S01-1 Fine circadian clock machinery composed of interlocked molecular feedback loops

Sato Honma[1], Ken-ichi Honma[1]
[1]Dept Physiol, Hokkaido Univ Grad Sch Med, Sapporo, Japan

Circadian rhythms of mammals are driven by the master clock in the suprachiasmatic nucleus (SCN). A transcription-translation feedback loop involving 4 clock gene families, ***Clock, Bmal, Per*** and ***Cry*** has been considered to work as a molecular machinery of the circadian clock. We found that DEC1 and DEC2, bHLH transcription factors, strongly suppressed CLOCK/BMAL1 induced activation of ***Per1*** promoter from E-box elements, indicating that ***Decs*** form the 5th clock gene family of the core feedback loop. Since transactivation of ***Dec*** by CLOCK/BMAL1 is inhibited by DECs, another molecular loop is considered to interlock with the core loop. Both ***Dec1*** and ***Dec2*** are strongly expressed in the SCN in a circadian fashion. A brief light pulse given during the subjective night rapidly induces ***Dec 1*** expression, suggesting a function in the light entrainment of the circadian clock. These interlocked transcription-translation feedback loops may be advantageous for the stable rhythm generation and fine phase-resetting of the mammalian circadian clock.

S01-2 The multiple CK1 ε -phosphorylation site of mPer1 are implicated in the nuclear translocation.

Atsuko Takano[1], Yasushi Isojima[1], Katsuya Nagai[1]
[1]Div of Protein Metabo, Inst for Protein Res, Osaka Univ, Osaka, Japan

Casein kinase 1 epsilon (CK1 ε) was known to be an essential component of the circadian clock in mammals. The phosphorylation of mPer proteins by CK1 ε was implicated in the mechanism of the subcellular localization of mPer proteins, but the precise mechanisms have remained unknown. In this study, we identified three putative CK1 ε -phosphorylation motif clusters by CK1 ε in mPer1, examined roles of the phosphorylation of Ser/Thr residues of the clusters, and found evidence suggesting following things. 1) Among the clusters, phosphorylation of serine residues Ser661 and Ser663, might be responsible for CK1 ε -dependent nuclear translocation of mPer1. 2) The serine residue in another putative CK1 ε -phosphorylation cluster, Ser714, might be involved in the regulation of the phosphorylation of other sites in mPer1 by CK1 ε . Considering these, it seems that multiple CK1 ε -phosphorylation sites are implicated in the mechanism of nuclear translocation of mPer1, thus in that of circadian clock.

S01-3 Systems-biological analysis of mammalian circadian rhythms

Hiroki R Ueda[1, 2, 3]
[1]Dept of Pharmacology, Grad Sch of Med, Univ of Tokyo, [2]RIKEN, CDB, [3]Yamanouchi Pharm. Co., LTD

Mammalian circadian clock is one of the complex and dynamic systems, which consist of complexly integrated feedback loops. Such a complex system cannot be elucidated without comprehensive measurement of system dynamics and extensive determination of network structures. We demonstrate system identification strategy for analyzing mammalian circadian rhythms. We profiled suprachiasmatic nuclei (SCN) and liver genome-wide expression patterns under light/dark (LD) cycles and constant darkness (DD). Extensive determination of transcription start sites (TSS) of human orthologues for newly identified cycling genes followed by bioinformatical searches for transcriptional factor responsive elements near TSS revealed the role of the Rev-ErbA/ROR responsive element in generating circadian oscillations antiphase to Per2 expression. This hypothesis was verified using a newly developed in vitro validation system, in which cultured fibroblasts transiently transfected with clock-controlled reporter vectors showed robust circadian bioluminescence.

S01-4 The role of phosphorylation and degradation of hPer proteins oscillation in normal human fibroblasts

Norio Ishida[1,2], Koyomi Miyazaki[1], Miho Mesaki[1]
[1]Clock Cell Biology, IBRF, AIST, Tsukuba, Japan, [2]Dept Biomol Engineer, Tokyo Institute of Japan, Yokohama, Japan

The circadian expression in ***Drosophila*** of clock gene products, such as PER and TIM, is thought to be important for driving overt rhythms. The circadian rhythmic expression of PER and TIM proteins is much more important than their rhythmic mRNA expression in ***Drosophila***. To compare molecular mechanism of circadian clock in diverged species, we report here cloning and circadian mRNA and protein expression profile of human clock genes in normal human fibloblasts. Circadian oscillations of ***hPer1, hPer2, hPer3, hBMAL1***, and ***hCry2*** mRNA expression were observed in serum-stimulated normal human fibroblasts. The serum shock of human fibroblasts also caused daily oscillations in apparent molecular amount and size of human PER proteins. Inhibitor studies indicate that phosphorylation and degradation of PER proteins is an important step in human molecular clock.

S01-5 Circadian system of molecular clock in mammals

Hitoshi Okamura[1]
[1]Div Mol Brain Sci, Dept Brain Sci, Kobe Univ Grad Sch Med, Kobe, Japan

A striking discovery of recent circadian biology is that the core transcription/translation oscillatory loop consisting of a small number of clock genes reflects the behavioral rhythm almost in a perfect state. We established the method of monitoring clock gene transcription at single cell level by applying the highly sensitive cryogenic CCD camera to the mPer1-luc incorporated slice cultured suprachiasmatic nucleus (SCN). Our real-time monitoring has revealed that several hundreds of clock cells synchronously express mPer1 genes with inherent periodicities of rhythm generation. Moreover, the individual oscillatory cells are arranged in topographic order. mPER and mCRY proteins are interacting each other to shut down the transcription of mPer genes. The cell-rhythm oscillation generated by the core clock oscillatory loop is coupled and amplified by the ordered cell-cell communications in the SCN. Harmonized and strongly oscillating activities are thus spread out from the SCN to the whole brain, and ultimately to any peripheral organs which contain peripheral clocks.

S02-1 Regulation of cell differentiation by the bHLH oscillator Hes1

Ryoichiro Kageyama[1]
[1]Inst Virus Res, Kyoto Univ, Kyoto, Japan

It has been suggested that embryogenesis is controlled by some biological clocks, although their molecular nature is not known. We have recently found that both mRNA and protein levels of the basic helix-loop-helix (bHLH) repressor Hes1 oscillate in a two-hour periodicity after serum treatment or activation of Notch signaling. Serum treatment or Notch activation upregulates mRNA and protein levels of Hes1, which in turn represses its own expression by directly binding to its own promoter. Since both Hes1 mRNA and protein have short half-lives, they disappear rapidly when synthesis is blocked. Thus, the negative feedback is transient, thereby allowing the next cycle of Hes1 expression. This oscillatory expression of Hes1 is observed in many cell types such as fibroblasts, myoblasts, and neuroblastoma cells, indicating that this oscillator regulates the timing of many biological systems. In the absence of Hes1, neural stem cells are not properly maintained and prematurely differentiate into neurons. Thus, Hes1 oscillator is essential for the normal timing of cell differentiation.

S02-2 The NRS-NRSF/REST system: Its target genes, molecular partners, repression mechanisms, and roles in neural development

Nozomu Mori[1]
[1]Dept Mol Genet, Natl Inst Longev Sci, Aichi, Japan

Neural restrictive silencer NRS is a cell-selective transcriptional silencing element that primarily determines the neuronal cell type specific gene transcription. During the last decade, there is growing evidence regarding a large set of NRS target genes. Most targets are neuronal-specific genes, but some are non-neuronal. The NRS-binding transcriptional repressor NRSF (also known as REST) is expressed mostly in non-neuronal cells; however, a slight expression occurs in neuronal cells, where a splicing variant NRnV/REST4 is also expressed. Both NRSF/REST and NRnV/REST4 retain a transcriptional repression domain RD1. The RD1-mediated repression utilizes corepressor mSin3 that recruits histone deacetylase (HDAC). RD1 also interacts with basal transcription factors including TBP, suggesting that the RD1 mediated repression occurs near or at the promoter region of NRS-target genes. Recent progress of the NRS-NRSF works from my own and other laboratories will be reviewed and its roles in neural development will be discussed.

S02-3 Notch signaling in the mammalian neural precursor cells

Yukiko Gotoh[1,3], Sachiko Kamakura[1,3], Koji Oishi[1], Takeshi Yoshimatsu[1], Masato Nakafuku[2], Norihisa Masuyama[1]
[1]Inst. Mol. Cell. Biosci., Univ. Tokyo, [2]Grad. Sch. Med., Univ. Tokyo, [3]PRESTO

The mammalian central nervous system consists of neurons and glia, which are derived from neural precursor cells. Recent studies have indicated that Notch promotes astrocyte differentiation, although the underlying mechanisms are largely unknown. Here we show that expression of NotchIC in neuroepithelial cell cultures promoted expression of the astrocyte marker GFAP. Hes1 and Hes5, two downstream effectors of Notch signaling, also induced GFAP expression in these cells, whereas expression of Notch mutants that are defective in activation of the Hes pathway had no effect on GFAP expression. We also found that Notch and Hes1/Hes5 induced the activation of STAT3, a key player in astrocyte differentiation. Finally, induction of GFAP by Notch and Hes proteins was blocked by a dominant negative mutant of STAT3. These data suggest that Notch promotes astrocyte differentiation through the Hes-mediated activation of STAT3.

S02-4 Transcriptional machinery regulating nerve regeneration

Hiroshi Kiyama[1]
[1]Dept Anatomy & Neurobiol, Osaka City Univ Grad Sch Med, Osaka, Japan

Following nerve injury, the injured neuron initiates an organized cascade of molecular expression to promote survival and nerve regeneration, and a failure or lack of the expression of some molecules leads the neurons to death. The question arises as to whether such fate of injured neurons is determined by a master gene such as a transcription factor, which serves several gene expression regulation? Probably things are not so simple. For instance JNK-cJun pathway, which is activated in some neurons under conditions of stresses is a well-known death signal, however the activation of cJun is probably necessary for nerve-injured motoneurons to elicit a survival signal. This inconsistency may reflect subtlety of the gene expression regulatory machinery. In an attempt to explain the inconsistency, multiple implications of transcription factors and epigenetic modifiers might be necessary. In this symposium an implication of multiple transcriptional machineries for nerve regeneration will be discussed.

S02-5 Cross-regulatory interactions of transcriptional networks involved in the development of mouse brain

Tetsuya Taga[1]
[1]Dept Cell Fate Modulation, Inst Mol Embryol & Genetics, Kumamoto Univ, Kumamoto, Japan

Neurons, astrocytes and oligodendrocytes arise from a common progenitor. We have shown that two different cytokines LIF and BMP2 synergistically induce astrocytic differentiation from neural stem cells via the formation of a complex comprising their respective downstream transcription factors STAT3 and Smad1 which are bridged by a transcriptional coactivator p300. We have also shown that BMP2 induces expression of negative regulatory HLH proteins, leading to inhibition of transcriptional activity of neurogenic basic-HLH transcription factors. Taken together with our recent finding that an oligodendrocyte-inducing tranacription factor Olig2 inhibits the activity of an astrocyte-inducing transcription factor STAT3, we suggest that the cell fate in the developing brain is determined by cross-regulatory interactions among transcription factors. We furthermore propose that lineage specification in the nervous system is regulated also by cell-intrinsic transcriptional regulatory programs such as DNA methylation.

S03-1 Regulation of exocytosis mediated by Ca^{2+}-dependent protein-protein interactions with syntaxin

Michiihiro Igarashi[1]
[1]Div Mol Cell Biol, Dept Signal Transduct, Grad Sch Med Dent Sci, Niigata Univ

Syntaxin 1A is a key molecule of the SNARE mechanism, i.e., the molecular processes of exocytosis. Recently, we found that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) binds to syntaxin at Ca^{2+} concentrations exceeding 10^{-6} M. Only autophosphorylated CaMKII was bound to the linker domain of syntaxin, to which no other known syntaxin-binding proteins bind. The microinjected CaMKII-binding domain of syntaxin specifically affected exocytosis in chromaffin cells and in neurons, indicating that binding of CaMKII to syntaxin is an important process in the regulation of exocytosis. The linker domain is known to be important for the conformational change of syntaxin 1A, which enables it to act as a SNARE for exocytosis. CaMKII-binding in several syntaxin isoforms revealed that each substitution of K146, R151, T159, and S162 attenuated CaMKII-binding activity. These results suggest that these residues are important to the role of the linker domain for the structural conversion of syntaxin.

S03-2 Regulation of exo/endocytosis in the function of central synapse

Kazuhiko Yamaguchi[1,2]
[1]Lab. for Memory and Learning, BSI, RIKEN, Wako, Japan, [2]CREST/JST

Exo/endocytosis play essential roles in regulation of both pre- and postsynaptic functions. For the presynaptic exocytosis, molecular step between vesicle docking and membrane fusion is a missing link. To investigate possible involvement of small G protein family in this step, hippocampal autaptic EPSC in Rab3 GEP, activator for Rab3A family, gene KO animal was examined. Results showed marked reduction of release probability without change in readily releasable pool size or Ca^{2+}-sensitivity, suggesting up-regulation of priming process of exocytosis by Rab3 GEP. Postsynaptic exocytosis regulates glutamate receptor (GluR) expression at dendritic spine. To examine involvement of SNARE proteins in GluR trafficking in cerebellar Purkinje cell, tetanus toxin (TeTX) was intracellularly applied. TeTX caused slow reduction in EPSC amplitude, suggesting involvement of VAMP in GluR-trafficking. Contrary, application of GTP γ S caused slow increase in EPSC amplitude, suggesting regulation of GluR expression by constitutive exo/endocytosis at dendritic spine.

S03-3 Role of the synaptotagmin family in calcium-regulated exocytosis

Mitsunori Fukuda[1]
[1]Fukuda Initiative Res. Unit, RIKEN

Synaptotagmins (Syts) represent a large family of putative membrane trafficking proteins found in various species from different phyla. All Syt family members consist of an N-terminal single transmembrane domain and C-terminal tandem C2 domains. Ca^{2+}-binding to two C2 domains of the Syt family is widely believed to be essential for Ca^{2+}-regulated exocytosis, and two C2 domains of Syt I, the best characterized Syt isoform abundant on synaptic vesicles, have been shown to regulate synaptic vesicle trafficking. However, role of Syt isoforms in dense-core vesicle exocytosis in endocrine cells was poorly understood and is still a matter of controversy. In this study, I showed that Syt IX, a closely related isoform of Syt I, regulates dense-core vesicle exocytosis in PC12 cells (J. Biol. Chem. (2002) 277: 4601-4604) and that Syt IV is a stimulus (e.g., NGF treatment)-dependent regulator for exocytosis of dense-core vesicles (J. Biol. Chem. (2003) 278: 3220-3226). Based on our findings, functional redundancy and diversity of the Syt family in calcium-regulated exocytosis will be discussed.

S03-4 New imaging method for exo- and endocytosis using an evanescence microscope

Susumu Terakawa[1]
[1]Photon Med Res Ctr, Hamamatsu Univ Sch Med, Hamamatsu, Japan

Exocytosis and endocytosis are rapid and minute responses taking place on the cell membrane. It has been difficult to capture dynamic images of these responses by optical means especially when the vesicles are the smallest. We developed an ultra high NA objective lens and applied it for evanescent field illumination. This optics is powerful in visualizing molecular events fluorescently in living cells. By this technique, rapid exocytosis of synaptic vesicles stained with acridine orange was captured in neurons of the spinal cord as a 5 ms-frame movie. The images showed complicated steps of exocytosis including translocation, priming, and release distinctively. Chimera of dynamin and GFP could be visualized also in PC12 cells during their responses of exocytosis and endocytosis continuously. The dynamic images reflected unique activity of dynamin in initiation of the endocytosis. Images of slow endocytosis were also captured in non-neuronal cells by using confocal or DIC optics, providing a spectrum of endocytic patterns.

S03-5 Regulation of clathrin-mediated endocytosis at the synapse

Volker Haucke[1]
[1]Center for Biochemistry and Molecular Cell Biology, Department of Biochemistry, University of Goetingen

Clathrin-mediated endocytosis is a vesicular transport event involved in the internalization of signal transduction and nutrient receptors as well as in the reformation of synaptic vesicles. Recent data have provided a number of unexpected and novel insights into the initial steps of clathrin-coated vesicle formation and the membrane factors involved in this process. These include both membrane proteins such as synaptotagmin and phosphoinositides, most notably phosphatidylinositol (4,5)-bisphosphate (PIP_2). PIP_2 binds to several endocytotic proteins including the α and μ2 subunits of the clathrin adaptor complex AP2 which is activated upon phosphorylation and recruitment to PIP_2-rich membranes. Moreover, accessory proteins including AP180, epsin, eps15, intersectin, and stoned B form a regulatory network of protein-protein interactions in coated pit assembly and cargo selection. The complexity of these interactions at the cytosol-to-membrane interface suggests that clathrin-coated vesicle assembly is a highly regulated process.

S03-6 Protein-lipid interactions in endocytosis

Kohji Takei[1], Masahiro Kinuta[1], Tadashi Abe[1], Kenta Araki[1]
[1]Dept Neuriscience, Okayama University Graduate School of Medicine and Dentistry

Endocytosis is highly regulated by interactions between endocytic proteins and the plasma membrane, and phosphatidyl inositol (4,5)-bisphosphate (PIP_2) is proposed to play a major role in the interactions. To elucidate functions and kinetics of PIP_2 in endocytosis, we established an in vitro system, in which endocytic vesicle formation was mimicked. We show that increased amount of PIP_2 enhanced the vesicle formation, and that PIP_2 was substantially degraded upon vesicle formation. Next, regulatory mechanism of PIP_2-mediated interaction with endocytic proteins was analyzed using dynamin 1 and amphiphysin 1, endocytic proteins that are dephosphorylated upon stimulation of nerve terminals. Phosphorylation of the endocytic proteins by cycline-dependent kinase 5 drastically reduced GTPase activity of Dynamin 1, binding of these proteins to the PIP_2-containing membrane, and vesicle formation activity. Thus, phosphorylation of endocytic protein is likely one of the mechanisms to regulate PIP_2-mediated interaction in endocytosis.

S04-1 Neocortical organization: from the gene expression point of view

Tetsuo Yamamori[1]
[1]National Institute for Basic Biology, Okazaki, japan

The neocortex can be divided into so-called "areas", but it has been disputed to what extent the areas are genetically determined. It is also puzzling how the neocortex evolved while the number of genes across mammalian phyla has changed so little. To access these questions, we employed differential display methods to find genes specifically expressed in the primate neocortical areas. We have found thus far three genes of this kind. One gene, designated occ1, was specifically expressed in the visual cortex, particularly in V1. Furthermore, the expression of occ1 was developmentally regulated and activity-dependent in the adult (Tochitani et. al., 2001, 2002). The second gene, gdf7, is specifically expressed in the motor cortex of the African green monkey (Watakabe et al., 2001). The third gene, 134g indicated, interestingly, a complementary expression pattern to that of occ1: high expression in the association and higher cortical areas and low in the primary sensory area (Komatsu et al. 2002SFN). The implication of these expression patterns will be discussed.

S04-2 Microcircuitry of the neocortex

Henry Markram[1]
[1]Brain Mind Institute

How does the neocortex carry out a spectrum of functions using seemingly stereotypical microcircuitry? The microcircuit sheet has no obvious boundaries, yet a mosaic of functional modules emerge during activity. We are exploring the genetic, structural and functional principles that underlie this apparent omnipotence, using the rodent somatosensory cortex. At the genetic level, we apply single cell RT-PCR to explore molecular principles that give rise to the diversity of neocortical neurons and connections. At the structural level, we explore morphological principles that characterize different types of neurons and their connections. At the functional level, we explore principles that determine single neuron behavior, dendritic integration, synaptic transmission and plasticity between specific types of neurons, and the emergent dynamics of small neuronal networks. Our overall objective is to reconstruct a neocortical microcircuit and explore the emergent dynamics by computational modeling, while considering differences seen during development, across cortical regions and species, and in neurological disorders.

S04-3 Neocortical organization: from the perspective of long distance connections

Kathleen Rockland[1]
[1]RIKEN Brain Sci. Institute

Based on features of parent neurons and their terminal arbors, corticocortical connections in primates are broadly subdivided into what have been called feedforward, feedback, and lateral connections. Compared with interneuron populations, however, much less data are available for these or other subdivisions; and how these systems interact or fit into the microcircuitry of particular areas is largely unknown. The reciprocity of feedforward-feedback connections has been used to construct hierarchical maps of cortical areas. The reciprocity, however, is at best approximate. Even early visual areas receive convergent connections from several areas; and parallel direct and indirect connections (such as homo- and heterotopic callosal connections) are the rule. There are many reports of neurons with divergent branches to multiple targets. For non-primate species, the criteria for hierarchical architectures are yet more problematic. Implications for micro and macro cortical organization will be discussed.

