating a cascade that leads to accumulation of toxic tau species, and targeting such tau species may be an effective strategy to block the cascade of events leading to neuron loss in diseased brains.

3P-40 PUFA-derived lipid peroxide enhances alpha-synuclein toxicity through perturbation of autophagy system

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Docosahexaenoic acid（DHA）is a major fatty acid composition of the neuronal membrane. DHA is α-Synuclein αSyn）has been proposed to be associated with the pathogenesis of Parkinson disease（PD）. and stabilizes conformation of αSyn in α-helical structure. The cytotoxicity of αSyn depends on its higher structure, including momomeric, oligomeric and aggregated forms. DHA is known as a potent anti-oxidant but simultaneously, it is oxidized and produces cytotoxic lipid-radicals. This paper reports that DHA peroxidation modified αSyn and induced the oligomerization and facilitated the amyloidogenesis in vitro. DHA induced cell death with increased αSyn adduct with N-acyl product from DHA peroxidation and cell death in SH-SY5Y cells overexpressing αSyn. Using this model, the proteolysis system was studied. αSyn overexprssion itself activated autophagy system. In addition, DHA-derived lipid-derived oxidative stress disturbed autophagy-lysosomal fusion and as a result, the accumulation of abnormal proteins in the cells was observed. This finding indicates that oxidation of DHA which is rich in neuronal membrane may enhance the toxic proteins by inhibiting proteolysis system.

3P-41 Analysis of contextual fear memory and hippocampal CREB phosphorylation in 1-methyl-4-phenyl-1, 2, 3, 6, tetrahydropyridine（MPTP）-induced mouse model of Parkinson's disease

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In order to clarify the mechanism of the cognitive deficits in Parkinson's disease（PD）, we investigated the mechanism of cognitive deficits in MPTP-induced PD model mice（PD mice）. PD mice were produced by MPTP（four injections at a single dose of 20 mg/kg every 2 h, i.p. ）, which destroys specifically the nigrostriatal dopaminergic neurons. The number of tyrosine hydroxylase positive cells in the substantia nigra pars compacta was significantly decreased in PD mice. We evaluated the cognitive function of PD mice using the contextual fear conditioning test. In the test, we conducted using a weak unconditioned stimulus（US）(1 mA/2 s, single) or an intense US (2 mA/2 s, twice), and evaluated fear consolidation, reconsolidation and extinction. Under the weak US in reconsolidation and extinction tests, there were no significant differences in the freezing rates between control and PD mice. When we conducted the tests under the intense US, memory reconsolidation of PD mice normally occurred, but the memory of PD mice was attenuated earlier than the control mice by brief exposures to CS（3 min）every 24 h. In the extinction test, the PD mice showed a significant reduction in freezing rate earlier than the control mice. Next, we examined the expression level of the phosphorylated CREB（p-CREB）that is critical for fear memory formation. After an extinction training under the intense US, PD mice showed significant reduction in the number of p-CREB positive cells in hippocampal dentate gyrus（DG）. The p-CREB expression was observed specifically in immature cells（Doublecortin）but not in mature cells（NeuN）. These results suggest that the enhancement of memory extinction observed in PD mice may cause from a decrease in the p-CREB positive immature cells in hippocampal DG.

3P-42 Expression of the huntingtin-associated protein 1-immunoreactive stigmoid body and its morphological relationship with androgen receptor in the spinal cord of adult rat