S04-4 Functional organization of visual association cortex, area TE, in macaque monkeys

Manabu Tanifuji[1]
[1]Brain Science Institute, RIKEN

Clustering of neurons with similar response specificity in a columnar organization is considered to be a universal characteristic of cortical areas. However, such clustering and formation of columns is not fully characterized in higher association cortices, including area TE. We have investigated the functional structure of area TE, using combinations of intrinsic signal imaging and extracellular recording. The results suggest that clustering of neurons with similar response specificity is partial. There are indeed regions where neurons having the same response specificity are clustered together, or where neurons are systematically arranged across cortical surface according to a definable parameter of visual stimuli. However, there are also regions where nearby neurons are different in response specificity. Is the difference in response specificity due to the way we are defining "similar specificity", or to the inherent nature of higher association cortices? The functional structure of area TE will be further discussed in relation to early visual areas.

S04-5 Action from thoughts: Building brain-machine interfaces to investigate large-scale cortical function

Miguel Nicolelis[1]
[1]Duke Univer. Med. Center

Real-time direct interfaces between the brain and electronic and mechanical devices could one day be used to restore the sensory and motor functions lost through injury or disease. Hybrid brain-machine interfaces also have the potential to enhance our perceptual, motor and cognitive capabilities by revolutionizing the way we use computers and interact with remote environments. In this talk, I will review recent advances in this field and discuss how future research in this area may impact systems and cognitive neuroscience.

S05-1 P2X receptor - its structure and function

Ken Nakazawa[1]
[1]Div Pharmacol, National Inst Health Sci, Tokyo, Japan

P2X receptor is an ion channel forming membrane protein. The channel is activated by extracellular ATP, and sodium and calcium permeate through the channel under physiological condition. Seven subclasses of P2X receptor subunits have been cloned, and they are termed P2X1 to P2X7. A functional channel is believed to be formed by three subunits, and the channel can be either homomeric or heteromeric. One subunit has two transmembrane regions termed TM1 and TM2, and one long extracellular region (E1) between them. Recent works have revealed that both TM1 and TM2 contribute to the forming of the channel pore, and basic amino acid residues close to the outer mouth of the channel pore bind to ATP molecules presumably through electrostatic interaction with the phosphate group. E1 involves a region homologous to the ATP binding domain of tRNA aminoacyl synthetases, and small changes in this region lead to loss of the channel function. P2X7 has a long intracellular carboxyl terminus, and the terminus appears to have a special role in cellular responses by interacting other functional proteins.

S05-2 Functional roles of presynaptic P2X receptors in spinal dorsal horn

Terumasa Nakatsuka[1,2], Daisuke Takeda[2], Jianguo Gu[2], Megumu Yoshimura[1]
[1]Department of Integrative Physiology, Graduate School of Medical Sciences, Kyushu University, [2]University Florida Brain Institute, Florida, USA

Recently, molecular mechanisms of pain has been greatly advanced following the identification of P2X receptors, VR1 receptors, and other molecules in nociceptive sensory neurons. The role of P2X receptors in sensory transmission at central synapses has been proposed based on a study in a cultured cell system, but has never been tested in actual spinal cord circuitry. In this study, we demonstrated that P2X receptors were highly expressed in central terminals of both superficial and deep dorsal horn neurons by using patch-clamp recordings from spinal cord slice preparations. In addition, distinct subtypes of P2X receptors are expressed in central terminals of primary afferents innervated into superficial and deep dorsal horn neurons, and modulate glutamate release differently. These distinct roles of presynaptic P2X receptors may contribute to the understandings of the various sensations mediated by P2X receptors.

S05-3 Intrinsic regulation of P2X receptor function in the hypothalamic neuron

Minoru Wakamori[1], Masaru Sorimachi[1]
[1]Dept Physiol, Kagoshima Univ Fac Med, Kagoshima, Japan

Anatomical studies have revealed the wide distribution of P2X receptors in the brain. Hypothalamus has been reported to release ATP upon nerve stimulation. These previous studies suggest that extracellular ATP is a neurotransmitter in the brain. In the present study, the modulation of P2X receptor function by widely distributed cations *in vivo* was studied in neurons freshly dissociated from hypothalamic arcuate nucleus of 16- to 21-day old rats. Currents were recorded with the patch-clamp method at -70 mV. ATP induced inward currents in a concentration-dependent manner (EC_{50} = 42 μM, in the external solution with 2 mM Ca^{2+}). (1) Ca^{2+} inhibited the ATP-induced currents in the range of milimolars (Ki = 6.9 mM). (2) Zn^{2+} in the range of 1 - 20 μM facilitated the currents but Zn^{2+} at the concentrations over 50 μM inhibited the currents. (3) Spermine, but not spermidine, inhibited the currents (K_i = 36 μM) in a non-competitive manner. The present results indicate these cations may contribute to fine-tuning of P2X receptor-mediated synaptic function.

S05-4 Functional and molecular diversity of P2X receptor expressed among the vagal preganglionic, superior cervical and dorsal root ganglionic neurons

Shinya Ueno[1], Junichi Nabekura[2]
[1]Dept Physiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan, [2]Dept Cellular & System Physiol, Grad Sch Med Sci, Fukuoka, Japan

In spite of the robust expression of P2X receptor mRNA in the CNS, the demonstration of post-synaptic response via P2X receptor was limited in several nuclei such as dorsal vagal preganglionic (DMV) neurons. We characterized the sensitivity to divalent cations of P2X receptor of DMV neurons using patch clamp technique and analyzed the P2X receptor expressions in mRNA levels among DMV, superior cervical (SCG) and dorsal root ganglionic (DRG) neurons using quantitative RT-PCR. In the DMV neurons, biphasic effects of P2X receptor mediated current (I_{ATP}) by Zn^{2+}, a potentiation by a low concentration of Zn^{2+} and an inhibition by its high concentration, were obtained. RT-PCR analysis revealed that P2X2 and P2X6 receptors mainly expressed in DMV neurons. These results suggest that P2X2 and P2X2/6 receptors function in DMV neurons.

S05-5 Purinergic receptors in the mouse retina

Makoto Kaneda[1], Katsuyoshi Ishii[2], Yosuke Morishima[3]
[1]Dept Physiol Keio Univ Sch Med, Tokyo, Japan, [2]Lab Neural Architecture, BSI, Riken, Wako, Japan, [3]Dept Biol Sci Kyoto Univ, Kyoto, Japan

In the rerina, extracellular ATP modulates ACh release from cholinergic neurons and ATP is released from amacrine-like cells. Although immunohistochemical studies of the P2X-purinoceptors have been carried out in the retina, the distribution of P2-purinoceptors in the cholinergic neurons has not been examined. In the present work, we immunohistochemically studied the relationship between P2X purinoceptors and the cholinergic neurons in the mouse retina. We found that P2X2-purinergic receptors selectively co-localize in the OFF pathway of cholinergic amacrine cells. We also examined whether P2X2-purinergic receptors exist presynaptic or postsynaptic by the selective ablation of cholinergic amacrine cells with immunotoxin treatment, together with the observation at the ultrastructural level. Our findings support the idea that the regulation of ACh release by ATP is mediated by the postsynaptic P2X2 purinergic receptor, especially in the OFF pathway.

S05-6 Presynaptic P2X receptors in the brainstem network

Fusao Kato[1], Eiji Shigetomi[1], Yoshinori Kawai[2]
[1]Lab Neurophysiol, Jikei Univ Sch Med, Tokyo, Japan, [2]Dept Anatomy I, JIkei Univ Sch Med, Tokyo, Japan

Despite their wide expression in the brain, synaptic transmissions mediated by ATP and postsynaptic P2X receptors (P2X-Rs) are reported in limited brain regions. Unlike NMDA-Rs, P2X receptors are permeable to Ca^{2+} even at negative membrane potentials, making it likely to underlie transmitter release facilitation at presynaptic terminals. In brainstem slices of the caudal part of the nucleus of the solitary tract (cNTS) of rats, we found that activation of P2X-Rs prominently facilitates glutamate release in a manner dependent on extracellular Ca^{2+} but independent of voltage-dependent Ca^{2+} channel activation. This release facilitation was so potent that postsynaptic cells could generate action potentials by EPSP accumulation in the presence of Cd^{2+}. Immunosignals for P2X4 proteins were localized presynaptically in the cNTS. We argue that the presynaptic release facilitation might be the most important function of the p2X-Rs at least in cNTS but probably in the whole brain.

S06-1 Function of brain HDL and its pathophysiology

Jinichi Ito[1], Shinji Yokoyama[1]
[1]Dept Biochem Cell Biol & Metabolism, Nagoya City Univ Grad Sch Med Sci, Nagoya, Japan

As direct exchange of cholesterol between the CNS and the plasma lipoproteins is blocked by the blood-brain barrier, there must be CNS-specific mechanism for intercellular cholesterol transport of the neural cells. Astrocytes secrete apoE as cholesterol-rich HDLs, so that the astrocytes appear to contribute actively to the intercellular cholesterol transport in the brain. The conditioned medium obtained from 4-weeks primary culture of rat fetal brain cells (4W-CM) stimulated rat astrocytes to increase secretion of apoE and cholesterol. FGF-1 increased secretion of cholesterol and apoE of rat astrocytes and an anti-FGF-1 antibody suppressed the activity of 4W-CM. Immunohistochemical analysis of the cryo-injury lesions of the mice brain revealed the production of FGF-1 in the reactive astrocytes, prior to the increase of apoE synthesis. FGF-1 thus appears to be produced and secreted by astrocytes after damage or stress of the CNS and exerts its effect such as the stimulation of apoE secretion by an autocrine mechanism.

S06-2 Regulation of membrane raft function and cholesterol in neurons.

Kohji Kasahara[1]
[1]Tokyo Metropolitan Institute of Medical Science / PRESTO, JST

Lipid rafts are glycosphingolipid- and cholesterol-enriched microdomains. Lipid rafts have been implicated in signal transduction because a variety of signaling molecules are associated with them. We previously demonstrated that GPI-anchored neuronal cell adhesion molecule TAG-1, src-family kinase Lyn and 80kDa phosphoprotein (p80) are associated with lipid rafts of cerebellar granule cells (K. Kasahara et al. J.Biol.Chem. 275 34701-34709, 2000, Neurochem.Res. 27 823-829, 2002). Antibody-mediated crosslinking of TAG-1 induced activation of Lyn and tyrosine phosphorylation of p80, a putative substrate for Lyn, in lipid rafts. Cholesterol depletion by methyl β cyclodextrin inhibits the tyrosine phosphorylation of p80 by TAG-1 crosslinking. Sucrose density gradient analysis shows distribution of TAG-1 and Lyn in raft fraction is not altered by methyl β cyclodextrin treatment. However, distribution of active src-family kinases in raft fraction is reduced. These observations suggest that cholesterol is important for signal transduction by TAG-1 in lipid rafts.

S06-3 Production of amyloid-β peptides and intracellular cholesterol

Tsuneo Yamazaki[1]
[1]Dept Neurology, Gunma Univ Sch Med, Maebashi, Gunma, Japan

Amyloid β protein (Aβ) is a component of senile plaques, which are characteristic brain structures of Alzheimer disease, and a putative causal molecule of the disease. Although the precise intracellular site of Aβ production is not known, cholesterol depletion strongly decreases the Aβ production in cultured cells. To investigate whether disturbance of intracellular cholesterol trafficking also affects the Aβ generation, we treated cultured cells with a compound which affects the cholesterol transport in late endosomes / lysosomes. These treatments caused cells to significantly accumulate Aβ, especially Aβ42 species, inside the cells. This accumulated Aβ was SDS insoluble but formic acid soluble, thus presumably an aggregated form. Cell fractionation studies revealed that the Aβ accumulated in the same fractions as free cholesterol. These results may indicate a strong interaction between free cholesterol and Aβ42 within the cell, and suggests that this particular metabolic pathway is responsible for intracellular accumulation of Aβ.

S06-4 ApoE-isoform-specific risk for Alzheimer ' s disease mediated by cholesterol

Makoto Michikawa[1]
[1]Dept Dementia Res, Natl Inst Longevity Sci, Aichi, Japan

Cholesterol is suggested to have dual functions in the pathogenesis of Alzheimer ' s disease. Cholesterol not only modulates Aβ generation and its aggregation, but also plays a central role in the amyloid cascade; that is, Aβ oligomers affect cholesterol metabolism (Michikawa et al, J Neurosci, 2001), reducing cholesterol levels in neurons, which in turn induces hyperphosphorylation of tau (Fan et al, J Neurochem, 2001), impairment of synaptic plasticity, and neurodegeneration. Since apoE modulates cholesterol metabolism, we examined how apoE is involved in this cascade. We found that the ability of apoE3 to generate HDL particles is 2.5-fold greater than that of apoE4 (Gong et al, J Biol Chem, 2002), suggesting that the lower ability of apoE4 to generate HDL and to supply cholesterol to neurons may result in earlier disruption of cholesterol homeostasis caused by Aβ oligomers, leading to tauopathy. In this symposium, the role of cholesterol in the amyloid cascade and the isoform-dependent involvement of apoE in this cascade will be reviewed and discussed.

S06-5 Aggregation of amyloid beta-protein and cholesterol

Katsuhiko Yanagisawa[1], Katsumi Matsuzaki[2]
[1]Dept Dementia Res, Natl Inst for Longevity Sci, Obu, Japan, [2]Kyoto Univ Grad Sch Biostudies, Kyoto, Japan

Regarding deposition of amyloid beta-protein (Abeta) in brains with Alzheimer ' s disease (AD), we previously identified a novel Abeta species that binds to GM1 ganglioside (GM1) in human brains that exhibit early pathological changes of AD. Based on the unique molecular characteristics of GM1-bound Abeta, we hypothesized that Abeta undergoes conformational alteration through its binding to GM1 and acts as a seed. We recently found that an increase in the cholesterol concentration in host membranes markedly accelerates the binding of Abeta to GM1. Furthermore, we found that the cholesterol concentration in the exofacial leaflet of synaptic plasma membranes of the human apolipoprotein E4-knock-in mice was approximately two fold higher than that of the apolipoprotein E3-knock-in mice. The results of our studies suggest that an increase in the cholesterol concentration in the neuronal membranes accelerates Abeta aggregation through the formation of an endogenous seed.

S07-1 Assessment of neuropsychological tests available in Japan

Yoshifumi Takai[1], Tetsumori Yamashima[2], Mie Matsui[3]
[1]Department of Neuropsychiatry, Faculty of Medicine, Hamamatu University, [2]Department of Neurosurgery, Division of Neuroscience, Kanazawa University Graduate School of Medical Science, [3]Department of Psychology, Toyama Medical and Pharmaceutical University School of Medicine

Neuropsychological tests are indispensable to evaluate higher brain functions. However, they have not been widely introduced in the daily clinical practice in Japan, because of the lack of appropriate standardized test domains that can provide comprehensive information. The test battery covering almost all the domains in the impairment is difficult and time-consuming for patients. We propose that the optimal test battery should: 1) be administered easily and briefly, 2) be repeatable with avoiding learning effect, 3) be sensitive to mild impairment, 4) allow extensive evaluation independent to the education level of subjects, and 5) correlate well with the previous tests.We would like to introduce a Japanese Version of RBANS which was originally developed by Randolph and revised by us.

S07-2 Neuropsychological assessment in neurology

Takayuki Taniwaki[1], Takao Yamasaki[1,2], Shozo Tobimatsu[1], Jun-ichi Kira[2]
[1]Dept Clinical Neurophysiol, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan, [2]Dept Neurol, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan

There are a lot of patients with dementia disorders (i.e. Alzheimer disease, Vascular dementia, Fronto-temporal dementia, Diffuse Lewy body disease, or Progressive supranuclear palsy) and with higher cerebral dysfuntion (i.e. aphasia, apraxia or agnosia after stroke) in neurology. In the diagnosis, treatment and care of these patients, simple and precise neuropsychological assessment is needed. Assessment has been done mainly using Hasegawa Dementia Scale, Mini-Mental State Examination, or Wechsler Adult Intelligence Scale (WAIS). We will discuss these assessments about their significance, advantage, disadvantage, and correlation with electrophysiological examination or neuroimaging.

S07-3 Assessment of cognitive impairment of the patients with schizophrenia

Yoshio Minabe[1], Tetsumori Yamashima[2], Mie Matsui[3]
[1]Department of Neuropsychiatry, Faculty of Medicine, Hamamatu University, [2]Department of Neurosurgery, Division of Neuroscience, Kanazawa University Graduate School of Medical Science, [3]Department of Psychology, Toyama Medical and Pharmaceutical University School of Medicine

Schizophrenia is typically associated with an acquired impairment of multiple cognitive domains. However, cognitive assessment has not become central to everyday clinical practice. The length and practice effects of existing neuropsychological tests also limit their utility in evaluating disease progression.The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) is designed to assess global cognitive functioning, and for repeat evaluations to assess disease progression or outcome of therapeutic trails. We administrated the existing neuropsychological tests and Japanese Version RBANS for the patient of schizophrenia. In this research, we present data suggesting that Japanese Version RBANS appears to be a useful screening measure of cognitive functioning in patients with schizophrenia.

S07-4 Neuropsychological assessment using the Japanese version of RBANS

Jiro Akimoto[1], Jo Haraoka[1], Tetsumori Yamashima[1]
[1]Dept Neurosurg, Tokyo Medical University,Tokyo, Japan, [2]Dept Neurosurg, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

The authors introduce a Japanese Version of RBANS (Repeatable Battery for the Assessment of Neuropsychological Status) which yielded scaled scores for five cognitive domains such as immediate memory, visuospatial/constructional ability, language, attention, and delayed memory. On RBANS, abnormal cognitive decline in the aged was easily detected, compared to MMSE and HDS-R. Normal volunteer subjects showed impairment of delayed and immediate memories due to ageing. Aged subjects with average scores of MMSE and HDS-R being over 25, showed impairment of immediate and delayed memory.In addition, cognitive impairment occurring on the post-traumatic brain injured cases was easily detected,being compared to MMSE and HDS-R. As RBANS is useful for both detecting and characterizing the cognitive impairment due to ageing or traumatic brain injury, it should be widely utilized for a neuropsychological screening battery in the clinical practice.

S07-5 Examination of the learning and memory ability of monkeys in manual experimental apparatus

Shigeya Yaginuma[1]
[1]Dept.Behav.Physiol.,Tokyo Metropol.Inst.for Neurosci. Fuchu, Japan

Compared with the lower animals, the monkey has a brain similar to the human brain. In addition, it tends to perform many learning and memory tasks with strategies similar to those of human. Thus the monkey makes a desirable subject for brain research. Introduction of the Wisconsin General Testing Apparatus (WGTA) made it possible for researchers to conduct systematically controlled experiments on monkeys. Thereafter, original and modified types of the WGTA have been widely used to examine the learning and memory ability of monkeys. In addition, they have been used in the ablation study, in which researchers have made efforts to identify the functional localization in the brain. I will talk on the principal procedure for testing and several typical learning and memory tests conducted by the use of the WGTA. Comparing manual experimental apparatus with automatized apparatus, I will discuss good and bad points of this apparatus in conducting experiments.

S07-6 Monkey behaviors as models for human higher cognitive functions

Chihiro Yokoyama[1], Hirotaka Onoe[2], Kenji Fukui[1], Hideo Tsukada[3], Yasuyoshi Watanabe[4]
[1]Dept Psychiat, Kyoto Pref Univ Med, Kyoto, Japan, [2]Dept Psychol, Tokyo Metropol Inst Neurosci, Tokyo, Japan, [3]Central Res Lab, Hamamatsu Photonics, Shizuoka, Japan, [4]Dept Physiol, Osaka City Univ Grad Sch Med, Osaka, Japan

Current functional neuroimaging studies have revealed specific neuronal circuits involved in psychological processes for higher cognitive functions in human. Animal studies, however, are still required to evaluate the neurobiological bases responsible for these functions. Recently, the neuroimaging technique started to be applied on animal studies, because of its advantage of noninvasively exploring functional localization in the whole brain. We performed PET studies using macaque monkeys and found the transformation of neuronal circuits for learning depending on a learning-set formation, closely related to abstract thinking that is essential superiority in human mentality. Monkey behaviors are valuable for explaining the development of higher cognitive functions in human.