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Huntingtin-associated protein 1 (HAP1) is a neural huntingtin interactor and is considered to be a determinant marker of the stigmoid body (STB). STB/HAP1 has putative protective functions against some neurodegenerative diseases (STB/HAP1 protection hypothesis). Although the expression of STB/HAP1 has been well described in the brain, little is known about its presence in the spinal cord which is also vulnerable to neurodegenerative diseases like spinal and bulbar muscular atrophy (SBMA) that is caused by mutation of androgen receptor (AR). We immunohistochemically determined the distribution of STB/HAP1 and its morphological relationship with AR in the spinal cord of adult Wistar rats of both sexes in light,fluorescence, and electron microscopy as well as western blotting. In this study, almost all STB/HAP 1-immunoreactive (ir) cells belonged to neurons, but not glial cells, as indicated by their co-expression with NeuN but not with GFAP, Iba1 or Olig2. About 90% of neurons in the lamina I-III, sympathetic and parasympathetic preganglionic cells, as well as in the lamina X, expressed STB/HAP1. In addition, about 50% of neurons in lamina IV and V expressed STB/HAP1, whereas STB/HAP1-ir cells were relatively sparse (15-30%) in lamina VI, VII and VIII. In contrast, no STB/HAP1-ir cells were found in the motoneurons of the lamina IX. Our present study suggests that STB/HAP1 in the spinal cord might play an important role in diverse spinal sensory and autonomic functions. Sensory and autonomic neurons in the spinal cord should be stable against stressful conditions as inducing neurodegeneration, due to putative STB/HAP1 protectivity, whereas the motoneurons might be vulnerable to such stresses due to the absence of STB/HAP1 in lamina IX. Interestingly, more than 80% of AR-ir cells in the dorsal horn or around the central canal contained STB/HAP1, but AR-ir cells in the ventral horn motoneurons were devoid of STB/HAP1. Our current results strongly support STB/HAP1 protection hypothesis in-vivo and might explain why the spinal motoneurons are major target in some neurodegenerative diseases including SBMA.

3P-43 TDP43 recognizes and transports G4-containing mRNAs into neurites for local translation

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Local protein synthesis within neurons is an essential mechanism for the establishment and conservation of synaptic plasticity, neural activities, and cell polarity. RNA-binding proteins(RBPs)are considered to play key roles in transport and distribution of specific mRNAs into target sites for the local protein synthesis. At present, however, little is known on the mechanism how these RBPs mediate selective transport of specific mRNAs into the target area.TDP-43 (TAR DNA-binding 43 kDa protein encoded by TARDBP), a ubiquitously expressed RBP in various tissues, contains two RNA recognition motifs and a Gly-rich domain, and forms a homodimer in normal cells under physiological conditions. In neurons of patients with amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD) or some neurodegenerative disorders, TDP-43 exists as a major component of the ubiquitin-positive inclusions, the pathological hallmarks of neuron diseases.In order to identify the binding target RNAs and the recognition sequences of TDP-43, we performed SELEX (systematic evolution of ligands by exponential enrichment) screening. The collection of RNA sequences contained G-quadruplex structure, which is formed from the stacking of two or more guanine tetrads. G-quadruplex structures have been identified in 3'-UTR of approximately 30% of the well-known dendritic mRNAs. We found that TDP-43 recognizes G-quadruplex containing mRNAs and transports them up to neurites for local translation. Furthermore we demonstrate that a TDP-43 with ALS-linked mutation is unable to co-localize with target RNAs. Finally TDP-43 was found to bind the G-quadruplex-forming RNA encoded by ALS and FTLD associated GGGGCC hexanucleotide repeat expansion of the C9orf72 gene. Taken together we propose that TDP-43 plays a key role in intracellular trafficking of G-quadruplex-containing mRNAs for the local protein synthesis in neurite.

3P-44 Cellular analysis of aberrant proteins derived from expanded GGGGCC repeat associated with ALS

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It has recently been recognized that amyotrophic lateral sclerosis (ALS) and frontotemporal dementia share common pathological and genetic features. For example, pathological inclusions containing TDP-43 are found in a majority of ALS and a subset of FTD cases. In 2011, an expansion of a GGGGCC repeat in the intron 1 of the C9orf72 gene was identified in autosomal dominant ALS-FTD families. This mutation is thought to be the most frequent cause of familial ALS. Interestingly, the pathology of ALS with C9orf72 expansion shows intracellular inclusions with and without TDP-43. Recent reports indicate that the repeat expansion leads to RNA toxicity associated with ribonuclear inclusions and protein toxicity of dipeptide repeat produced from the expanded RNA through repeat associated non-ATG translation (RAN translation). However, it is still unclear how these abnormal products cause diseases and which of these products is the most responsible for the pathogenesis. Here, we tried to establish an experimental system of GGGGCC repeat expression in cultured cells. While short GGGGCC repeats could be maintained stably in E. coli, (GGGGCC) 50 was highly unstable and even plasmids from a single bacterial colony harbored variable lengths of the repeat. We determined the condition that stabilizes long repeat tracts. We inserted (GGGGCC) 51 into the coding region downstream of EGFP with different reading frames. Upon transfection of these constructs into mammalian cells, we observed differential intracellular localization and aggregation properties depending on the reading frame of the repeat tract. Some aggregates were positive for ubiquitin or p62. Short Gly-Ala repeat protein alone did not show apparent aggregation, while it was detected in protein aggregates when co-expressed with a long repeat tract. These results not only reproduced some of recent results by others but also provided novel information on the properties of repeat-derived products. Our repeat expression system can be used for various cellular analyses, including the relationship between RNA foci formation and RAN translation and identification of dipeptide-associated proteins.

3P-45 Adenovirus-induced neuronal TDP-43 and FUS aggregates demonstrated by time-lapse imaging

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Formation of TDP-43- or FUS-positive cytoplasmic aggregates in neuronal and glial cells is one of the pathological hallmarks of amyotrophic lateral sclerosis (ALS). We have previously demonstrated that proteasome inhibition enhanced adenovirus-induced neuronal cytoplasmic aggregate formation of TDP-43 and FUS in vitro and in vivo, suggesting that impairment of protein degradation pathways accelerates formation of TDP-43 and FUS-positive aggregates in ALS. However, the relationship between the cytoplasmic aggregate formation and the cell death remains unclear. In this study, we performed time-lapse imaging analysis of neuronal cells infected with adenoviruses encoding TDP-43 and FUS cDNAs under conditions of proteasome inhibition. Rat neural stem cell lines stably transfected with EGFP or Sirius under the control of tubulin beta III (TBB3p), HB9, choline acetyl transferase (ChAT), or vesicular acetylcholine transporter (VAChT) promoter were differentiated in the presence of retinoic acid with or without smoothened agonist SAG, followed by infection of neurogenin-2, Islet-1 and Lhx3 adenoviruses in case of motoneuron differentiation. The differentiated neuronal/motoneuronal cells were then infected with adenoviruses encoding DsRed-tagged human wild type and C-terminal fragment (CTF) TDP-43 or mutant P525L FUS in the presence of proteasome inhibitor MG-132 or an adenovirus encoding shRNA for proteasome PSMC1. Time lapse imaging analysis revealed growing DsRed-positive cytoplasmic aggregates in the infected neuronal/motoneuronal cells followed by the cell collapse within 72 hours. Released cytoplasmic aggregates composed of WT and CTF TDP-43 remained insoluble in the culture media over 30 hours of the time course. We are also attempting to develop time lapse imaging of cell to cell spreading of cytoplasmic aggregates.

3P-46 Astrocyte-derived TGF-β1 accelerates disease progression in ALS mice by regulating the neuroprotective inflammatory response of microglia and T cells

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Neuroinflammation, consisted of both neuroprotective and neurotoxic reactions mediated by activated glial cells and infiltrated immune cells, is involved in the pathomechanism of amyotrophic lateral sclerosis （ALS）. However, the cytokines which regulate neuroprotective inflammatory response in ALS are not clarified. This study aims to elucidate the roles of TGF-β1, the elevated levels of which has been observed in the cerebrospinal fluid of ALS patients, in the context of neuroinflammation of ALS. We found that TGF-β1 levels were elevated in astrocytes of both murine and human ALS. By crossbreeding of $SOD1^{G93A}$ and GFAP-TGF-β1 mice, astrocyte-specific overproduction of TGF-β1 in $SOD1^{G93A}$ mice accelerated disease progression with reduced IGF-I production in deactivated microglia and fewer infiltrated T cells with a deregulated IFN-γ/IL-4 balance. Moreover, astrocyte-specific deletion of mutant SOD1 in $loxSOD1^{G37R}$ mice resulted in slowing disease progression with a decreased level of TGF-β1 in astrocytes. Pharmacological administration of TGF-β signaling inhibitor after onset extends survival time of $SOD1^{G93A}$ mice. In summary, we identify astrocytic TGF-β1 as a detrimental factor in accelerating disease progression of ALS through inhibiting the neuroprotective inflammatory response by microglia and T cells. Inhibition of TGF-β signaling in these cells may represent a novel therapeutic target for slowing disease progression of ALS.