S08-1 Glypican-1: An Aβ binding heparan sulfate proteoglycan and its possible role in the pathogenesis of Alzheimer ' s disease

Norifumi Watanabe[1], Takeshi Tabira[1]
[1]National Institute for Longevity Sciences

Previous studies have suggested that heparan sulfate proteoglycans (HSPGs) play a role in deposition of β-amyloid protein (Aβ) in the Alzheimer ' s disease brain. In this study, we analyzed the binding of HSPGs from the human brain with Aβ in vitro. The results showed that glypican-1 can bind fibrillar Aβ in a heparan sulfate chains dependent manner. Further, immunoblot analyses of human brain samples revealed that glypican-1 is the major HSPG localized in detergent-insoluble glycosphingolipid-enriched (DIG) fractions where all machinaries for Aβ production exist and Aβ is accumulated as monomeric and oligomeric forms. Immunohistochemical studies demonstrated that glypican-1 is co-localized in primitive plaques as well as classical plaques. The results suggest that glypican-1 interacts with Aβ in such a specific domain as the lipid raft and plays a role in the initial phase of amyloid plaque formation. Detailed analyses using glypican-1 and APP double transfectants are now ongoing.

S08-2 Chondroitin sulfate proteoglycan phosphacan/RPTPβ in the central nervous system: its activity-related regulation with structural plasticity in vasopressin and oxytocin neuronal system

Seiji Miyata[1]
[1]Dept Appl Biol, Kyoto Inst Technol, Kyoto, Japan

Phosphacan is one of chondroitin sulfate proteoglycans. We examined localization of phosphacan in adult rat brains. Phosphacan immunoreactivity (ir) was seen around the somata of some cortical and hippocampal neurons. The puncta of phosphacan-ir were colocalized with TAG-1-ir. Ultrastructural analysis further revealed the phosphacan-ir at extracellular surface of most axons and some somata and dendrites, but not at synaptic junctions. Phosphacan is also highly expressed around vasopressin neurons in the SON with frequent overlap to TAG-1 ir. Moreover, chronic physiological stimulation induced drastic structural changes in the SON and concomitant decrease in phosphacan ir was observed. The phosphacan-ir returned to normal level 3 weeks after the cessation of stimulation. Thus, we demonstrated that phosphacan was downregulated with physiological stimulation, suggesting that phosphacan may be responsible for the structural plasticity in the brains.

S08-3 Chondroitin sulfate proteoglycans in formation of the retinotectal pathway

Hiroyuki Ichijo[1]
[1]Dept Anatomy, Inst Basic Med Sci, Univ Tsukuba, Tsukuba, Japan

Formation of neuronal circuit is a fundamental problem in developmental neurobiology; one of the most feasible experimental models is the retinotectal projections. The axons of the retinal ganglion cells run on the diencephalotelencephalic boundary on their way to the tectum; however, they do not invade the telencephalon anteriorly. A series of experiments shows that the telencephalic cells delimit the anterior border of the optic tract with their chondroitin sulfate proteoglycans (CSPGs), especially with their carbohydrate chains (CS-GAGs), and prevent the retinal axons from aberrantly entering the anterior territory. Although it has been shown that the CS-GAGs were broadly distributed in the embryonic brains, removal of the CS-GAGs from the embryonic brains in vivo by the treatment with the chondroitinase ABC induced the restricted effect on the anterior enlargement of the optic tract at the diencephalotelencephalic boundary; it suggests that specific types of CS-GAGs cause the anterior delimitation of the retinal trajectory.

S08-4 Proteoglycan expression in damaged brains of adult and neonatal rats

Atsuhiko Oohira[1,2], Fumiko Matsui[1], Takuya Shuo[1,2], Yosihito Tokita[1], Sachiko Aono[1]
[1]Dept Perinatol, Inst Dev Res, Aichi Human Service Cnt, Kasugai, Aichi, Japan, [2]Dept Neurochem, Nagoya Univ Grad Sch Med, Nagoya, Japan

To study the involvement of proteoglycans in brain repair, we examined their expression patterns in various brain injury models of adult and neonatal rats. When the adult rat brains were injured by a surgical incision or by kainate-induced seizures, a juvenile-type of neurocan (NC), a brain-specific chondroitin sulfate proteoglycan (CSPG), was transiently up-regulated in the lesioned cerebral tissue. The amount of phosphacan/RPTPβ/ζ (PC/PTPζ), another brain-specific CSPG, tended to be reduced in either injury model of adult rats. By contrast, neither NC nor PC/PTPζ was up-regulated by neonatal hypoxic-ischemic brain insult, although the immunoreactivity for PC/PTPζ was intensified on neuronal cells in the cerebral infarct area. These findings suggest that the pathological process of neuronal degeneration varies depending on the developmental stage of the brain at which the lesion is given and/or on the type of brain injury.

S08-5 The function of glycosaminoglycans in the cerebellar development

Nobuaki Maeda[1], Masahiko Tanaka[2], Jue He[1], Tomio Yabe[1]
[1]Dept Dev. Neurosci., Tokyo Metro Inst Neurosci, Fuchu, Japan, [2]Inst Comprehens Med Sci, Fujita Hlth Univ, Toyoake, Japan

There is increasing evidence that the glycosaminoglycans (GAGs) with particular microstructural domains are synthesized in a cell- and/or tissue-specific manner, regulating the activities of various growth factors and extracellular matrix molecules. Using various monoclonal antibodies against specific sequences within intact GAGs, we observed highly dynamic changes in the structures of these polysaccharides during development of the cerebellum. In situ hybridization analysis of the various GAG sulfotransferases also revealed the distinct expression patterns of each enzymes during development. We further revealed that the chondroitin sulfate with specific structure are involved in the morphogenesis of Purkinje cells. We will discuss the possibitity that the specific sets of GAG sulfotransferases, the specific GAG microstructural domains generated by these enzymes, and the corresponding binding partners are involved in the particular phases of neural network formation.

S09-1 Analyses of neural cell lines

Yasuhiro Tomooka[1]
[1]Dept. Bilogical Science and Technology, Tokyo University of Science

We established clonal cell lines from brains of p53-deficient mice. Studies defined them as stem cells producing all types of neural cells or producing neurons and astrocytes, precursor cells producing neurons or glia, and myocyte cell lines. Precursors proliferate in serum-containing medium. While in serum-free medium, they cease mitosis and differentiate into neurons or glia. Many genes are up-regulated when precursors become differentiated neurons. 2Y-5o2b cells plastically change phenotypes between undifferentiated and differentiated. Aggregation formation in suspension culture change the phenotype once defined in monolayer culture. Multipotency of stem cells can be confirmed by clonal assay. Clonal cell lines are good tools for transplantation experiment to investigate behaviors of donor cells. Precursors were transplanted into the ventricle of fetal brains. Postnatal analysis showed that transplanted cells were identified in various regions of brain. Supported by Specific Coordination Founds (SPSBS) of the Ministry of Education, Sports, Science and Technology of the Jap. Government

S09-2 Differentiation of various types of neurons from ES-derived neural stem cells

Takuya Shimazaki[1,2], Hideyuki Okano[1,2]
[1]Dept physiology, Keio Univ. School of Medicine, Tokyo, Japan, [2]JST, CREST

The identification and characterization of neural stem cells (NSCs) have brought increasing interest in NSCs and neural progenitor cells from the aspects of both basic developmental biology and therapeutic applications to the damaged brain. Transplantation of NSCs or their derivatives into the host brain, and proliferation and differentiation of endogenous stem cells by pharmacological manipulations may possibly be applied to treatment of many neurodegenerative diseases and brain injury. On the other hand, sources of NSCs are crucial for both basic research and novel approaches toward treating neurological disorders. Embryonic and adult NSCs may have some limitation for their application. The differentiation potential of NSCs is likely to be spatially and temporally restricted during the development and in vitro expansion. The Plasticity and differentiation potential of adult NSCs are still mysterious. We have been developing systematic protocols to generate various types of neurons from ES-derived NSCs.

S09-3 Adeno-associated viral vectors for treating neurodegenerative diseases

Shin-ichi Muramatsu[1], Kunihiko Ikeguchi[1], Ken-ichi Fujimoto[1], Lijun Wang[1,2], Imaharu Nakano[1], Keiya Ozawa[2]
[1]Dept Neurology, Jichi Med Sch, Tochigi, Japan, [2]Div Gene Therapy, Jichi Med Sch, Tochigi, Japan

The recombinant adeno-associated viral (rAAV) vector is a powerful vehicle for delivering therapeutic genes into mammalian brains and muscles. Efficient and long-term expression of genes for the dopamine (DA) -synthesizing enzymes in the striatum restored local DA production and achieved behavioral recovery in animal models of Parkinson ' s disease. Gene transfer of aromatic L-amino acid decarboxylase in combination with oral administration of L-DOPA offers a shortcut towards reaching clinical trials because DA production can be controlled by a dose of L-DOPA. Intramuscular delivery of the glial cell line-derived neurotrophic factor gene mediated by a rAAV vector prevented degeneration of motoneurons and prolonged the lifespan in a mouse model of amyotrophic lateral sclerosis. These results suggest that gene therapy using rAAV vectors may offer a novel and feasible protocol for the treatment of neurodegenerative diseases.

S09-4 Upregulated trophic factor in the dopamine-depleted striatum to promote the survival and/or differentiation of neural stem cells

H Hida[1], C-G Jung[1], H-J Kim[1], S Urakawa[1], H Nishino[1]
[1]Dept Neuro-physiol & Brain Sci, Nagoya City Univ Grad Sch Med Sci, Nagoya, Japan

Neural stem cells and embryonic stem (ES) cells are considered as good donor candidates to repair injured brain function in neural transplantation. Practically, trophic support for grafted donor cells seems to be an important problem to get good functional recovery. Recently, we found that pleiotrophin expression are upregulated in dopamine (DA)-depleted striatum at 1-3 week after DAergic denervation into the striatum. In this study, we focused on the effect of pleiotrophin on the donor candidates cells (primary DAergic neurons, neurospheres expanded in serum-free medium containing bFGF, ES-derived neural stem cells) in vitro. Pleiotrophin promoted the survival of primary DAergic neurons, and promoted the differentiation of glial progenitor cells, promoted the differentiation and /or survival of ES-derived neural stem cells into DAergic neurons. Data suggest that pleiotrophin is a promising trophic factor to donor cells in neural transplantation.

S09-5 Intracerebral grafting of cell lines secreting neurotransmitters and neurotrophic factors

Isao Date[1]
[1]Dept of Neurol Surgery, Okayama Univ Grad Sch Med & Dent, Okayama, Japan

We have investigated encapsulated cell grafting techniques, in which neurotransmitter- and neurotrophic factor-secreting cell lines were enveloped in the hollow fiber consisted with semipermeable membrane and were grafted into the brain. In order to deliver dopamine and GDNF simultaneously, PC12 cells were genetically modified to secrete GDNF (PC12-GDNF). Intrastriatal grafting of PC12-GDNF cells showed therapeutic effects on parkinsonian model rats. Human amniotic epithelial cells were immortalized, encapsulated and grafted into the striatum of parkinsonian model rats. The capsules secreted dopamine, bFGF and TGF beta continuously and therapeutic effects were observed in the host animals. VEGF secreting cells showed neuroprotective effect on the ischemic host brain when encapsulated and grafted into the striatum of MCA occlusion model rats. These data demonstrate that grafting of neurotransmitter and neurotrophic factor secreting cells is a promising approach for the treatment of certain neurological disorders.

S09-6 Clinical trials of transplantation of autologous sympathetic neurons in patients with Parkinson disease

Naoyuki Nakao[1], Toru Itakura[1]
[1]Dept of Neurol Surg, Wakayama Medical University

This study investigated the clinical effect of unilateral intrastriatal grafting of autologous sympathetic neurons in patients with Parkinson disease (PD). Four PD patients that had been followed for one year after grafting of autologous sympathetic neurons were selected for analyzing the graft effect. Sympathetic ganglion tissue was endoscopically excised from the thoracic sympathetic trunk, and grafted into the unilateral caudate head and putamen of the PD patients. Whereas the sympathetic neuron grafts failed to affect clinical scores reflecting the motor performance evaluated in either the on or off phases, the grafts significantly increased the duration of the levodopa-induced on period. This beneficial effect may be explained by the present in vitro and in vivo experiments showing that human sympathetic neurons have the ability to convert exogenous levodopa to dopamine, and to store the synthesized dopamine. Further studies are needed to determine whether the grafts may provide long-lasting clinical benefits.

S10-1 Intrinsic optical imaging of functional organization in mammalian primary auditory cortex

Shigeru Tanaka[1]
[1]Lab for Visual Neurocomputing, RIKEN Brain Science Institute, [2]Lab for Visual Neurocomputing, RIKEN Brain Science Institute

Recently it is widely accepted that intrinsic optical imaging is a powerful tool for investigation of functional organization in sensory cortices. We used this technique to study how the sound frequencies and interaural time differences (ITDs) are represented in the primary auditory cortex (AI) of rats and cats. In the recording, we presented sequences of pure tones or binaural clicks with interaural delays ranging from 0 to 250 μs. We could visualize tonotopic organization of sound frequencies and confirmed that neurons firing maximally in response to a particular sound frequency were located in a domain optically responding to the same frequency. We also found that acoustic stimuli with different ITDs activated different localized domains in AI. Considering that ITD is an important cue to detect where a sound comes from, we suggest that AI is involved in information processing with respect to ITD for sound source localization.

S10-2 Intrinsic optical analysis of postnatal maturation of whisker barrel responses

Katsushige Sato[1]
[1]Dept Physiol & Cell Biol, Tokyo Med Dent Univ Grad Sch & Fac Med, Tokyo, Japan

Using an intrinsic optical imaging technique, we examined postnatal changes in the rat barrel responses in control and *de*-whiskered rats, from which whiskers were removed except for one whisker just after birth. In the control rats, the area of the barrel response decreased gradually as postnatal development proceeded from 2 to 7 weeks, until reaching the adult pattern. The trial-to-trial variations in the responses were studied under the same conditions of whisker stimulation, and we observed that the extent of the variations decreased with postnatal development. On the other hand, in the *de*-whiskered rats, the barrel response area did not change during development, and showed a larger size than in the control rats. The extent of the trial-to-trial variations was also unchanged during development. These results suggest that the decrease in the area and variations are caused by whisker interactions, and that these interactions are necessary for the developmental stabilization of the intracortical connections.

S10-3 Spatial aspects of optical intrinsic signal responses in the cortical taste area elicited by gustatory stimulation on the tongue

Hiroshi Yoshimura[1]
[1]Dept Oral and Maxillofacical Surgery, Kanazawa Med.Univ.

To examine whether cortical taste neurons use spatial codes for discrimination of taste information, we investigated spatial aspects of optical intrinsic signal (OIS) responses in gustatory insular (GI) cortex elicited by delivering solution of sucrose and NaCl on the tongue. OIS responses to sucrose appeared in rostral part of GI cortex, whereas OIS response to NaCl appeared central part of GI cortex. Anesthetization of the tongue by local injection of lidocaine abolished OIS responses to tastants, and delivering distilled water elicited no OCI response. Thus, information of tastant from the peripheral sensory organs is segregated in the level of GI cortex, suggesting that taste information is assembled as spatial codes in primary cortical taste area through the process of taste perception.

S10-4 Regional location of odor-intensity in the olfactory cortex

Tokio Sugai[1]
[1]Dept Physiol. Kanazawa Med.Univ.

Using the optical imaging method for detecting intrinsic signals, we carried out to explore the organization of olfactory information in the dorsal part of the anterior PC (aPCd) of the guinea-pig. Odor-induced activation showed a narrow band in the aPCd. Lower concentration odors elicited active spots located within the rostral aPCd; higher ones generated activation extending up to the caudal aPCd. Therefore, odor stimuli produce the spatial extent of cortical activations. In addition, increasing odor concentrations increased the total area of the activation in proportion to stimulus intensity raised to a power of 0.5 to 1.5. Further, single-unit recording data indicated a rostro-caudal gradient in odor-sensitivity among neuron populations. These results suggest the aPCd detects the concentration gradient.

S11-1 Development of a novel voltage-sensitive probe and its application to neuronal systems.

Toshio Iijima[1]
[1]Dep Developmental Biol & Neurosci, Tohoku Univ Grad Sch Life Sci, Sendai, Japan

Real-time optical imaging of neural activity using voltage-sensitive dyes enabled us to visualize the dynamics of neural activity in the cerebral cortex and brain slice with a high spatial and temporal resolution. However, the conventional method had two problems in its use. First, the dye stains all neurons and other cells in the neural tissue non-selectively. So, it was very hard in the analysis to separate the optical signal into segments of different origin. Second, the change in the dye signal is very small in size, in general, resulting in the low signal to noise ratio. To solve these problems we developed a novel voltage-sensitive probe which can be applied only to the neuron pool of our interest, and causing a larger response than the conventional voltage-sensitive dyes.

S11-2 Genetically-encoded indicators of neuronal functions

Atsushi Miyawaki[1], Satoshi Shimozono[1], Hirofumi Katayama[1], Chikako Hara[1], Hiroshi Hama [1]
[1]Lab for Cell Function Dynamics, Brain Science Institute, RIKEN, Saitama, Japan

The discovery and cloning of naturally fluorescent proteins has enabled the construction of genetically encoded fluorescent indicators of intracellular biochemistry. During recent years, a variety of GFP-based sensors for physiological parameters such as pH, membrane voltage, calcium and chloride concentrations have been developed. These genetically encodable sensors opened a new approach to dynamically image the functions of neuronal circuits. Recent examples of indicator design and biological application will be presented.

S11-3 Flavoprotein fluorescence imaging of learning-induced rat somatosensory plasticity

Katsuei Shibuki[1], Kentaro Ono[1], Ryuichi Hishida[1], Masaharu Kudoh[1]
[1]Dept Neurophysiol, Brain Res Inst, Niigata Univ, Niigata, Japan

Green autofluorescence (500-550 nm) of flavoproteins in blue light (450-490 nm) is useful for functional brain imaging. We used this method to visualize learning-induced neural plasticity in the rat primary somatosensory cortex. Rats were trained to discriminate between the frequencies (20 Hz or 40 Hz) of floor vibration in a Skinner box. Vibration at 20 Hz or 40Hz (S+) was paired with reward, while vibration at 40 Hz or 20 Hz (S-) was not. After the rats learned to discriminate between S+ and S-, they were anesthetized with urethane (1.5 g/kg, i.p.). Somatosensory neural activities in response to S+ and S-, which were applied on the plantar hindpaw, were visualized as the changes in green autofluorescence in blue light. In all of the 8 rats tested, the neural responses to S+ were larger than that to S-. No significant difference was observed in control rats trained with S+ only. These results indicate the usefulness of flavoprotein fluorescence for investigating learning and memory.

S12-1 Dynamic redistribution of the postsynaptic density molecules in and out of the spines

Shigeo Okabe[1]
[1]Dept Anat & Cell Biol, Sch Med, Tokyo Med & Dent Univ, Tokyo, Japan

A variety of proteins are recruited to the postsynaptic density (PSD) and play critical roles in the modulation of synaptic functions. Recent imaging experiments revealed surprisingly rapid redistribution of PSD proteins in and out of the spines in hippocampal pyramidal neurons. To further explore the molecular mechanism of PSD dynamics, we performed time-lapse fluorescence microscopy of four PSD proteins, PSD-95, GKAP, cortactin-binding protein, and PSD-Zip45. Dynamic behavior of PSD proteins showed distinct kinetics, with the highest turnover rate of PSD-Zip45. Although PSD-95 clusters were the most stable among four PSD proteins, fluorescence recovery after photobleaching (FRAP) analysis revealed the presence of the slowly exchangeable fraction. The FRAP kinetics was different among four PSD proteins and the molecules closer to the NMDA receptor complex were less exchangeable than those away from the NMDA receptors. Thus, hierarchy of the domain interactions determines the remodeling process of the PSD scaffolding proteins.