3P-47 Methylcobalamin Protects Motor Neuron Loss in Mutated Human SOD1 and ES Cell Mediated in vitro ALS Model

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Amyotrophic lateral sclerosis（ALS）is a neurodegenerative disease characterized by loss of upper and lower motor neurons and progressive decline of muscle function resulting in death caused by respiratory defects. The approved drug for the ALS treatment is only riluzole but its effect on ALS is slight and limited. Therefore, more effective ALS drugs are in great expectations. Most ALS cases are sporadic, which are thought to be caused by multiple factors like environmental stress, life style habits, genetic and epigenetic failures, but 5-10% are familial cases. About 20% of familial cases link with mutations in the SOD1 gene. Causal link of the mutation was proven by the transgenic mice expressing ALS-linked mutant of human SOD1（G93A）that die around age 140-150 days with drastic motor dysfunction like ALS. It has been also known that the astrocytes expressing mutant human SOD1（G93A）have killing activity to motor neurons in in vitro co-culture. Here we established the mutant human SOD1-mediated in vitro ALS model using pluripotent stem cell technologies and examined that the effect of methylcobalamin（MBL）treatment in this model. MBL is an activated form of vitamin B12 known as an effective drug for the wide variety of neuronal diseases like palsy and diabetic neuropathy through regulating Akt/mTOR signaling pathway. In the present study, MBL treatment significantly prevented the motor neuron loss induced by mutant human SOD1-expressing astrocytes in a concentration dependent manner in an effective range at 10-100nmol/L. This result suggests that the MBL prevents ALS-like death in the motor neurons and will be a new choice for the treatment of ALS.

3P-48 Induced pluripotent stem cells-derived neurons of the patients with discordant schizophrenia

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Schizophrenia is one of the major psychiatric disorders with the onset between late adolescence and early adulthood. Despite its high lifetime prevalence, about 1%, its pathobiology is still unclear. While many kinds of comprehensive researches had revealed a number of aspects of this disorder, there still existed some difficulties because of the methodological limitations such as inaccessibility of the live brain. However, a technology of induced pluripotent stem cells（iPSCs）allows us to investigate living brain cells from patients with neuropsychiatric disorders. Here, we present our studies using iPSCs-deprived neurons of discordant schizophrenia and the healthy controls. Human iPSCs were established via electroporation of episomal plasmid vectors into dermal fibroblasts obtained from skins of 3 subjects；schizophrenia patient, his monozygotic twin sibling without schizophrenia, and the healthy control subject. There was no difference between the subjects in the expression pattern of pluripotent markers of established iPS clones. Neurons were differentiated from iPSCs via neurosphere formation in suspension culture, and subsequent adherent culture on coverslips. There were also no differences in the induction property and the proliferation rate of neural stem cells. Most neurons were considered glutamatergic pyramidal neurons with PAX6 and vGlut1 expression. All of these neurons showed electrophysiologically mature features such as multiple action potentials and spontaneous post synaptic currents recorded with a patch-clump method after 3 months culture in vitro.

3P-49 Human iPS cells-derived neuron ReproNeuro for electrophysiological assay

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Human induced pluripotent stem（iPS）cells can proliferate infinitely and differentiate into most cell types in the human body. In addition, production of human iPS cells can be scaled to support large-scale experiments, and human iPS cells are a native cellular source similar to primary cell cultures. And human iPS cells are good source to make primary cell types from them. These features of human iPS cells, and differentiated cells derived from them, are attractive for evaluating pipeline compounds and elucidating pathological conditions in a variety for therapeutic areas.We have developed a comprehensive workflow inclusive of patient specific primary somatic cell isolation, cellular reprogramming, and genetic modification with directed differentiation to the neural lineage. These derived neurons carry Alzheimer's disease specific mutations. In addition, we have created developed patient-specific neurons that specifically carry the PS1 gene mutation, by using gene-recombination technology starting with healthy cells. By regulating the differentiation conditions for these neurons, the proportion of neuronal subtypes can be controlled, and the resulting neurons can be analyzed functionally and phenotypically such as MEA assays, Ca imaging, and ELISA.ReproNeuroTM has a mixed population of neurons, including dopaminergic, glutamatergic, cholinergic, and GABAergic neurons. ReproNeuroTM is available for Patch clump, Ca imaging, and toxicity assay such as LDH assay. ReproNeuro MQTM is designed for MEA analysis, and these neurons show higher-frequency spikes and better sensitivity to antagonists of glutamate receptors such as AP5 or CNQX.This comprehensive workflow capability enables us to generate customized disease models that target specific neurological disease requirements.

3P-50 Astrocyte-secreted factors promote neurite length of human iPSCs-derived neurons in early developmental stage

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Human induced pluripotent stem cells（hiPSCs）have an enormous potential for biomedical research and clinical applications. Differentiated neurons from hiPSCs are expected to be good tools for developing new methods of treatments for various neurological diseases. However, the detailed processes of neuronal development from hiPSCs have not been shown. In this study, we cultured iCell neuron（Cellular Dynamics International）, Repro Glu and Repro Neuro（ReproCELL）under various conditions. We found that neurite outgrowth of hiPSCs-derived neurons is promoted by using culture medium conditioned by astrocytes at 2 days in vitro. In contrast, the number of neurites was not altered by using the medium. These results indicate that astrocytes secrete some factors promotes neurite outgrowth. Therefore, we focused on TSP-1（thrombospondin-1）, which is extracellular-matrix glycoprotein secreted by astrocytes. Previous studies showed that TSP-1 promotes synaptogenesis in cultured neurons. We are planning to examine whether TSP-1 promotes neurite outgrowth as well as synaptogenesis.

3P-51 Induction and characterization of synaptic transmission induced synchronized population bursts of the induced pluripotent stem cell-derived neurons

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Many drugs have been reported to cause seizures. It has been reported that the causes of drug-induced seizures are GABAA antagonism, GABAB agonism, adenosine antagonism, and enhanced excitation through NMDA in the neurons. So far, there is no good in vitro assay system for predicting drug-induced unexpected seizure-risks. Spontaneous neuron activity recordings by multi-electrode array (MEA) system from networks of cultured neurons could be a good risk evaluation system for such drug-induced seizure events [1]. It was reported that long-term electrophysiological activity and pharmacological response of human induced pluripotent stem cell (hiPSC)-derived neurons were accelerated by co-culture with rat astrocytes [2]. In this study, we observed time course generation of population burst spikes from iCell neurons with conditioned medium of mouse primary astrocytes by MEA system. Humoral factor (s) from mouse primary astrocytes was sufficient to generate synchronized population burst spikes in the iPSC-derived neurons. GABA antagonism enhanced the periodic synchronized burst spikes in a dose-dependent manner. P/Q-type and N-type calcium channel blockers eliminated the periodic synchronized burst spikes, suggesting that the burst spikes are mediated by synaptic transmission. We concluded that the observed astrocyte-induced population bursts by MEA system are mediated by synaptic transmission and the periodic synchronized population burst signals could be a good prediction marker of GABAA antagonism.

3P-52 Relevance between the expression of nur family genes and the neurite outgrowth through the histone modification

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Nerve cells are specialized to transduce an electric signal via the axon, thus it is necessary to construct the neuronal network by the elongation of neurites.

In this process, specific genes are expressed during neurite formation. Identification and functional analyses of these genes are important in developing a new strategy for regenerative therapy.

We have previously analyzed that forskolin (FSK), an intracellular cAMP producer, or valproic acid (VPA), a histone deacetylase inhibitor, both are involved in neurite outgrowth. As a result, we have revealed that Nur77 protein induces neurites in PC12 cells. In addition, we have demonstrated that both FSK and VPA induce other genes, which belongs to the Nur nuclear receptor family, along with the nur77 gene, within 4 hours in PC12 cells. FSK was induced nurr1 gene and nor1 gene while VPA alone was induced nurr1 gene only.

In present study, we investigated which genes belonging to the nur family are important in elongation of the neurites in the presence of FSK or VPA. Knock-down experiments showed that siRNA against each of nur family mRNA suppressed neurite outgrowth in response to treatment with FSK or VPA, suggesting that nur77 gene and nurr1 gene are essential for neurite outgrowth in the presence of FSK or VPA. We also found that epigenetic regulation via histone H3 modification was important for the FSK- or VPA-induced neurite outgrowth. These results show that up-regulation of nur77 gene and nurr1 gene are involved in neurite outgrowth induced by FSA or VPA through acetylation of histone H3 at the lysine residue, and suggest that different mechanisms are also involved the nur77 family genes during neurite outgrowth.