S12-2 Cupidin/Homer links the glutamate-Ca^{2+} signaling complex and actin cytoskeleton in postsynapses

T Furuichi[1], Y Shiraishi[1], S Shoji[1]
[1]Mol Neurogenesis, RIKEN Brain Sci Inst

Homer represents a prominent component of glutamatergic postsynaptic density protein complexes. The N-terminal EVH1 region binds to proteins containing a Pro-rich motif, including mGluR1a/5, IP3R, RyR, and Shank, a scaffold protein for the NMDAR complex. The C-terminal coiled-coil and Leu zipper region is involved in multimerization. We found that an isoform Cupidin/Homer2 binds to F-actin and Drebrin, an actin binding protein in dendritic spines, at the N-terminal region, and to Cdc42 at the C-terminal region. Cupidin acts as a mobile scaffold that changes the postsynaptic clustering localization in cerebellar granule cells in response to Ca2+ influx via NMDAR. In hippocampal neurons, we showed a coincidence in dendritic clustering and synaptic targeting between Homer and the NMDAR complex during development and a morphological change in spine shapes by overexpression of exogenous Cupidin. Homer would therefore be a good candidate for forming a postsynaptic protein cluster, which involves in synaptic signaling and spine morphology.

S12-3 Actin dynamics in filopodia: Possible roles of actin-binding proteins

Ryoki Ishikawa[1], Kato Kaoru[2], Kazuhiro Kohama[1]
[1]Dept Pharm, Gunma Univ Grad Sch Med, Maebashi, Japan, [2]Neurosci Res Institute, AIST, Tsukuba, Japan

Filopodia contain bundles of F-actin, with all the barbed ends face the filopodial tip, and their dynamics is implicated in elongation and path finding of axons. To understand the mechanism of actin dynamics in filopodia, we have characterized the major actin-binding proteins in filopodia. Fascin crosslinked F-actin and formed tight bundles with polarity. The bundles slid and disassembled on myosin, the dynamics of which was quite similar to that in vivo, suggesting that fascin is an actin bundling factor of filopodia. Tropomyosin coupled with caldesmon, inhibited the actin-binding and -bundling activities of fascin. Overexpression of tropomyosin inhibited the neurite outgrowth. Drebrin also inhibited the fascin activities. The dynamics of these proteins in vivo will also shown. These results are discussed in possible roles of actin-binding proteins on filopodial dynamics.

S12-4 Functional roles of actin cytoskeleton in dendritic spine morphogenesis during development

Hideto Takahashi[1], Tomoaki Shirao[1]
[1]Dept Neurobiol & Behavior, Gunma Univ Sch of Med, Gunma, Japan

Dendritic spines have two major structural elements: PSD and actin cytoskeleton. However, how the postsynaptic molecular constituents target to spines during development is unclear. Here we show that synaptic clustering of drebrin, an actin-binding protein highly enriched in spines, is an essential early step for spine morphogenesis. Clustering of drebrin with F-actin occurs at postsynaptic sites in dendritic filopodia. During spine morphogenesis via dendritic filopodia, synaptic clustering of drebrin precedes that of PSD-95. By suppression and restoration of drebrin adult isoform expression, we found that synaptic clustering of drebrin with F-actin is necessary for that of PSD-95 in developing neurons. Our data suggest that drebrin-associated actin cytoskeleton establishes fundamental postsynaptic structures, which are required for synaptic targeting of postsynaptic molecules, while PSD components modify the established postsynaptic structures.

S12-5 Dendritic spine and adhesion proteins

Hidekazu Tanaka[1]
[1]Dept Pharmacol, Osaka Univ Sch Med, Osaka, Japan

CNS synapse is an adhesive junction specified for intercellular communication. Among various types of adhesion molecules implicated in synaptic function, cadherins at least in part supply the adhesive force to maintain the synaptic adhesion. Cadherins are homophilic adhesion proteins, which are linked to actin-cytoskeleton that shapes the structures of presynaptic terminal and postsynaptic spine. In parallel with the arrangement of actin during spinal morphogenesis, N-cadherin is recruited to the developing spine, and is required for its morphological integrity. In mature synapse, synaptic activity redistributes and functionally modulates N-cadherin. The disruption of N-cadherin function impairs the morphological plasticity of spines. Furthermore, another synaptic cadherin, arcadlin, is recruited to the N-cadherin-mediated synaptic junction, and modulates the function of N-cadherin. Thus multiple cadherins cooperate to regulate synaptogenesis and plasticity in combination with the dynamics of actin-cytoskeleton.

S12-6 Regulation of dendritic spine morphogenesis by the PDZ domain-containing protein afadin/AF6 and the Rac1-GEF kalirin

Peter Penzes[1], Richard L Hugnair[1]
[1]Howard Hughes Medical Institute and Department of Neuroscience, Johns Hopkins University, Baltimore, USA.

Changes in the number and shape of dendritic spines during development and plasticity are important for the formation of neuronal circuits and synaptic plasticity. We previously identified the Rac1-GEF kalirin-7 as a key regulator of spine morphogenesis and showed that kalirin is regulated by EphB2 receptors. We report that the protein afadin/AF6, a component of adherent junctions, and a binding partner of EphB2, is also present in the dendritic spines of cultured hippocampal neurons, where it co-localizes with kalirin. Afadin/AF6 interacts with kalirin in brain and heterologous cells. When over-expressed in neurons, afadin/AF6 is targeted to spines and induces kalirin-dependent spine morphogenesis. We identified structural determinants of afadin/AF6 synaptic targeting and spine morphogenic activity which led us to potential mechanisms for upstream regulation.

S13-1 Not only fear --the role of amygdala in social recognition--

Etsuro Hori[1,2], Taketoshi Ono[1,2], Hisao Nishijo[1,2]
[1]Dept Physiol, Fac Med, Toyama Med & Pharmaceu Univ., [2]CREST, JST

Recent human neuropsychological studies suggested a new role of the amygdala in non-verbal social recognition; understanding of other person's mental states. Faces emit most powerful social signals that can be used to guess mental states of the persons with the faces. To investigate a role of the amygdala in social recognition, we analyzed monkey amygdalar neuron activity during presentation of human photos of various facial expressions. The results indicated that some amygdalar neurons responded differentially to certain emotional expressions, while others responded differentially to specific persons. Activity of the amygdalar neurons was not directly correlated to behavioral responses. The results suggest amygdalar involvement in discrimination and evaluation of other's facial expression. Based on these results, this presentation will discuss about recent progresses in understanding of a role of the amygdala in social recognition.

S13-2 The spatiotemporal dynamics of the face inversion effect: A magneto- and electro-encephalographic study

Ryusuke Kakigi[1], Shoko Watanabe[1], Kensaku Miki[1]
[1]Dept. Integrative Physiol., Natl Inst. for Physiol. Sci., Okazaki, Japan

The neurophysiological basis of the face inversion effect was studied with magneto- (MEG) and electro-encephalography (EEG). Inferior temporal cortex (IT) i.e. fusiform gyrus, and lateral temporal cortex (LT) near the superior temporal sulcus were activated simultaneously, but independently, at 140-200msec post-stimulus to upright and inverted unfamiliar faces. Right hemisphere IT and LT were active in all subjects, and in the left hemisphere in half the subjects. Latencies to inverted relative to upright faces were longer in the right hemisphere, and shorter in the left hemisphere. We did not identify clear differences in anatomical location of activated regions to upright and inverted faces. We believe that the differences in processing upright versus inverted faces are attributable to temporal processing differences rather than to processing of information by different brain regions.

S13-3 Brain imaging studies of recognition of familiar and own faces

Ryuta Kawashima[1]
[1]NICHe, Tohoku Univ, Sendai, Japan

The functional organization of human brain involved in recognition of familiar faces and own face, which determined by our functional imaging studies, will be discussed in this session. In our previous study using PET, brain activity was measured during face familiar/unfamiliar discrimination task. We found that the right temporal pole plays an important role in recognition of familiar faces. The results of our additional MEG study indicated that activation of the right temporal role occurred approximately 270 msec after the presentation of the faces. Then, we compared brain activity involved in recognition of familiar faces and own face using an event-related fMRI techniques. We found that the different brain networks were activated in relation to the presentation of own face and familiar faces. Namely, the rightand left occipital, parietal and frontal cortices were activated for own face and familiar faces, respectively. The similar right-left functional segregation was also found in our previous MEG study.

S13-4 Neural correlates of facial expression recognition as revealed by fMRI

Tetsuya Iidaka[1], Hideki Ohira[1]
[1]Dept Psychology, Nagoya University, Grad Sch Environmental Studies

Recognizing and memorizing facial expressions are important cognitive function for appropriate social behavior in human. How these complex processes are modulated in the brain is a topic of cognitive neuroscience. In this symposium, we present data of fMRI activation studies involving several aspects of facial expression recognition. In a study of supraliminal and subliminal presentation of faces, negative expression (angry and disgust) significantly activated the left and right amygdala, respectively. Asymmetry in the medial temporal lobe activity extends to a face retrieval task in which the left hippocampus was predominantly involved in identity task and the right counterpart in both emotion and identity tasks. These results may be attributable to finding that part-based information processing of visual images is left hemisphere dominant in temporo-occipital lobe. Finally, in amygdaloid regions healthy elderly subjects had reduced, and schizophrenic subjects had increased activity while judging facial expressions.

S14-1 Prefrontal participation in decision-making processes

Shintaro Funahashi[1], Saori Igaki[1]
[1]Dept Cog Behav Sci, Grad Sch Human & Environment Std, Kyoto Univ, Kyoto, Japan

To examine whether prefrontal (PF) neurons participate in decision-making processes, PF activity was recorded while a monkey performed two tasks. In ODR, the saccade direction was informed by a visual cue, whereas in S-ODR, 4 visual cues were presented simultaneously and the monkey freely selected one position as a saccade target. Using neurons exhibited cue- or delay-period activity, we performed an ROC analysis to examine temporal change of directional selectivity. In neurons having cue-period activity, ROC values increased phasically only during the cue period in ODR, but showed no change in S-ODR. In neurons having delay-period activity, ROC values increased rapidly during the cue period and was maintained throughout the delay period in ODR. However, ROC values increased gradually along the delay period in S-ODR. These results suggest that the gradual increase of directional selectivity observed during the delay period in S-ODR reflects neuronal processes for decision making regarding where to make a saccade.

S14-2 Prefrontal cortex contributes selection of an object from memorized objects

Masato Inoue[1], Akichika Mikami[1]
[1]Department of Behavioral and Brain Sciences, Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan

We can memorize multiple objects simultaneously for temporal use, and can select one object from the memorized objects. This process is one of possible functions of the working memory. To investigate neuronal mechanism of this process, we examined single neuronal activities in the prefrontal cortex while monkeys were performing a serial probe reproduction task. In the task, two sequentially presented objects were memorized and then a target object was selected from memorized objects based on a color stimulus. During the color cue period, a class of ventrolateral prefrontal neurons exhibited the object-selective response. Since no objects were presented during this period, this response must be correlated with the process of selecting an object from memorized multiple objects. These results suggest that the ventrolateral prefrontal cortex plays a role in selection of an object from memorized objects.

S14-3 Two aspects of prefrontal participation in behavioral decision

Eiji Hoshi[1], Jun Tanji[1]
[1]Dept Physiol, Tohoku Univ, Sendai, Japan

Behavioral decision involves various aspects of information processing to which the prefrontal cortex (PFC) plays a part. We have studied two aspects of behavioral decision, examining neuronal activity in PFC. First, we required subjects to sort out a reach target by processing two sets of visual signals. In PFC of monkeys performing this task, we found three groups of neurons in ventral PFC, reflecting past-, present-, and future-information when they were making a motor decision. Second, we asked subjects to make a behavioral decision by integrating two motor instructions given with visual signals: information about a spatial target and a body part to use. We found a ventro-dorsal trend in activity properties within PFC. Ventral-PFC neurons were predominantly involved in detecting and processing visual information, whereas dorsal-PFC neurons were involved more in retrieving information out of visual signals, and in integrating two sets of information. Taken together, our study revealed two aspects of PFC involvement in processing information toward a goal of behavioral decision.

S14-4 Cellular substrates of decision making in the primate prefrontal cortex

Christos Constantinidis[1]
[1]Wake Forest Univ. Sch. of Med., Winston-Salem, NC, USA

The primate prefrontal cortex (PFC) plays a critical role in the evaluation of sensory information and planning of appropriate actions. The underlying circuit organization that allows the PFC to perform all these cognitive operations is poorly understood. In order to address the question, monkeys were trained in a discrimination task that required them to evaluate the relative brightness of two stimuli and, after a delay period, to saccade towards the brighter of the two. Simultaneous recordings from multiple isolated neurons were performed. Each neuron has spatial tuning and temporal envelope of response was evaluated. Neurons were further classified as regular spiking (RS), putative excitatory and fast spiking (FS), putative inhibitory, based on their action potential characteristics and firing rate patterns. Both RS and FS neurons were activated during the cue presentation, delay, and response periods of the task and carried information about both the sensory attributes of the stimuli and the perceptual decision that the animal was required to perform.

S15-1 Heat-evoked activation of the ion channel TRPV4 and its possible roles as a warmth sensor

Hyosang Lee[1], Ali Guler[1], Tohko Iida[2], Isao Shimizu[1], Makoto Tominaga[2], Michael Caterina[1]
[1]Dept Biol Chem, Johns Hopkins Sch Med, [2]Dept Cell & Molec Physiol, Mie Univ Sch Med

We show that a related protein, TRPV4, previously described as a hypoosmolarity-activated ion channel, also can be activated by heat. In response to warm temperatures TRPV4 mediates cationic currents in Xenopus oocytes and both currents and calcium influx into human embryonic kidney 293 cells. In both cases responses are observed at temperatures lower than those required to activate TRPV1 and can be inhibited by ruthenium red. Heat-evoked TRPV4-mediated responses are also sensitive to osmolarity. Consistent with these findings, we observed TRPV4 immunoreactivity in anterior hypothalamic structures involved in the integration of thermal and osmotic information. Curiously, TRPV4 is not detectably expressed in sensory neurons, but is highly expressed in skin keratinocytes. We propose that TRPV4 participates in neuronal detection of warm temperatures within the hypothalamus and epithelial detection of warmth at the skin surface.

S15-2 Identification of a cold receptor reveals a general role for TRP channels in thermosensation

Werner M Neuhausser[1], David D McKemy[1], Katerina Nydahl[1], David Julius[1]
[1]Dept Cell & Molec Pharmacol, Univ Calif San Francisco, USA

The cellular and molecular mechanisms that enable us to sense cold are not well understood. Insights into this process have come from the use of pharmacological agents, like menthol, that elicit a cooling sensation. We have characterized and cloned a menthol receptor from trigeminal sensory neurons that is also activated by thermal stimuli in the cool to cold range. This cold- and menthol-sensitive receptor, CMR1, is a member of the TRP family of excitatory ion channels, and we propose that it functions as a transducer of cold stimuli in the somatosensory system. These findings, together with our previous identification of the heat-sensitive channels, VR1 and VRL-1, demonstrate that TRP channels detect temperature over a wide range and are the principle sensors of thermal stimuli in the mammalian peripheral nervous system. Immunohistochemical analysis suggests that CMR1 is expressed in a distinct subset of sensory neurons and has a role in cold allodynia.

S15-3 Cold receptors acting as comparators of temperatures

Shigeo Kobayashi[1], Makoto Okazawa[1]
[1]Grad Sch Inf, Kyoto Univ, Kyoto, Japan, [2]Grad Sch Inf, Kyoto Univ, Kyoto, Japan

When temperature (T) decreases stepwise, cold receptors evoke impulses with threshold and transient responses. Since T and discharge frequency are not in a one-to-one ratio, the concept that a cold receptor is a sensor is doubtful. Instead, we have proposed that a cold receptor is a comparator that judges whether T is below threshold. Here, we studied mechanisms of the comparator action in cultured cold-sensory neurons of rats with patch-clamp recordings. In current-clamp recordings, when T decreased stepwise, cold receptors evoked a receptor potential with adaptation, eliciting impulses only at the onset of cooling. In voltage-clamp recordings (-60 mV), step cooling induced an inward current with adaptation. Reversal potential showed that cooling-induced current was non-selective cation current. In single-channel recordings, when T decreased gradually, a phase transition from a silent phase to an active phase occurred in cold receptors at threshold. We conclude that cold receptors are comparators that directly compare T with threshold.

S15-4 Dissecting thermosensation through behavioral genetic analysis in C. elegans

Ikue Mori[1]
[1]Dept Mol Biol, Div Biol Sci, Nagoya Univ, Nagoya, Japan

After cultivation at certain temperatures ranging from 15- to 25-degrees, C. elegans migrates to and moves isothermally around the cultivation temperature on a temperature gradient. This thermotaxis behavior is an ideal behavioral paradigm to dissect thermosensation at molecular and cellular levels. Laser ablation experimetns identified thermosensory neurons and their downstream interneurons, which are required for normal thermotactic responses. The molecular genetic analysis of thermotaxis-defective mutants revealed several molecules important for thermosensory signaling. The cyclic nucleotide-gated cation channel is likely to be essential for thermosensory neurons to transduce temperature signals into changes in membrane potential. Calcineurin appears to be a gain controller of thermosensory neurons, by negatively regulating their neuronal activities. The recent studies suggest that PKC as well as trimeric G protein coupled signaling cascades are also involved in thermosensation.

S15-5 Molecular mechanisms of heat sensation

Makoto Tominaga[1], Tohko Iida[1], Mitsuko Numazaki[1,2], Tomoko Moriyama[1], Kazuya Togashi[1], Tomohiro Higashi[1], Namie Murayama[1], Tomoko Tominaga[1]
[1]Dept Cell & Molec Physiol, Mie Univ Sch Med, Tsu, Mie, Japan, [2]Dept Anesth, Univ Tsukuba Sch Med, Tsukuba, Ibaraki, Japan

We feel a wide range of temperature. However, molecular mechanisms of thermosensation have not well been elucidated. Recently, several TRP (transient receptor potential) channels have been reported to function as thermosensors. Among them, capsaicin receptor TRPV1 (VR1) is the first molecule to detect heat stimulus and known to play a pivotal role in detection of noxious stimuli including heat within sensory neurons. Temperature threshold for TRPV1 activation is about 43 degree, a temperature causing pain in vivo. The threshold temperature could be reduced down to 30 degree upon PKC-dependent phosphorylation so that non-elevated temperatures such as body temperature are capable of activating TRPV1, thereby causing pain. Another heat activated ion channel, TRPV2, would also be discussed.

S16-1 Hypocretin/orexin system and narcolepy

Seiji Nishino[1]
[1]Center for Narcolepsy, Stanford University

Using forward (i.e. positional cloning) and reverse genetics (i.e. mouse gene knockout), genes involved in the pathogenesis of narcolepsy in animals have been identified. Human narcolepsy is a chronic disabling sleep disorder affecting 1:2000 individuals. Contrary to findings in animals, mutations in hypocretin related-genes are extremely rare in humans, but hypocretin-ligand deficiency is found in many cases. This discovery is likely to lead to the development of new diagnostic tests and treatments in human narcolepsy. Hypocretins/orexins are novel hypothalamic neuropetides discovered outside the sleep research field by searching endogenous ligands for orphan receptors and by the subtractive PCR techniques. Hypocretins are also involved in various fundamental hypothalamic functions such as energy homeostasis, and neuroendocrine functions. Hypocretin-deficient narcolepsy thus appears now to be a more complex condition than a simple sleep disorder. Pathophysiological aspects of hypocretin-deficient narcolepsy in human and canine will be discussed further.

S16-2 Hypothalamic Orexin Neurons Regulate Arousal According to Energy Balance

Takeshi Sakurai[1]
[1]Dept Pharmacol, Inst of Basic Med Sci, Univ Tsukuba, Tsukuba, Ibaraki, Japan

Mammals respond to declining food availability by becoming more wakeful and active, yet the central pathways regulating arousal and instinctual motor programs (such as food seeking) according to homeostatic need are not well understood. We demonstrate that hypothalamic orexin neurons monitor indicators of energy balance and mediate adaptive augmentation of arousal in response to fasting. Activity of isolated orexin neurons is inhibited by glucose and leptin, and stimulated by ghrelin. Orexin expression of normal and ob/ob mice correlates negatively with changes in blood glucose, leptin, and food intake. Transgenic mice, in which orexin neurons are ablated, fail to respond to fasting with increased wakefulness and activity. These findings indicate that orexin neurons provide a link between energy balance and arousal.

S16-3 Is orexin a determinant of the selection of motor behaviors induced by emotional stimuli?

Kaoru Takakusaki[1], Kazumi Takahashi[2], Hirohumi Harada[1], Kazuya Saioh[1], Yoshimasa Koyama[2], Toshikatu Okumura[3]
[1]Dept Physiol, Ashikawa Med Coll, Asahikawa, Japan, [2]Dept Physiol, Sch Med, Hukushima Med Univ, Hukushima, Japan, [3]Dept General Medicine, Ashikawa Med Coll, Asahikawa, Japan

Signals from the hypothalamus to the brainstem evoke emotional behaviors. Emotional stimuli normally increase muscle tone and elicit locomotion. On the other hand, those to narcolepsy patients, whose orexin level is reduced, induce muscular atonia associating with rapid eye movement (REM-with-atonia). However, there is no rationale how emotional signals elicit locomotion in normal and REM-with-atonia in narcolepsy. Orexin terminals are observed in the mesopontine tegmentum, where the mesencephalic locomotor region (MLR) and REM-with-atonia region in the pedunculopontine tegmental nucleus (PPN) are located. Both areas receive GABAergic efferents from the substantia nigra pars reticulata (SNr). The present study is designed to elucidate how orexinergic projections to these areas in cats contribute to the selection of emotional motor behaviors.

S16-4 Involvement of histaminergic system in the orexin-induced wakefulness

Kazuki Honda[1]
[1]Institute of Biomaterials and Bioengineering,Tokyo medical and Dental University

Orexins A and B are novel neuropeptides which are known to regulate the control of feeding and arousal state. Orexin-containing neurons are localized in the lateral hypothalamic area and densely project to the locus coeruleus, ventral tegmental area, dorsal raphe nuclei and tuberomammillary nucleus (TMN). Recent studies showed direct synaptic connectivity between orexin nerve terminals and histaminergic neurons in the TMN, and that mice lacking the H1 receptor do not show orexin A-induced wakefulness. Intracerebroventricular infusion of pyrilamine (an H1 receptor antagonist) caused a dose-dependent decrease in the orexin A-induced arousal effect. Orexin A-induced suppression of non-REM sleep was dose-dependently reversed by pyrilamine treatment. Since orexin neurons densely innervate TMN where the orexin-2 receptor is highly expressed, orexin neurons might regulate the arousal state through the modulation of histaminergic neurons activity in the TMN.

S16-5 How do orexins regulate sleep and wakefulness?

Yoshimasa Koyama[1], Kazumi Takahashi[1], Yukihiko Kayama[1]
[1]Dept Physiol, Fukushima Medical Univ Sch Med, Fukushima, Japan

Neurons containing orexins are located in the perifornical hypothalamic area (PFH), send their axons to the various brain structures including brainstem sleep regulating areas and cause excitation on the target neurons, such as noradreneregic, serotonergic and cholinergic neurons. Based upon these facts, it has been hypothesized that the orexinergic system regulates the sleep-waking cycles by influencing the aminergic and cholinergic neurons in the brainstem. To evaluate this hypothesis, it is required to clarify the activity of the orexinergic neurons across sleep-waking cycles. Using head-restrained, unanesthetized rats, the neuronal activity was recorded from the PFH where orexin-immunoreactive neurons are distributed and the activity pattern was compared with that of the noradrenergic neurons in the locus coeruleus and the cholinergic neurons in the laterodorsal tegmental nucleus. How the orexinergic neurons are involved in the regulation of sleep and wakefulness in relation with brainstem neurons would be discussed.

S17-1 Molecular basis for the Cl homeostasis underlying functional differentiation of GABA and glycine in the developing neocortex

Atsuo Fukuda[1]
[1]Dept Physiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan

At the time when GABAergic neurons tangentially migrate into the cortical plate (CP), most cortical cells, e.g., neural progenitors in the ventricular zone (VZ), radially migrating CP cells, later-disappearing subplate cells and Cajal-Retzius cells in the marginal zone (MZ), seem to be depolarized (or excited) by GABA due to high [Cl]i. Glycine receptors are transiently expressed in CP and Cajal-Retzius cells, which are also depolarized by glycine. Thus, depolarizing GABA and/or glycine actions may play a crucial role in corticogenesis. We have studied roles and molecular basis for the Cl homeostasis enabling these specific GABA and glycine functions. The observed rank order for [Cl]i (VZ＞MZ= CP＞ Layer V/VI) were consistent with expression levels of Cl transporter mRNAs for NKCC1 (inward) and KCC2 (outward). The immature stage of Cl homeostasis rendering GABA/glycine excitatory, as regulated by the differential expression of NKCC1 and KCC2, may modulate such events as CP cell migration and synaptogenesis.

S17-2 Transmitter switching of inhibitory synapses on developing auditory neurons

Junichi Nabekura[1], Yoshito Mizoguchi[1], Shumei Shibata[1]
[1]Dept Cell & System Physiol, Kyushu Univ Grad Sch Med Sci, Fukuoka, Japan

IPSC in lateral superior olive (LSO) neuron, receiving auditory inputs from both ears, was change GABAergic to glycinergic with maturation. The switch of inhibitory synapse was associated with 1) In transient period of development, bicuculline decreased the amplitude of miniature IPCP (mIPSP) without affecting the frequency and preferentially affected the slow phase of mIPSP decay. 2) Focal stimulation applied on a single bouton revealed evoked IPSC was partially inhibited by bicuculline and completely suppressed by additional strychnine. This result suggests co-release of GABA and glycine on developing LSO neurons as a single vesicle event. Electron microscopic immunogold analyses with anti-GABA and anti-glycine antibodies revealed that GABA and glycine co-localized in single presynaptic terminal at P8 and the density of GABA at glycine terminal reduced prominently at P14. Thus, transmitter switching from GABA to glycine is achieved by transmitter change in a single terminal attached on LSO neurons rather than by GABAergic synapse elimination. In addition, possible importance of GABAergic synapses in immature LSO, e.g. GABAb receptor will be discussed.

S17-3 Development of GABAergic neurons and glutamate decarboxylase gene expression

Yuchio Yanagawa[1,2,3], Satoe Ebihara[1,2], Naoko Tsunekawa[1,2], Kunihiko Obata[1,2]
[1]Lab Neurochem, Natl Inst Physiol Sci, Okazaki, Japan, [2]The Graduate University for Advanced Studies, Hayama, Japan, [3]CREST & SORST, JST, Kawaguchi, Japan

Glutamate decarboxylase (GAD) 67 is a rate-limiting enzyme that catalyzes the production of GABA from glutamic acid and is selectively expressed in GABAergic neurons. We generated the GAD67-GFP knock-in mice to tag the GABAergic neurons with EGFP and analyzed the migration pattern of GABAergic neurons during development. Tangential migration was observed in cerebral cortex of the knock-in mice, while radial migration was observed in midbrain superior colliculus. These results indicate that the use of the knock-in mice facilitate the analysis of development of GABAergic neurons in the other regions of the brain. The investigation of the molecular mechanism controlling GAD gene expression pave the way for elucidating the differentiation pathway of GABAergic neurons. We focused on the Pitx2 transcription factors and revealed the involvement of Pitx2 gene in the induction of GAD gene expression in cultured cells.

S17-4 Immigration of the GABAergic neuron progenitors in the mouse telencephalon

Nobuaki Tamamaki[1], Kouichi Nakamura[1], Kazuhiro Nakamura[1]
[1]Department of Morphological Brain Science, Graduate School of Medicine, Kyoto University, Kyoto Japan

Pyramidal neurons in the neocortex are produced in the neocortical ventricular zone and migrate radially to form the neocortical cell layers. In contrast, majority of GABAergic neurons in the neocortex are supplied by cell migration from the medial ganglionic eminence. Finally the GABAergic neurons constitute 20% of the neocortical neurons. Here we investigated the mechanisms that keep the ratio even across the neocortex and found that GABAergic neuron progenitors proliferate in the parenchyma of the developing mouse neocortex. However, all the investigated GABAergic neuron progenitors were Emx1 negative. We further investigated the origin of the Emx1-negative GABAergic neuron progenitors in the neocortex by a cre-loxP system and found that they originate in the ganglionic eminence and immigrate to the neocortex. The immigrants seemed to persist in the mouse neocortex through its life and proliferate to supply GABAergic neurons in case of necessity.

S18-1 Functional roles of Cdk5 in the brain development

Toshio Ohshima[1]
[1]Develop. Neurobiol. Lab., Brain Science Institute, RIKEN

Cdk5/p35 is a serine/threonine kinase and its activity is detected mainly in the post-mitotic neurons. We have been studying the functions of Cdk5/p35 using gene targeting method in mice. Cdk5-/- mice exhibit perinatal lethality and defects of neuronal positioning including later-generated neurons in cerebral cortex, Purkinje cells in the cerebellum and facial branchiomotor neurons in the brain stem. Although histological defects of neuronal positioning of Cdk5-/- mice are somewhat similar to reeler-phenotype, our study of double transgenic mice indicates that linear-relationships are not likely to occur between Cdk5/p35 and Reelin-signal pathway. To study of the functions of Cdk5 in postnatal brain development, we generated a transgenic mouse line which expresses Cdk5 under nestin-promoter and utilized to rescue the embryonic lethality of Cdk5-/- mice. We also established a neocortex-specific Cdk5 knockout mouse line using Cre-loxP system. Further characterization of these mice is underway to determine the roles of Cdk5 in brain development.

S18-2 Role of Cdk5 in the regulation of neurotransmitter release

Kazuhito Tomizawa[1]
[1]Dept. of Physiol. Okayama Univ. Graduate Sch. of Med. and Dent.

Neurotransmitter release from specialized active zones in presynaptic terminals is a critical step in synaptic transmission and consists of exocytosis and endocytosis processes. Both processes are triggered by an influx of calcium through voltage-dependent calcium channels (VDCCs). We have shown that Cdk5 directly phosphorylates the rbA isoforms of P/Q-type VDCC. The phosphorylation inhibited the interaction of the VDCC with SNAP-25 and synaptotagmin I, resulting in the down-regulation of the channel activity. Furthermore, Cdk5 phosphorylated amphiphysin I and dynamin I, which were essential for clathrin-mediated endocytosis of synaptic vesicles. Cdk5-dependent phosphorylation of these proteins inhibited the association with the binding proteins and disrupted the co-polymerization into a ring formation. FM-dye experiments revealed that Cdk5 inhibitors enhanced the electric stimulation-induced endocytosis in hippocampal neurons. I will review these finding and discuss the role of Cdk5 in the regulation of neurotransmitter release.

S18-3 Role of Cdk5 in drug abuse and plasticity

James A. Bibb[1]
[1]Dept of Psy. Univ. of Texas Southwestern Med. Center

Plasticity associated with contextual, motor, and reward based learning provides selective advantages by enforcing survival behavior. Drug addiction may be viewed as self-administration of pharmacological reagents that target reward perception and cause adaptive plasticity-mediated changes. Substantial evidence indicates that the neuronal protein kinase Cdk5 is involved in both functional and structural plasticity. We have previously shown that Cdk5 controls dopamine neurotransmission through regulation of the protein phosphatase-1 inhibitor, DARPP-32. Furthermore, Cdk5 is targeted by chronic cocaine and mediates the effects of the psychomotor addictive drugs, cocaine and caffeine. Moreover, the effects of chronic cocaine on neuronal morphology are dependent on Cdk5. We will review these findings and discuss the role of Cdk5 in neuronal plasticity. Finally, we will report on new studies that are being conducted to better understand the function of Cdk5 and how it contributes to the regulation of plasticity in the brain, including that associated with drug addiction.

S18-4 Inducible overexpression of the aberrant Cdk5 activator, p25, in transgenic mice results in neuronal loss, tau aggregation and increased beta-cleavage of APP

Li-Huei Tsai[1]
[1]Dept of Pathology, Harvard Med. School

Various neurotoxic insults lead to proteolytic cleavage of a Cdk5 activator, p35, that generates a toxic p25 species. The accumulation of p25 has been implicated in neurodegenerative diseases such as Alzheimers Disease, amyotrophic lateral sclerosis and Niemann-Pick type C. The deregulation of cdk5 by p25 leads to neuronal apoptosis and tau hyperphosphorylation. Recently, we generate inducible transgenic (Tg) mice overexpressing GFP tagged p25 restricted to the post-natal forebrain. The resulting aberrant cdk5 activity leads to progressive neuronal loss in the cerebral cortex and hippocampus of the Tg mice, Hyperphosphorylation and insoluble aggregation of endogenous mouse tau are also evident in Tg mice. Furthermore, the processing of endogenous amyloid precursor protein (APP) is altered in Tg mice, resulting in accumulation of APP C-terminal fragments. These findings indicate that aberrant cdk5 activation is neurotoxic and initiates pathogenic events associated with neurodegenerative disease.

S18-5 The regulation mechanism of Cdk5 activity in physiology and pathology

Shin-ichi Hisanaga[1], Taro Saito[1]
[1]Dept Sci, Fac Sci, Tokyo Metro Univ, Hachiohji, Tokyo, Japan

Cdk5/p35 is a neuron specific cyclin-dependent kinase, playing various roles in neuronal development, synaptic transmission and neuronal cell death. While Cdks in proliferating cells are activated and inactivated coordinately with cell cycle progression, however, how Cdk5 activity is regulated in neurons is largely unknown. Major determinant of the Cdk5 activity is the amount of p35 activation subunit, although phosphorylation of Cdk5 is indicated to simulate the kinase activity. The amount of p35 is mainly determined by degradation with proteasome. The degradation of p35 by proteasome is induced by NMDA receptor stimulation through autophosphorylation of p35 in active neurons. On the other hand, the degradative turnover rate of p35 slows down with aging, concomitantly with the increased chance to be cleaved to p25 by calpain. Deregulation of Cdk5 by the cleavage of p35 to p25 is suspected to induce neuronal cell death. This age-dependent increase in p25 generation may explain the vulnerability of neurons.

S19-1 Autophage formation during ER stress-mediated cell death

Takashi Momoi[1], Yoriko Kouroku[1], Eriko Fujita[1], Yasuo Uchiyama[2], Eiki Kominami[3]
[1]Division of Development and Differentiation, National Institute of Neuroscience, NCNP, Tokyo, Japan, [2]Department of Biology and Neuroscience, Osaka University Graduate School of Medicine, Osaka, Japan, [3]Department of Biochemistry, Juntendo University School of Medicine, Tokyo, Japan

When ER chaperon proteins are unable to refold the misfolded proteins into normal configuration and ER associated degradation system (ERAD) can not degrade the unfolded proteins sufficiently, the unfolded proteins are accumulated and apoptotic pathways are finally activated. Recently, the relationship between ER stress and pathogenesis of the conformational diseases such as polyglutamine diseases and parkinson disease has been focused. We have shown that polyglutamine aggregates induce ER stress and activate multiples apoptotic pathways including caspase-dependent and -independent pathways. Autophagic vesicles also detected in the cells showing polyglutamine aggregates. Here we show the ER stress-mediated apoptotic pathways and the relation between ER stress and autophage formation.

S19-2 ASK1 is essential for ER stress-induced JNK activation and apoptosis

Hideki Nishitoh[1,2], Hidenori Ichijo[1]
[1]Lab. Cell Signaling, Tokyo Univ. Grad. Pharmaceut. Sci. and CREST, JST, Tokyo, Japan, [2]Lab. Cell Signaling, Tokyo Med. and Dent. Univ. Grad., Tokyo, Japan

Accumulation of misfolded proteins within the ER lumen induces cellular stress and cell death, and ER stress has been implicated in human neurodegenerative disorders. However, the molecular mechanism of ER stress-induced cell death is controversial. Recently, we found a fundamental role of Apoptosis Signal-regulating Kinase (ASK) 1, one of the MAPKKK family proteins, in the ER stress signaling. Upon ER stress, an ER-resident type I transmembrane serine/threonine protein kinase termed IRE1 recruited TRAF2 and ASK1 and thereby activated ASK1. By using ASK1-/- cells, ASK1 was shown to be required for the ER stress-induced JNK activation and apoptosis. These results indicate that IRE1-TRAF2-ASK1 axis is essential for the ER stress-induced apoptosis. In this presentation, we will provide evidence that ASK1-dependent ER stress pathway plays an important role in the pathogenesis of human neurodegenerative disorders.

S19-3 ORP150, a novel stress protein rescues neurons from ischemia-induced cell death

Satoshi Ogawa[1], Kenntaro Ozawa[1], Osamu Hori[1], Yasuko Kitao[1]
[1]Dept of Neuroanatomy, Kanazawa Univ, Schol. Med., Kanazawa, Japan

Oxygen-regulated protein 150 kDa (ORP150) is a novel ER-associated chaperone induced by oxygen deprivation/ischemia. In cultured neurons, exposure to either hypoxia or glutamate induced the expression of ORP150, and this was also observed by treating the culture with either thapsigargin or breferdin-A, indicating that both glutamate and hypoxia can cause stress in the ER. Overexpresion of ORP150 in neurons suppressed the Ca2+ elevation induced by glutamate, and this was accompanied by a suppression of proteolytic activity as well as the eventual neuronal cell death. Cultured neurons overexpressing ORP150 were resistant to hypoxemic stress, whereas astrocytes with inhibited ORP150 expression were more vulnerable. Neurons with increased ORP150 demonstrated suppressed caspase-3-like activity and enhanced elaboration of neurotrophic BDNF under hypoxia. These data indicate that ORP150 is an integral participant in ischemic cytoprotective pathways.

S19-4 Sustained calpain activation and lysosomal rupture execute necrosis of CA1 neurons after ischemia

Tetsumori Yamashima[1]
[1]Dept Neurosurg, Kanazawa Univ Grad Sch Med Sci, Kanazawa, Japan

This is to report that calpain-cathepsin cascade prevails caspase-3-CAD cascade in delayed (day 5) neuronal death of the monkey CA1 sector undergoing 20min ischemia. Expression, activation, and translocation of key proteins as well as morphology of the postischemic neurons and DNA electrophoresis, were studied. On Western blot, sustained (until day 5) and maximal (days 2-3) activation of μ -calpain was found. Immunoreactivity of activated μ-calpain was remarkable at lysosomes until day 2 while throughout the cell on day 3. LAMP-1 showed a similar translocation being maximal on days 2-3. These indicated disruption of the lysosomal membrane, and the resultant leakage of lysosomal enzyme cathepsins B, D and L was confirmed. Caspase-3 activation was maximal a few hours after ischemia, but became negligible on day 3. Expression of CAD was very little, although its tranlocation to the nucleus was seen on days 2-3. Light and electron microscopy showed eosinophilic coagulation necrosis while DNA electrophoresis showed smear pattern.

S20-1 Imaging of membrane permeability transition in single mitochondria

Yoshihiro Ohta[1]
[1]Dept Biotech and Life Sci, Tokyo Univ Agricult and Tech

Mitochondrial permeability transition is the increase in the permeability of mitochondrial membrane to solutes and is accompanied by the loss of the mitochondrial membrane potential. To examine the permeability transition, we have observed membrane potentials of individual mitochondria with time-resolved fluorescence microscopy. Mitochondria were prepared from porcine hearts. During respiration, the mitochondrial membrane potential repeated the sudden collapse and the quick recovery. The frequency of the sudden collapse significantly decreased in the presence of ADP. These results suggest that the permeability transition occurs transiently during respiration. Consistent with this idea, under the condition where the transient permeability transition occurred frequently, the enhancement of efflux of water-soluble molecules from matrix was observed, indicating the increase in the permeability of inner mitochondrial membrane. The physiological significance of the transient permeability transition will be discussed in relation to generation of reactive oxygen species.

S20-2 Free Ca^{2+} dynamics in mitochondria

Takeharu Nagai[1,2], Atsushi Miyawaki[1]
[1]Lab for Cell Function Dynamics, BSI, RIKEN, [2]PRESTO, JST

Mitochondria are active participants in cellular Ca^{2+} signaling. They sequester Ca^{2+} from cytosolic microdomains of high Ca^{2+} concentration generated by the entry or release of Ca^{2+} from intracellular stores. Although it is interesting to know how the $[Ca^{2+}]c$ increases are relayed into mitochondria, only few studies have succeeded in simultaneous measurements of $[Ca^{2+}]c$ and $[Ca^{2+}]m$ in single intact cells. We measured $[Ca^{2+}]m$ and $[Ca^{2+}]n$ using ratiometric-pericams targeted to mitochondria and nucleus, respectively, in HeLa cells. This allows for simultaneous observations of intra- and extra-mitochondrial Ca^{2+} signals to be performed, which were well separated spatially. We shows that a sustained increase in $[Ca^{2+}]n$ is evoked, which is accompanied by a transient and synchronized increase in $[Ca^{2+}]m$. However, the peak of $[Ca^{2+}]m$ lags behind $[Ca^{2+}]n$ by 5 to 10 seconds. In addition we found that during the falling phase, some mitochondria transiently take up more Ca^{2+} than others. We will discuss the mitochondrial role in regulation of cytosolic Ca^{2+} dynamics.

S20-3 Morphological changes of mitochondria by a large GTP binding protein mOPA1

Takumi Misaka[1,2], Yoshihiro Kubo[1,3]
[1]Dept Physiol and Cell Biol, Tokyo Med and Dent Univ Grad Sch, Tokyo, Japan, [2]Dept Appl Biol Chem, Grad Sch Agric Life Sci, Univ Tokyo, Tokyo, Japan, [3]CREST, JST, Kawaguchi, Japan

It is generally accepted that mitochondria play key roles in cell stress responses and apoptosis. The regulation of mitochondrial morphology has been focused recently as an important factor controlling these phenomena. We observed that mOPA1, a novel large G protein, localized to mitochondria in the transfected cells and the overexpression of mOPA1 induced dramatic morphological changes of mitochondria from a tubular to a fragmented shape. By triple staining of transfected cells using mOPA1 antibody and mitochondrial markers, we observed that the intermembrane space (IMS) was concentrated in a small vesicular pattern at one end of the ring-shaped matrix, and that mOPA1 was localized in the IMS. The concentrated IMS could be also observed by immunoelectron microscopy. These results suggest that the overexpression of mOPA1 induce not only the mitochondrial fragmentation, but also the concentration of IMS within mitochondria.

S20-4 A novel mitochondrial cell death factor HtrA2 and neuronal death

Ryosuke Takahashi[1], Yasuyuki Suzuki[1], Haruhisa Inoue[1]
[1]Lab Motor System Neurodeg, RIKEN BSI, Saitama, Japan

Inhibitor of apoptosis (IAP) family proteins play an important role in regulating apoptosis. Several members of the human IAP family proteins including XIAP are potent direct inhibitors of cell death proteases, i.e., caspases. We identified a serine protease named Omi/HtrA2 as a novel XIAP binding protein.. HtrA2 binds and inhibits IAPs in a similar manner to Smac/DIABLO, an inhibitor of IAP. Although HtrA2 proteins are usually confined to mitochondria, they are released into the cytosol as a result of apoptotic stimulus, where they are thought to inhibit IAPs. Extramitochondrially expressed HtrA2 induces cell death in a caspase-independent, serine protease-dependent manner. Taken together, HtrA2 is a novel Smac-like inhibitor of IAP with a serine protease activity-dependent cell death inducing effect. The involvement of HtrA2 in neurodegeneration will also be discussed.

S21-1 Remodeling of neural circuits during metamorphosis of *Drosophila*

Takeshi Awasaki[1,2,3], Kei Ito[1,2,4]
[1]IMCB, Univ of Tokyo, [2]Nat Inst Basic Biol, [3]PRESTO, JST, [4]BIRD, JST

Remodeling of neural circuits is a general phenomenon in the development of neural network. One of the prominent feature of neural remodeling is axon pruning. In order to understand the molecular mechanisms that regulate the axon pruning, we analyzed the larval mushroom bodies (MB) of *Drosophila*, which remodels their axon branches during metamorphosis defined program. Single neuron analysis using MARCM system shows that pruning of the MB axon branches consists of at least two separable events. Synaptic boutons on the axon branches are eliminated at the first phase, and axons degenerate at the second phase. Local degradation of FasciclinII (FasII) occurs prior to the elimination of boutons in the axon branches. Over-expression of FasII suppresses this elimination. Expression of dominant negative form of Rac suppresses the degeneration of axons without affecting the elimination of boutons. These indicate that the elimination of synaptic boutons and the degeneration of axons are induced by the degradation of FasII and activation of Rac, respectively.

S21-2 Role of a semaphorin in guiding spinal motor neurons in zebrafish larva

Wataru Shoji[1]
[1]Dept Cell Biology, Institute of Development, Aging, and Cancer, Tohoku Univ, Sendai, Japan

Semaphorin gene family encodes secreted and transmembrane proteins and some members of the family are known to navigate specific growth cones in repulsive or attractive manner. We previously reported zebrafish Sema3A is expressed by dorsal and ventral portion of the somite in the trunk, and repulses the growth cones of the spinal motor neurons toward their intermediate target cells in between. Pausing of the growth cones take place on the intermediate target, where the growth cones lose their repulsive response against Sema3A. Here, we report receptor down-regulation as well as additional signal transduction are involved in this process. After the pausing, the growth cones migrate toward Sema3A positive region and terminate with axonal arbors. We will also address a potential role of Sema3A at the later stages.

S21-3 Plexin-A2 and Plexin-A4 regulate development of hippocampal mossy fiber projection.

Fumikazu Suto[1], Miu Tsuboi[1], Makoto Sanbou[2], Takeshi Yagi[3], Hajime Fujisawa[1]
[1]Div Biol Sci, Nagoya Univ Grad Sch Sci, Nagoya, Japan, [2]Nat Inst Physiol Sci, Okazaki, Japan, [3]Grad Sch Frontier Biosci, Osaka Univ, Japan

Plexin-A subfamily has been shown to make receptor complexes with neuropilins and mediate chemorepulsive activities of class 3 semaphorins. Hippocamal dentate granule cells (DGCs) express plexin-A2, plexin-A3, plexin-A4 and pyramidal cells in CA3 express all members of plexin-A subfamily (plexin-A1, -A2, -A3, -A4). To understand functions of plexin-A subfamily in hippocampal mossy fiber (MF) projection, we generated plexin-A2 and plexin-A4 mutant mice. The laminated MF projection was disrupted in these mutant mice. Co-cultures of dentate gyrus slices with CA3 slices derived from wild type or mutant mice revealed that plexin-A2 expression in the CA3 pyramidal cells and plexin-A4 expression in the DGCs are prerequisite to establish laminated MF projection. Our results suggest that plexin-A2 and plexin-A4 play roles in laminated MF projection.

S21-4 Building the neural network by intra-cellular patterning

Yasushi Hiromi[1,2,3], Masaki Hiramoto[1,4], Takeo Katsuki[1,2]
[1]Dept Dev Genet, Nat Inst Genet, Mishima, Japan, [2]Dept Genetics, SOKENDAI, [3]CREST, JST, [4]PRESTO, JST

Construction of the neuronal network requires positioning of axonal guidance information at strategic locations within the nervous system. Classical models on axonal guidance assumed that guidance cues are provided by secreted molecules that act by forming concentration gradients, read by growth cones. Indeed, several secreted molecules, such as Netrin and Slit, have the property to alter the growth cone behavior when presented in a gradient form. However, this notion has been challenged by the observation that halving the gene dose of secreted molecules, which should have a profound effect on the shape of the gradient, has little effect on the guidance behavior in vivo. We describe an alternative strategy to provide positional information within the nervous system, using intra-cellular patterning. Because neurons possess axons that can span a wide area within the nervous system, localization of specific molecules within the axonal segment can provide global positional information for axon guidance.

S21-5 Roles of BMP signaling in the topographic retinotectal projection

Masaharu Noda[1,2]
[1]National Institute for Basic Biology, [2]CREST

Regional specification in the retina is the basis for the topographic retinotectal projection. We have been studying the molecular mechanisms underlying these two sequential developmental events.The regional specification along N-T axis is controlled by the region specific expression of CBF-1, GH6 and SOHo-1 in the nasal, and CBF-2 in the temporal retina. We found that CBF-1 specifies the expression of all of the topographic molecules along N-T axis through DNA binding-dependent and -independent mechanisms. Along D-V axis, complementary expression of BMP-4 in the dorsal and Ventroptin in the ventral retina leads to the expression of Tbx5 and cVax, respectively. Ventroptin next turns to a double-gradient pattern with V/N high-D/T low gradient from E6, concomitantly with the disappearance of BMP-4. We found that BMP-2 instead begins to be expressed double-gradiently, complementarily to the Ventroptin expression.I will discuss about molecular cascades of the region-specific molecules in the retina and roles of BMP signaling in the topographic retinotectal projection.

S21-6 Regulation of odorant receptor gene expression

Hitoshi Sakano[1]
[1]Dept Biophys & Biochem, Tokyo Univ Grad Sch Sci, Tokyo, Japan

The mouse has over one thousand odorant receptor (OR) genes clustered at multiple loci on many different chromosomes. Each olfactory sensory neuron (OSN) appears to express only one member of the OR gene family in a monoallelic manner. The OSNs expressing a given OR gene, project their axons to a specific set of glomeruli on the olfactory bulb (OB). Using the YAC transgenic expression system, we have been studying the mouse OR gene cluster containing MOR28. It was found that the 2kb DNA region, 75kb upstream of the MOR28 gene, is highly homologous between the mouse and human, and is necessary for the expression of not only the MOR28 but also the downstream OR genes. Surprisingly, when this homology region was added to a MOR28 minigene, choice of the minigene was greatly enhanced: In the zone 4 of the OE, almost two thirds of the OSNs expressed the MOR28 minigene. Furthermore, on the OB, more than 50 glomeruli were targeted by the axons of the minigene-expressing OSNs. Regulations of OR gene expression and projection of OSNs will be discussed.

S22-1 Visualization of spatio-temporal regulation of Rho-family G proteins in growth cone dynamics

Takeshi Nakamura[1], Kazuhiro Aoki[1], Michiyuki Matsuda[1]
[1]Dept Tumor Virol, Res Inst Microbial Diseases, Osaka Univ

Rho-family G proteins (RhoA/Rac1/Cdc42) function as molecular switches that transduce signals from extracellular stimuli to the actin cytoskeleton. Recent evidence indicates that Rho GTPases play a central role in the regulation of neuronal morphogenesis including growth cone dynamics steered locally by guidance cues; however, the spatio-temporal activity-change of Rho GTPases has not been elucidated in a living cell. In this study, we imaged the activities of Rho GTPases during growth cone navigation in sensory neurons and neuronal cell lines, by the use of probes based on fluorescent resonance energy transfer (FRET). The activities of Rac1 and Cdc42 were high at the peripheral domain of growth cones; Rac1 was uniformly activated throughout the peripheral domain, whereas higher Cdc42 activity was observed near the edge of growth cones. Unexpectedly, high RhoA activity was also observed at the extending region of growth cones. The activity change of Rho GTPases upon guidance cues are under investigation.

S22-2 Functions of CRMP-2 in advancing growth cone

Nariko Arimura[1], Kozo Kaibuchi[1]
[1]Ins Adv Res & Dept Cell Pharm, Nagoya Univ Grad Sch Med, Nagoya, Japan

Neurite elongation to target cells is a fundamental step to form complex neuronal network. Growth cones act as a motile organ in guidance to the target cells. We have previously reported that phosphorylation of CRMP-2 by Rho-kinase plays an essential role in LPA-induced growth cone collapse. Moreover, verexpression of CRMP-2 enhances axonogenesis in hippocampal neurons. However, the molecular mechanisms underlying mode of actions of CRMP-2 are unclear. We have recently found two novel activities of CRMP-2: tubulin-dimer binding and microtubule-assembly promoting activities. A CRMP-2 mutant defective in microtubules-assembly activity suppresses axonal elongation, suggesting that CRMP-2 governs axonal growth by promoting microtubule assembly. We also identified Numb as a CRMP-2 interacting molecule. CRMP-2-Numb interaction is involved in endocytosis of L1, adhesion molecule. This time, we summarize and discuss functions of CRMP-2 and its interacting molecules in growth cone regulation.

S22-3 CRMP-2: an intracellular mediator protein for Semaphorin3A signaling

Yoshio Goshima[1]
[1]Dept Molecular Pharmacol & Neurobiol, Yokohama City Univ School of Med, Yokohama, Japan

Semaphorin3A (Sema3A) regulates axon and dendrite guidance in the nervous system. An intracellular CRMP-2 is required for Sema3A-induced growth cone collapse response, but its molecular mechanism remains ill-defined. We have provided evidence that Fyn and Cdk5 mediate Sema3A signaling. In *fyn*- and *cdk5*-deficient mice, Sema3A-induced growth cone collapse responses were attenuated compared to their heterologous controls. Cdk5 was associated with plexin-A2 through the active state of Fyn. Sema3A promoted Cdk5 activity through phosphorylation of Tyr15, a phosphorylation site with Fyn. A Cdk5 mutant (Tyr15 to Ala) showed a dominant-negative effect on the Sema3A-induced collapse response. Cdk5 phosphorylated CRMP-2 at Ser-522 in vitro. Overexpression of a mutant CRMP2 S522A inhibited Sema3A-induced growth cone collapse. With anti-pS522 CRMP-2 antibody, the level of pCRMP-2 in the growth cones was increased during Sema3A-induced collapse response. We here propose a Fyn-Cdk5-CRMP pathway for Sema3A signaling.

S22-4 Phosphorylation of N-WASP and Neurite extension

Shiro Suetsugu[1], Sun Joo Park[1], Tadaomi Takenawa[1]
[1]Dept Biochemistry, Institute of Medical Science, Univ Tokyo

Neurite extension is a key process for constructing neuronal circuits during development and remodeling of the nervous system, in which actin cytoskeletal dynamics is essential. Here we show that Src family tyrosine kinases and proteasome-degradation signals synergistically regulate actin regulatory protein N-WASP, leading to neurite extension. Src family kinases activate N-WASP through tyrosine phosphorylation, which induces nucleation of actin polymerization through the Arp2/3 complex. Tyrosine phosphorylation of N-WASP initiates its degradation through ubiquitination, providing the tight control of activated N-WASP levels. When neurite growth is stimulated in culture, degradation of N-WASP is markedly inhibited, leading to accumulation of the phosphorylated, activated N-WASP and resulting in neurite extension. Collectively, neurite extension appears to be regulated by the balance of N-WASP phosphorylation (activation) and degradation (inactivation), which are induced by tyrosine phosphorylation.

S22-5 Migration of nerve growth cones requires detergent-resistant membranes in a spatially defined and substrate-dependent manner

Hiroyuki Kamiguchi[1]
[1]Dev Brain Sci Group, RIKEN Brain Sci Inst, Saitama, Japan

Motility of nerve growth cones is regulated by cell adhesion molecules (CAMs) such as L1, N-cadherin, and β1 integrin. CAM activities could be modified by its localization to detergent-resistant membranes (DRMs), specialized microdomains enriched in signaling molecules. We demonstrate that L1 and N-cadherin are present in DRMs while β1 integrin is exclusively detected in non-DRMs of neurons and that localization of L1 and N-cadherin to DRMs is developmentally regulated. Growth cone migration mediated by L1 and N-cadherin, but not by β1 integrin, is inhibited after DRM disruption by micro-scale chromophore-assisted laser inactivation (micro-CALI) of GM1 gangliosides or by pharmacological treatments that deplete cellular cholesterol or sphingolipids, essential components for DRMs. Micro-CALI within the peripheral domain of growth cones, or even within smaller areas such as the filopodia and the lamellipodia, is sufficient to impair their migration. However, micro-CALI within the central domain does not affect growth cone migration. These results demonstrate the region-specific involvement of DRMs in CAM-dependent growth cone migration.

S22-6 Interplay between cyclic nucleotide and Ca^{2+} signaling: the polarity of bidirectional netrin-induced axon turning

Kyonsoo Hong[1]
[1]Department of Biochemistry, New York University School of Medicine

Signaling by cyclic nucleotides and Ca2+ is known to regulate the attractive and repulsive guidance of axons by guidance cues. However, the interplay between these second messengers in determining bidirectional guidance responses is largely unknown. Here, we report that the ratio of cAMP/cGMP activities sets the polarity of netrin-1 induced axon guidance: High ratios favor attraction and low ratios favor repulsion. Whole-cell recording of Ca2+ currents at the growth cone indicates that cyclic nucleotide signaling modulates the activity of L-type Ca2+ channels in the axonal growth cone. Furthermore, cGMP signaling via 12-lipoxygenase suppresses the Ca2+ channel activity triggered by netrin-1, and is required for DCC/UNC5-mediated growth cone repulsion. By linking the cAMP/cGMP ratio to Ca2+ signaling via Ca2+ channels in the growth cone membrane, our findings delineate an early membrane-associated event responsible for signal transduction during bidirectional axon guidance.

S23-1 Molecular mechanism of directed migration start out of the cortical ventricular zone

Makoto Sato[1]
[1]Dept Anat 2, Fukui Med Univ, Fukui, Japan

Precise control of migration start out of the ventricular zone and the following directed migration toward the cortical plate are essential steps for the neocortex formation. Here we show a novel mechanism that can tether ventricular zone cells in situ. A FILIP interacts with Filamin A, an indispensable actin-binding protein for cell motility, and induces its degradation in COS-7 cells. Degradation of Filamin A is indicated in the cortical ventricular zone where filip mRNA localize. Most ventricular zone cells that overexpress FILIP fail to migrate in explants. These results signify that FILIP acts through a Filamin A-F-actin axis to control the start of neocortical cell migration from the ventricular zone. Furthermore, we show the evidence that the following directed migration toward the pial surface is potentially controlled by the polarized distribution of intracellular signalling molecules within a cell. Involvement of Filamin A in this polarized localization of signalling molecules will be discussed.

S23-2 Area specification and map formation of mammalian cerebral cortex

Shun Nakamura[1], Nobuo Funatsu[1], Kanae Ohsaki[1], Takayoshi Inoue[1]
[1]Natl. Inst. Neurosci., Tokyo, Japan

The mammalian cerebral cortex develops regional differences primarily represented by a few classes of gene expressions in the embryonic cortical plate. These regional differences are converted to distinct areal cyto-architectures with sharp borders and later to the adult functional cortical map, yet the number of genes identified so far is not enough to explain such intricate processes. Here we divided the mouse E16.5 cerebral cortex into five pieces and performed an extensive gene expression analysis per each division using the Affymetrix U74Av2 DNA microarray with probes for 12,500 genes. Combining this with a real time quantitative RT-PCR and in situ hybridization analyses, we could finally identify several genes specifically expressed in the medial, dorsal, and lateral cortex, respectively. Possible roles of these genes in the cortical arealization and/or map formation will be discussed in the light of our recent results from in utero gene manipulations suggesting roles of extrinsic mechanisms in functional map modifications.

S23-3 Local GABA circuit regulation of developing columnar architecture in visual cortex

Takao Hensch[1]
[1]RIKEN BSI

Afferents from the two eyes are developmentally segregated into discrete ocular dominance columns in visual cortex. Their final width is set by intracortical interactions in self-organizing computational models. We have previously shown that GABA circuits within visual cortex establish the "critical period" for plasticity. In mice genetically engineered to maintain low GABA release uniformly (GAD65 KO), or specifically from a subset of interneurons (Kv3.1 KO), ocular dominance plasticity is impaired unless inhibitory transmission is enhanced with benzodiazepines. We further examined a cortical influence upon anatomical column spacing by similar local modulation of intrinsic inhibition in kittens. Near an agonist (diazepam) infusion site, column periodicity in flattened cortex was wider than in distant areas or throughout control hemispheres. An inverse agonist (DMCM) produced the opposite effect. Intracortical circuits, thus, shape plasticity and the overall layout of incoming thalamic arbors, supporting an activity-dependent development of cortical columnar architecture.

S23-4 Patterning of the cerebral cortex by the roof plate

Edwin S Monuki[1]
[1]Dept Pathology & Develop & Cell Biol, Univ of California Irvine

The roof plate is a source of signaling molecules that are known to regulate dorsal patterning in the developing spinal cord. Previous studies using genetic fate mapping and ablation suggested similar signaling functions for the roof plate in the developing cerebral cortex, as well as an unexpected progenitor function for migratory cortical neurons. Although previous ablations were complicated by an open forebrain defect, more recent ablations in a different mouse strain background have resulted in closed forebrains with expression defects within the developing cortical field, thus confirming a role for the roof plate in cortical patterning. Interestingly, the mutant cortex resembles that seen in holoprosencephaly (HPE), the most common congenital brain malformation in humans, and possesses histologic and molecular abnormalities suggestive of more global functions for the roof plate in regulating tangential and radial patterning of the developing cortex. (Supported by the NIMH and March of Dimes)

S23-5 Horizontal axons in the monkey visual cortex: regional differences and postnatal development

Ichiro Fujita[1]
[1]Lab Cogni Neurosci, Osaka Univ Grad Sch Frontier Biosci, Osaka, Japan

The striate (V1) and inferior temporal (TE) cortices in adult monkeys markedly differ in the topographic features of horizontal axons in layers 2 and 3. Patches of axonal arbors in the TE are larger, more widely spaced, and more irregularly distributed than those in V1. Horizontal axons in V1 link nearby cortical sites more strongly than distant sites, while the strength of the connection in the TE depends less on the distance. The differences in axonal patches between the 2 areas already exist in animals injected with a neuronal tracer on postnatal day 3 and perfused on day 7. Patches with area-specific features are formed early in life, presumably in utero. Prolonged, area-specific changes in axon terminals, however, do occur within patches after birth, to refine the two areas into their adult organization. The pattern of the changes, together with the known functional architecture of the 2 areas, suggests that this refinement depends on visual experience. Supported by CREST and MEXT.

S24-1 What does zebrafish teach us about development of our hindbrain ?

Hitoshi Okamoto[1]
[1]Lab. for Devel. Gene Regulation, RIKEN Brain Science Inst.

Taking advantage of the structural conservation of hindbrain in evolution, we can find novel mechanisms regulating pattern formation and neural differentiation in this tissue using simple zebrafish brain as a starting material. Islet-1 and Islet-3 are both LIM/homeodomain-type transcription factors. Islet-3 is critically involved in the reciprocal inductive signaling between the midbrain and the midbrain-hindbrain boundary (MHB). Systematic search for the downstream target genes has lead to identification of novel families of proteins which play critical roles for the patterning of both MHB and hindbrain. We identified two highly conserved short enhancer elements sufficient for recapitulating the Islet-1 expression specific to the cranial motor and sensory neurons, and opened a possibility for identifying novel genes by positionally cloning which are disrupted in the mutants defective in tangential and radial migration and axonogenesis of the cranial motor neurons using transgenic zebrafish expressing GFP under control of the Islet-1 enhancer.

S24-2 The role of Pax6 in specification of neurons in the hindbrain

Noriko Osumi[1], Masanori Takahashi[1]
[1]Dept. of Developmental Neuroscience, Tohoku Univ. Grad. Sch. of Med.

In the developing neural tube, various types of neurons are generated in precise positions along the dorsoventral and anterioposterior axes. Recent studies have shown that transcription factors with homeodomain (HD) expressed by the progenitor cells specify neuronal cell identity. We have been studying the role of paired type HD protein Pax6 in differentiation of subtypes of neurons in the hindbrain. In the *Pax6* homozygous mutant rat, most of somatic motor (SM) neurons and V1 interneurons were missing. Expression boundaries of other HD protein genes, Nkx2.2, Nkx6.1, Nkx6.2, Irx3, Dbx1 and Dbx2, which define distinct progenitor domains, were shifted and became blurred in the *Pax6* mutant hindbrain. Overexpression of *Pax6* altered expression of HD protein genes, and introduction of exogenous *Pax6* into the mutant hindbrain rescued expression of maker genes for SM neurons and V1 interneurons. These findings suggest that Pax6 regulates the specification of the ventral neurons by establishing the correct progenitor domains.

S24-3 Tangential migration of rhombic lip-derived neurons

Fujio Murakami[1]
[1]Lab Neurosci, Grad Sch Frontier Biosci, Osaka Univ, Toyonaka, Osaka, Japan

Neuronal migration is required for the establishment of specific neural structures, such as layers and nuclei. Neurons migrate along specific routes toward their final destinations, sometimes across long distances. Neurons that form the hindbrain precerebellar neurons originate from the rhombic lip and migrate tangentially. These neurons are initially attracted by the floor plate (FP) at the ventral midline. A subset of these neurons reach ipsilateral destinations, but other subsets cross the midline. These neurons then lose their responsiveness to the FP and become attracted by the alar plate (AP). Although the loss of responsiveness to FP cues is caused by an encounter of migrating cells with the FP, the gain of responsiveness to AP cues occurs irrespective of their encounter with the FP. These findings identify a crucial change in the response of migrating cells to attractive guidance cues during thetangential migration of rhombic lip-derived neurons.

S24-4 Migration of rhombic lip-derived cells and their differentiation in the chick embryo brainstem

Katsuhiko Ono[1]
[1]Dept Anat, Shimane Med Univ, Izumo, Japan

Migration of cells derived from the rhombic lip (RL) was examined in the brainstem by labeling cells with an in ovo electroporation of EGFP gene. The labeling was carried out on fourth or fifth day of incubation (E4 or E5), and the labeled cells were examined sequentially by E9. The RL-derived cells migrated tangentially in the superficial part of the caudal brainstem. They sometimes apposed to each other, showing a neurophilic or chain migration. Tangential unipolar cells subsequently extended a process interiorly, and eventually changed their polarity from tangential to radial. At E9, the labeled cells were distributed in the raphe nuclei, reticular formation and lateral reticular nucleus. Transfection at E5 resulted in more labeled cells in the contralateral brainstem than that at E4. Some of these cells showed glutamate immunoreactivity. Results indicated that early-generated cells settle in the ipsilateral brainstem whereas some of the late-generated cells colonize contralaterlly, and that RL-derived cells may differentiate into glutamatergic neurons.

S24-5 Molecular mechanism of neuronal migration in the rat medulla oblongata

Hitoshi Kawano[1], Masao Horie[1]
[1]Dept Dev Morphol, Tokyo Metro Inst Neurosci, Fuchu, Tokyo, Japan

Neuronal migration in the medulla oblongata was examined in rats.
(1) Neurons generated in the lower rhombic lip express Pax-6 and migrate circumferentially in the subpial region to form the precerebellar nuclei in the medulla oblongata. In Pax-6 mutant rats, both delay in migration and abnormal migratory route of these neurons are observed. The latter defect may be attributed to the change in expression pattern of the cell adhesion molecule TAG-1. TAG-1 as well as netrin, an attractive molecule secreted from the floor plate, is implicated in this migratory process.
(2) Substance P (SP)-containing neurons of the raphe nuclei are situated along the midline of the adult medulla oblongate. SP neurons are first appeared in the ventroalteral medulla at embryonic day 14 (E14) and by E16 they array in two rows closely parallel to the midline glia which are immunoreactive for SP receptor (SPR). SPR in the midline glia, a descendant of the floor plate, may have a special role for the formation of the raphe nuclei.

S25-1 Negative affective aspects of drug withdrawal: reversal by antidepressant treatments

Athina Markou[1]
[1]Dept of Neuropharmacology, The Scripps Research Institute

Drug withdrawal in humans is associated with a syndrome reminiscent of a major depressive episode. In rats, withdrawal from drugs of abuse leads to elevations in brain reward thresholds reflecting anhedonia. Treatment with antidepressants, such as co-administration of a selective serotonin reuptake inhibitor (fluoxetine, paroxetine) and a serotonin-1A receptor antagonist, or bupropion (inhibitor of dopamine and norepinephrine re-uptake, receptor antagonist at nicotinic acetylcholine receptors) reversed the threshold elevations associated with amphetamine and/or nicotine withdrawal. Amphetamine withdrawal led to depression-like behavior (immobility) in two other models of antidepressant activity (rat forced swim test, mouse tail suspension test). The reversal of reward deficits associated with drug withdrawal by clinically proven antidepressants suggests that common substrates, involving decreased monoaminergic neurotransmission, mediate the symptom of anhedonia characterizing drug- and non-drug-induced depressions.

S25-2 Neural basis of emotional disturbance in the Fyn-deficient mice: implication of the amygdalar dysfunction

Shigeki Yuasa[1]
[1]Dept Ultrastructural Res, Nat Inst Neurosci, NCNP

Fyn tyrosine kinase-deficient mice display various emotional defects. The functional- neuroanatomical study was carried out to study the neural basis of the behavioral phenotype. By fear conditioning, Fyn-deficient mice represented increased freezing behavior and c-Fos expression increased in the several areas related to emotional neural circuit such as medial amygdaloid nuclear group, hypothalamus and midbrain central gray. These areas are involved in the expression of amygdala-related emotional responses and their increased excitability is well correlated to the behavioral characteristics of the mutant. This mutant also displayed developmental defects in the amygdaloid nuclei. Thus, the developmental defects and the defective signal transduction in the amygdaloid neural circuits might be responsible for the defective behavior of this mutant. To get insights into the molecular mechanisms of this neural disorder, comprehensive analysis of gene expression in the amygdaloid neurons of the mutant is also to be represented.

S25-3 Regulation of emotional behavior by nuclear receptors

Satoshi Kida[1]
[1]Dept Biosci, Tokyo Univ Agriculture

Many family members of nuclear receptors (NRs) express in adult mammalian brain. However, the function of NRs in adult brain remains unknown. To understand of the role of NRs in brain function, we are trying behavioral analysis using pharmacological and genetics approaches. We found that the injection of ligands activating estrogen receptor (ER) or retinoic acid receptor (RAR) into mice causes significant increase in aggression and anxiety. Currently, we are generating transgenic mice expressing mutant nuclear receptors. Additionally, we found that the level of serotonin transportor (SERT) mRNA is decreased after the injection of ligands for ER or RAR. The reporter assay of mouse SERT promoter showed that the activity of SERT promoter is repressed by retinoic acid (RA), suggesting that RA regulates trasncription of SERT gene through RARs. From these results, we are concluding that RAR and ER signaling pathway regulates emotional behavior through the control of target gene expression.

S25-4 Drug-induced mesolimbic and behavioral activation

Niall Murphy[1]
[1]Neural Circuit Mechanisms Research Group, RIKEN Brain Science Institute, Wako, Japan

Despite a huge variability in their structures and mechanisms of action, addictive drugs have several common properties. That is, they (1) are rewarding (2) stimulate locomotion, and (3) activate the mesolimbic dopamine system. The precise relationship between these properties has been debated for some time. In particular, the psychostimulant theory of addiction posits that ability to stimulate mesolimbic dopamine release and locomotion ought to be a hallmark of all rewarding drug. We have studied the relationship between parameters through use of inbred mice and novel pharmacological agents. We find that although rewarding drugs do consistently stimulate mesolimbic activity, correlations between locomotor and mesolimbic activity are often weak. These studies call the importance of the locomotor stimulatory effects of rewarding drugs into question and caution against using locomotion as an index of mesolimbic activation.

S25-5 Roles of the basolateral (BLA) and central (CeA) amygdaloid nuclei in pain-related aversion

Masabumi Minami[1], Masamichi Satoh[1]
[1]Dept of Mol Pharmacol, Grad Sch of Pharmaceut Sci, Kyoto Univ, Kyoto, Japan

Pain is a complex experience composed of sensory and affective components. The neural system of the sensory component has been extensively studied, while that of the affective component remains to be elucidated. In this study, we examined the roles of the BLA and CeA in the affective component. Place-conditioning paradigm revealed that both somatic (i.pl. formalin) and visceral (i.p. acetic acid) pain induced aversion. Chemical lesion of the BLA suppressed the aversion induced by somatic, but not visceral, pain. On the other hand, CeA-lesion abolished that induced by the both pains. BLA- or CeA-lesion failed to reduce the nociceptive behaviors (sensory component) produced by formalin or acetic acid. These results suggest the differential contributions of distinct amygdaloid nuclei to the affective components of somatic and visceral pain. Additional experiments using receptor antagonists and a microdialysis method revealed the involvement of NMDA receptors in the BLA in the formalin-induced aversion.

S25-6 Roles of opioid and dopamine systems in intracranial self-stimulation

Kazutaka Ikeda[1,2], Ichiro Sora[1,3], Yukio Takamatsu[1], Takehiro Takahashi[1], Hiroaki Niki[2]
[1]Dept Mol Psychiatry, Tokyo Inst Psychiatry, Tokyo, Japan, [2]Lab Neurobiol Emotion, RIKEN-BSI, Wako, Japan, [3]Div Psychobiol, Tohoku Univ Grad Sch Med, Sendai, Japan

Opioid and dopamine systems play crucial roles in reward system. However, similarities and differences in neural mechanisms of reward produced by these two systems remain largely unknown. We recently found differences in reward functions between opioid and dopamine systems, by analyzing intracranial self-stimulation (ICSS) in mice lacking mu-opioid receptor (MOR) and dopamine transporter (DAT). MOR-knockout (KO) increased lateral hypothalamic ICSS responses, suggesting that MOR is involved in inhibition of lateral hypothalamic ICSS. In contrast, DAT-KO mice showed maintained ICSS responses even when the electrical stimulation can not be obtained easily or immediately, suggesting that DAT is involved in craving for reward. These results support the hypothesis that opioid and dopamine systems are involved in distinct aspects of rewards, drive-reducing and drive-increasing aspects, respectively.

S26-1 Reward expectancy and executive control in the primate prefrontal cortex

Masataka Watanabe[1]
[1]Dept Psychol, Tokyo Metropol Inst Neurosci

The lateral prefrontal cortex (LPFC) plays important roles in cognitive operations such as retaining information in working memory (WM) while the orbitofrontal cortex (OFC) is more concerned with motivational operations such as processing reward information. However the LPFC is also related to motivational operations; in neurophysiological studies, reward- and reward expectancy-related neurons are found in both the LPFC and OFC while WM-related neurons are observed only in the LPFC. Furthermore, many LPFC neurons are related to both reward expectancy and WM. For survival, monkeys work to obtain a goal, such as food, liquid and a mate. Reward expectancy-related activity and WM-related activity in LPFC neurons are considered to be concerned with representing what is the goal of a behavior, and how the goal can be attained, respectively. Thus, it appears that executive control function for goal-directed behavior is supported not solely by cognitive operations but by the integration of cognitive and motivational operations in the LPFC.

S26-2 Neuronal signals in rhesus monkey anterior cingulate and ventral striatum during the multi-trial reward schedules.

Munetaka Shidara[1], Barry J Richmond[2]
[1]Neurosci. Res. Inst., AIST, Japan, [2]NIMH/NIH, USA

Feelings of expectancy increase as a predicted reward's arrival becomes more imminent. To search for related neuronal signals we recorded single neurons from anterior cingulate and ventral striatum in monkeys performing a multi-trial reward schedule task. In the task, monkeys must successfully complete 1-4 simple visual color discrimination trials to obtain a reward. A visual cue's brightness indicates progress of the schedule. The monkey's reaction time to bar release and error rate decreased as the cue brightened, suggesting the monkey's larger reward expectancy as approaching the rewarded trials. A large proportion of neurons in the ventral striatum showed phasic responses that were selective to a cue signaling the stages of progress through the reward schedules. In the anterior cingulate, about one-third of the recorded neurons' firing progressively increased or decreased through the schedules, suggesting that these responses seem related to the degree of reward expectancy.

S26-3 Dual neural pathways for decision-making in monkey brain

Masamichi Sakagami[1,3], Shunsuke Kobayashi[1,2], Takuro Ikeda[1,2]
[1]Brain Science Research Center, Tamagawa University, [2]Department of Neurology, University of Tokyo, [3]PRESTO, JST

Cognition and motivation are key factors for decision-making. To understand how brain processes these two factors, we compared activity patterns of cells in lateral prefrontal cortex (LPF), caudate nucleus (CD) and superior colliculus (SC), while Japanese monkeys performed the same oculomotor delayed response task with asymmetric reward schedule. Based on signal detection theory, we calculated spatial discriminability (SD) and motivational bias of each cell. Majority of LPF cells changed their SD dependent on expected amount of reward, on the other hand CD cells tended to alter their general activity level based on reward expectation with keeping their SD. The functional segregation was still preserved by different groups of SC cells. Results suggest that parallel pathways are for a saccade generation system in different ways: the LPF-FEF-SC circuit for execution of saccade under a cognitive demand; the CD-SNr-SC circuit for execution of saccade under an appetitive drive.

S26-4 Contribution of the prefrontal cortex and amygdala to reward processing in human and non human primates

Angela C Roberts[1]
[1]Dept of Anatomy, University of Cambridge, UK

Reward processing, a fundamental brain mechanism critical for the control of a large range of emotional and motivated behaviour,is dependent upon a distributed network of structures within the mammalian forebrain and brainstem. Rewards can enter specifically into a number of different associations with both environmental stimuli and responses in order to control behaviour or can act more generally to energise behaviour by strengthening associations between environmental stimuli and responses without explicitly entering into the associations. Taking these different types of mechanism into account, the present series of experiments specifically compare the contribution of the orbital and medial prefrontal cortex and amygdala to food rewarded behaviours. In marmosets, the effects of excitotoxic lesions of these structures on food preferences and on responding for primary and secondary reinforcement will be described. Secondary reinforcement is explored further in a functional neuroimaging study in humans.

S26-5 Adaptation of reward expectation during learning in orbitofrontal cortex and striatum

Wolfram Schultz[1], Leon Tremblay[1], Jeffrey R Hollerman[1]
[1]Department of Anatomy, University of Cambridge, UK

Lesioning and psychopharmacological studies suggest that the basal ganglia and orbitofrontal cortex are involved in learning reward-directed behavior. Learning in a number of situations can be conceptualized as change in the prediction of outcome, and learning subsides when all outcomes occur as predicted. Neurons in the orbitofrontal cortex process information about the prediction, expectation and reception of liquid and food rewards. Some neurons in the striatum are also active during the expectation of reward and integrate information about expected rewards into activity related to the behavior leading to the reward. The different forms of reward expectation activity adapt to changes in reward expectation when learning new reward-predicting stimuli. The neuronal changes occur in parallel with the adaptation of behavioral reactions to the new reward contingencies. These data reveal neuronal mechanisms involved in mediating behavioral changes to modified expectations of future outcomes.

S27-1 Genetic sex of brain cells influences brain sexual phenotype

Arthur P Arnold[1]
[1]Department of Physiological Science and Brain Research Institute, UCLA

Research on mice and songbirds suggests that the genetic sex of brain cells contributes to sex differences in brain structure and function. We analyzed mice in which the genetic sex of brain cells is independent of the gonadal sex of the animal. XY cultures of embryonic mesencephalon develop more dopamine neurons than XX cultures irrespective of the gonadal sex of the embryo, showing that XX and XY cells have a different phenotype. In adult mice, the density of vasopressin fibers in the lateral septum is greater in XY than XX animals of the same gonadal sex, suggesting a direct action of X or Y genes on the brain. In zebra finches, genetic females induced to develop testes have a feminine brain. We analyzed a gynandromorphic finch which was genetically male on the right side of its body and genetically female on the left side of its body. Measures of brain sex showed that the right brain was more masculine than the left, a difference that could not have been caused by gonadal secretions but is attributed to the genetic differences between the two sides.

S27-2 Genes, sex and aggression: differential roles of estrogen receptor α and β in the regulation of neuroendocrine functions and socio-sexual behaviors

Sonoko Ogawa[1]
[1]Laboratory of Neurobiology and Behavior, The Rockefeller University

Estrogen plays a major role in the regulation not only of female sexual behavior but also an array of social and emotional behaviors in both sexes, by acting through intracellular estrogen receptor (ERs). Recent studies using knockout mice for the two types of ERs (ER-α or ER-β) have advanced our knowledge of how ERs are involved in the control of these behaviors. These studies revealed that activation of ER-α and ER-β differentially regulate a number of neuroendocrine functions and behaviors. Specifically, activation of ER-β in the hypothalamic and limbic brain areas as well as in the midbrain dorsal raphe nuclei may play a significant modulatory role to fine-tune the final outcome of the behavior. Our previous findings of behavioral characteristics of ER-α and ER-β knockout mice will be first overviewed. Recent data investigating the role of ER-β in the regulation of socio-sexual behaviors along with possible molecular and physiological mechanisms will then be presented.

S27-3 Sexual plasticity of behavior and LH secretion in goldfish and crucian carp

Makito Kobayashi[1]
[1]Dept Biology, Division of Natural Sci, International Christian Univ

In mammals, sex-typical patterns of behavior and luteinizing hormone (LH) secretion are considered to conform to the sex of the brain, which is determined during neonatal development. Our studies in goldfish, Carassius auratus and gynogenetic crucian carp, C. a. langsdorfii suggest that unlike mammals, teleost species retain a bipotential brain sex. Goldfish, a gonochoristic teleost, exhibits sex-typical behaviors and LH secretion during spawning. Heterotypical sexual functions do not normally occur, but can be induced in adults by hormone treatments that do not inhibit the occurrence of homotypical functions. Male-type sex behavior and LH secretion can also be induced by androgen treatment in the crucian carp. Such sexual plasticity is also observed in hermaphroditic teleosts during their lifetime. Existence of naturally occurring hermaphroditism and the results of our studies suggest that teleosts, regardless of their reproductive strategy (hermaphroditism, gonochorism, and gynogenesis), may commonly posses a sexually bipotential brain.

S27-4 Cellular mechanisms mediating hormonal specification of limbic-hypothalamic neural pathways

Richard Simerly[1]
[1]Division of Neuroscience, Oregon & Health Science University

Developmental mechanisms specifying numbers of neurons that reside in hypothalamic and limbic nuclei, and the pattern of connections that form between them, are major determinants of the functional properties of neural circuits.. The anteroventral periventricular nucleus (AVPV) plays a critical role in the neural control of ovulation and is unusual among sexually dimorphic nuclei in that it is larger in females. The results of both in vivo and in vitro experiments indicate that estrogen acts on the AVPV to specify neuronal number through an ERa dependent mechanism of caspase dependent, apoptotic like programmed cell death. Estrogen appears to induce formation of sexually dimorphic afferents to the AVPV through release of a diffusible target-derived factor, while at the same time it promotes collapse of efferent projection axons. Thus, estrogen acts at the level of the AVPV to sexually differentiate its input/output relationships by regulating programmed cell death and axonal targeting through ERa dependent signaling pathways.

S27-5 Voltage-gated calcium channels in GnRH neurons visualized by green fluorescent protein in transgenic rats

Yasuo Sakuma[1], Masakatsu Kato[1]
[1]Dept Physiol, Nippon Medical School, Tokyo, Japan

GnRH neurons were visualized by enhanced green fluorescence protein in transgenic rats. The brain of neonatal (1-7 d) or pubertal (35-40 d) rat of either sex was excised under ether anesthesia. Neurons in the diagonal band and preoptic area were dispersed. Voltage-gated Ca^{2+} currents were recorded by perforated patch clamp. In neonatal GnRH neurons, high voltage activated Ca^{2+} currents were observed while low voltage activated Ca^{2+} currents were negligible. Nimodipine (L-blocker) and ω-conotoxin GVIA (N-blocker) attenuated the current by 20%. SNX-482 (R-blocker) attenuated the current by 55%. Inhibition by P/Q-blocker ω-agatoxin IVA was small. At puberty, both high and low voltage activated Ca^{2+} currents were present. Nifedipine, ω-conotoxin GVIA and SNX-482 inhibited the currents as in the neonate. In addition, ω-agatoxin IVA inhibited the current in many GnRH neurons. No sex difference was observed. GnRH neurons express L-, N-, P/Q-, R- and T-channels. P/Q- and T-channels were developmentally regulated.

S27-6 GFP-tagged steroid receptor dynamism in the hypothalamus and hippocampus in vitro and in vivo

Mitsuhiro Kawata[1]
[1]Department of Anatomy and Neurobiology, Kyoto Prefectural University of Medicine

GFP-tagged Steroid Receptor Dynamism in the Hypothalamus and Hippocampus in vitro and in vivo. Department of Anatomy and Neurobiology, Kyoto Prefectural University of Medicine. Steroid hormones regulate several important functions of the brain including neuronal development and plasticity through their receptors. Steroid receptors can be divided into three categories based on their unliganded distribution: those that (ERs and PR) are in the nucleus, those (GR, MR, and AR) in the cytoplasm, and those with mixed cytoplasmic and nuclear distribution. In all cases, addition of ligand leads complete nuclear translocation of the receptors. Hormonal stimulation induces changes of nuclear receptor distribution pattern and GFP-FRAP analysis demonstrates dynamism of liganded receptors in the nucleus than it has been thought. Transgenic animal model of ER a-GFP and fluorescent dye-injection into the cell show that cell bodies and dendritic profiles are plastic in the hypothalamus and hippocampus on the hormonal milieu

S28-1 Essential role of leptin in the higher brain function

Yutaka Oomura[1], Shuji Aou[2], Nobuaki Hori[3], Kazuo Sasaki[4], Koji Fukunaga[5], Matthew Wayner[6]
[1]Dept Integrated Physiol, Kyushu Univ, Fukuoka, [2]Kyushu Inst Tech Brain Sci, Kitakyu-shu, [3]Univ New York, Pub Health, Albany, USA, [4]Toyama Univ Bio-Inform, Toyama, [5]Touhoku Univ Pharmacy Sch, Sendai, [6]Texas Univ., San Antonio

Physiological increase of leptin in the rat brain facilitates spatial learning and memory associated with enhancement of LTP and calmodulin kinase II (CaMKII) activity in hippocampal CA1 neurons. Zucker rats and db/db mice have abnormal leptin receptors and display impaired spatial learning and memory accompanied by a lack of hippocampal LTP and low level of CaMKII activity. Ob/ob mice have normal leptin receptors but produce abnormal leptin. Ob/ob mice also display impaired spatial learning and memory. When 50 μg/kg/day leptin was applied iv for 3 weeks, spatial performance recovered normally. Body weight and food intake remained at original levels. LTP and the level of CaMKII activity were not affected by this treatment. These results support an essential role for leptin in spatial learning and memory.

S28-2 Energy signals regulates learning and memory function

Shuji Aou[1], Yutaka Oomura[2], Xue-Lieng Li[2]
[1]Dept Brain Sci & Engineer, Kyushu Inst Tech, [2]Dept Integr Physiol, Grad Sch Med Sci, Kyushu Univ

The effects of orexin-A on the performance of Wistar rats during the Morris water maze test and the long-term potentiation in Schaffer collateral/commissural-CA1 synapses in hippocampal slices wre examined. The results of the Morris water maze test show that 1.0 and 10 nmol of orexin-A, when administered intracerebroventricularly, retarded both spatial learning and memory. A probe test also showed an impairment. The results of an electrophysiological study using hippocampal slices demonstrated that 1.0 to 30 nM of orexin-A applied to the perfusate produces a dose-dependent and time dependent suppression of the long-term potentiation that was not affected by 6-cyano-7-nitroquinoxaline-2,3-dione, CNQX, which is a non-NMDA receptor antagonist, thereby implicating NMDA receptors. These results show that orexin-A impairs spatial and these impairments can be attributed to a suppression of long-term potentiation in the Schaffer collateral-CA1 hippocampal synapses.

S28-3 Involvement of stem cell factor/*c-kit* signaling in spatial learning and hippocampal synatpic plasticity

Toshihiko Katafuchi[1]
[1]Dept Integr Physiol, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan

Homozygous mutant rats (*Ws*/*Ws*) carrying deficit in 4 a.a. in the tyrosine kinase domain of *c-kit* receptor, showed an impairment of spatial learning, and long-term potentiation (LTP) in the mossy fiber (MF)-CA3 pathway. We found a negative correlation between the initial paired-pulse facilitation (PPF) and changes in the PPF measured after the tetanus-induced LTP in this pathway in normal mouse slices. Bath application of SCF, a intrinsic ligand for *c-kit*, for 30 min produced a similar correlation between PPFs before and after SCF. The effect of SCF was blocked by K252a, a receptor tyrosine kinase inhibitor, wortmannin, a phosphatidylinositol3'-kinase inhibitor, and mepacrine, a PLA_2 inhibitor. Preincubation of slices with *c-kit* antibody blocked LTP, suggesting an involvement of the intrinsic SCF in the induction of LTP. Since *c-kit* is expressed only on the postsynaptic CA3 neurons, the effects of SCF may be mediated by a retrograde messenger, such as the PLA_2-induced arachidonic acid.

S28-4 Endocannabinoid-mediated retrograde signaling at hippocampal synapses

Masanobu Kano[1], Takako Ohno-Shosaku[1]
[1]Dept Cellular Neurophysiol, Grad Sch Med Sci, Kanazawa Univ, Kanazawa 920-8640, Japan

Recent studies have revealed that the activity of postsynaptic neuron can influence presynaptic functions retrogradely through endocannabinoids. In this presentation, we summarize our recent studies on this phenomenon at hippocampal synapses. We have found: (1) Endocannabinoids can be released from postsynaptic neurons by depolarization or by activation of group I metabotropic glutamate receptors (mGluR). (2) Endocannabinoid release is also induced by activation of the M_1 and M_3 subtypes of muscarinic acetylcholine receptors. (3) Postsynaptic depolarization and activation of group I mGluRs or M_1/M_3 receptors work in a cooperative manner to release endocannabinoids. (4) The released endocannabinoids act retrogradely onto presynaptic cannabinoid CB1 receptors and suppress the transmitter release from presynaptic terminals. These results suggest that endocannabinoids mediate retrograde signals by which the postsynaptic neuronal activity influences the transmitter release from presynaptic terminals.

S28-5 Endogenous neuropeptides that impair hippocampal LTP and memory

M Wayner[1], D Armstrong[1], C Phelix[1]
[1]Univ Texas

Several neuropeptides producing a variety of behavioral effects have been studied for many years. Some of behavioral changes include enhancement of learning and memory, impaired learning and memory, changes in attention and anxiety. Deficits in these cognitive processes indicate impaired hippocampal functions. Conflicting results due to using different behavioral tests and different routes of administration of the neuropeptides have been difficult to interpret. This study began when it was discovered that angiotensin II inhibited LTP in medial perforant path dentate granule cells in the hippocampus and impaired 24 retention of a single trial step through passive avoidance response. These effects were prevented by pretreatment with an angiotensinI I AT1 antagonist. The purpose of present experiments was to examine other neuropeptides utilizing the same animal model. Earlier results as well as data on oxytocin, vasopression, will be presented. Peptides were administered directly into the dentate gyrus. Also appropriate peptide receptors vasopressin enhances and inhibits LTP dependent upon the dose.

S28-6 Novel central effects of complement C3a and C5a

Kousaku Ohinata[1], Yunden Jinsmaa[1], Masaaki Yoshikawa[1]
[1]Division Food Biosci & Biotechnol, Grad Sch Agri, Kyoto Univ, Kyoto, Japan

Complement C3a (77 amino acids) and C5a (74 amino acids) are released from C3 and C5, respectively, on activation of complement system and play important roles in host-defense system. Recently, C3a, C5a and their receptors have also been reported to exist in the central nervous system (CNS). However, little is known about their functions in the CNS. We found that C3a had anti-analgesic activity in tail-pinch test after central administration in mice. Since anti-analgesic drugs generally showed anti-amnesia and/or learning enhancement, we investigated the effects of C3a and C5a on learning. Central administration of C3a ameliorated amnesia induced by scopolamine and ischemia in the step-through passive avoidance test. C5a also had anti-amnesic effect. In addition, complements were associated with the regulation of food intake via prostaglandin system. We propose that complement C3a and C5a are regulators not only for the immune system but also for the CNS.

S29-1 Promotion of survival and axonal regeneration of axotomized retinal ganglion cells

Masami Watanabe[1]
[1]Dept. Physiol., Inst. Develop. Res.

Retinal ganglion cells (RGCs) of mammals regenerate their axons into a grafted peripheral nerve segment, but numbers of regenerated RGCs are 2% in average. Methods increasing the numbers should be developed to intend functional recovery of impaired vision, or to guide axons of stem cell-derived RGCs into the CNS. I tested several drugs which protect death of axotomized RGCs since surviving axotomy is the first requisite for regeneration. 1. Combination of BDNF, CNTF and forskolin facilitated both survival and axonal regeneration of cat RGCs, especially beta cells, but did not increase axonal regeneration of cells which survived axotomy but failed regeneration. 2. Nipradilol (NP), an anti-glaucoma drug, protects injured RGCs from death. I found that NP at 10[-7] mol increased numbers of regenerated RGCs into 3-4 fold, and shortened time for 20 mm axonal elongation by 7 days. Since NP is an NO donor and blocker of α and β adrenoreceptors, it must be clarified whether one or both of them are effective for regeneration.

S29-2 Functional restoration of visual pathway by means of electrical stimulation of the retina and optic nerve

Hajime Sawai[1]
[1]Dept Physiol, Osaka Univ Grad Sch Med, Suita, Japan

In adult mammals with damaged visual pathway, survival of retinal ganglion cells (RGCs) is a prerequisite for optic nerve (ON) regeneration and restoration of vision. Our recent studies on rats demonstrated that electrical stimulation (ES) to the sectioned ON significantly enhanced the RGC survival at the postoperative day 7. Such survival promoting effect was obtained even by the ES for 30 min just after the ON section, but the ES at 3 hr before or after the section resulted in no such effect. These results suggest that ES could overcome some intracellular death signals that operate immediately after ON section. On the other hand, we have developed a novel stimulating method for artificial retina, i.e. suprachoroidal-transretinal ES (STES), to restore vision from damage of the inner retina. Focal STES to the retina in RCS rats successfully evoked field responses from retinotopically predicted areas of the superior colliculus. Advantages of STS will be discussed in comparison with the other types of ES for artificial retina.

S29-3 Electric optic nerve stimulation induces ocular dominance plasticity in kitten visual cortex

Yoshio Hata[1]
[1]Div Neurobiol, Sch Life Sci, Fac Med, Tottori Univ, Yonago, Japan

Binocular visual responses of neurons in visual cortex can be changed by monocular visual deprivation in the critical period of early life. It is hypothesized that afferents from each eye compete with one another for synaptic connections with cortical neurons so that less active inputs from the deprived eye lose their connections. This hypothesis predicts that an increase in inputs from one eye instead of decrease due to deprivation should also change binocular responsiveness of cortical neurons. However, the hypothesis has not successfully been tested with experimental activation of afferents from one eye. We activated one of the optic nerves by electric stimulation in behaving kittens for 2 days. After such a monocular activation, visual cortical neurons showed a significant ocular dominance shift in favor of the electrically activated eye. These results support the synaptic competition hypothesis and further implicate that chronic electric stimulation of nerve fibers can be used to change physiological properties of cortical neurons.

S29-4 An artificial vision system for visual prosthesis

Tetsuya Yagi[1], Seiji Kameda[1], Liming Li[2]
[1]Osaka Univ Grad Sch Eng, Suita, Japan, [2]Kyushu Inst Tech, Grad Sch Life Sci and Sys Eng., Kitakyushu, Japan

The externally applied electrical stimulus has been considered as a means to excite retinal or visual cortex neurons to restore a partial vision in blind patients. For this attempt, electronic devices to transmit the visual information appropriately to those neurons are to be designed. We have designed an Analog Very Large Scale Integrated (VLSI) chip, a silicon retina, which emulats the parallel structure of the retinal circuit. The silicon retina can provide two fundamental classes of response found in the vertebrate retina, e.g., the sustained and the transient responses and therefore is suitable for transmitting visual signals to electrodes to be implanted in the eye to excite retinal ganglion cells. Combining the silicon retina and Field Programmable Gate Array (FPGA) circuit, we further developed an electronic device which generates the response mimicking the orientation selective neuron ' s in the visual cortex V1. The device can be used to provide signals to electrodes stimulating V1 neurons.

S29-5 Topographic map reorganization in cat area 17 after early monocular retinal lesions

Kazuki Matsuura[1]
[1]Dept Ophthalmology, Saiseikai-Goutsu Hosp

Neither discrete peripheral retinal lesion nor the optic disc produces obvious holes in one's percept of the world because the visual brain appears to perceptually "fill in" these blind spots. Since the map reorganization does not typically occur unless retinotopically matched lesions are made in both eyes, our experiments were designed to maximize the probability that monocularly deprived neurons would acquire new receptive field. We found that deprived neurons in cat area 17 can acquire new receptive fields if the lesion occurred early in life (8 weeks of age) followed by a substantial period of recovery (>3years). The monocular and binocular response properties of reactive units were remarkably robust, and the properties for the two eyes were generally similar. Moreover, excitatory or inhibitory binocular interaction was found in the majority of units. These results support the hypothesis that map reorganization require experience-dependent plasticity and may be involved in filling in of blind spots due to retinal lesions early in life.

S29-6 Visual cortical plasticity after localized retinal lesions

U.T. Eysel [1], D.V. Giannikopoulos[1]
[1]Dept. Neurophysiol., Ruhr-Univ. Bochum, Germany

The filling-in of the cortical lesion projection zone (LPZ) of binocular retinal lesions is a well studied phenomenon in cat visual cortex. We focus on neurophysiological and molecular changes associated with this neuronal plasticity. When recording from visual cortical neurons in anesthetized cats at different stages of reorganization (2,4,12wk), we observed a travelling wave of neuronal hyperexcitability associated with the filling-in process that moved with time from the border towards the center of the LPZ. The receptive field (RF) properties (size, orientation & direction preference) of reconnected cells in the LPZ indicate a continuous process of reorganization with a characteristic spatio-temporal pattern: RFs close to the front of the filling-in process showed large RFs and low orientation specificity, while both properties became increasingly normal towards the border of the LPZ. This time-dependent increase of specificity is associated with a downregulation of the glutamatergic excitation and an upregulation of GABAergic inhibition in the reorganized LPZ.

S30-1 Roles of oxidized galectin-1 in nerve regeneration -from PNS to CNS-

Hidenori Horie[1,2], Toshihiko Kadoya[3], Mitsuhiro Hasegawa[4]
[1]Adv Res Cent Biol Sci, Waseda Univ, Nishitokyo, Japan, [2]Corp Res Cent, Fuji Xerox, Kanagawa, Japan, [3]R&D Cent, Prod Dept, Pharmceut Div, Kirin Brewery Co,Ltd, Maebashi, Japan, [4]Dept of Neurosurg , Grad Sch Med Sci, Kanazawa Univ, Kanazawa, Japan

Oxidized galectin-1 (GA-1/Ox) has been proved to be an essential factor to initiate axonal regrowth after axotomy in PNS. Application of GAL-1/Ox to injured peripheral nerves promotes Schwann cell migration together with axonal regeneration resulting with functional recovery in in vivo models. We identified a macrophage to be a target cell of GAL-1/Ox. The GAL-1/Ox-activated macrophage conditioned medium promoted both axonal regeneration and Schwann cell migration in an in vitro PNS nerve regeneration model. This medium also enhanced nerve regeneration from retinal explants or cultured transected optic nerve sites with a retina These results suggest that the factors secreted from GAL-1/Ox-stimulated macrophages may be expected candidates to perform the axonal regeneration in injured CNS.

S30-2 Surface-localization and externalization of galectin-1 in adult rat DRG neurons and Schwann cells in culture

Kazunori Sango[1], Akiko Tokashiki[1], Kyoko Ajiki[1], Masao Horie[1], Hitoshi Kawano[1], Hidenori Horie[2], Toshihiko Kadoya[3]
[1]Dept Dev Morphol, Tokyo Metr Inst Neurosci, Tokyo, Japan, [2]Adv Res Center Biol Sci, Waseda Univ, Tokyo, Japan, [3]Pharmaceu Res Lab, Kirin Brewery Co., Maebashi, Japan

Galectin-1 (Gal-1) in the oxidized form is a novel factor to enhance axonal growth in peripheral nerves after axotomy. Although Gal-1 lacks signal leading peptide, the extracellular release of Gal-1 from neuronal cells is suggested. In our immunohistochemical analysis on the sections of adult rat dorsal root ganglia (DRG), Gal-1 was distributed diffusely throughout the cytoplasm in both neurons with smaller diameter and Schwann cells. In contrast, the immunoreactivity for Gal-1 was restricted to the surface and extracellular region of most DRG neurons and Schwann cells after 4 and 7 days in culture. Western blot analysis revealed that both reduced and oxidized forms of Gal-1 were detected in the culture medium of DRG cells. These results suggest that some of Gal-1 released from DRG neurons and Schwann cells is converted to the oxidized form.

S30-3 Galectin-1 facilitates regeneration of sensory fibers into the CNS after rhizotomy

John McGraw[1], Hidenor Horie[2], Toshihiko Kadoya[3], John D Steeves[1], Matt S Ramer[1], Wolfram Tetzlaff1[1]
[1]ICORD (Int. Collaboration on Repair Discoveries), University of British Columbia, Vancouver, BC, Canada, [2]Waseda University, Tokyo, Japan, [3]Pharmaceutical Res. Lab., Kirin Brewery Co.,Japan

Sensory axons typically fail to regenerate into the spinal cord after dorsal rhizotomy, i.e. after a dorsal root crush. Since galactin-1 has been previously reported to promote peripheral nerve regeneration, we tested the hypothesis that intrathecal application of galectin-1 promotes regeneration of sensory fibers through the dorsal root entry zone (DREZ) into the cervical spinal cord. Here we show regeneration of CGRP positive axons into the DREZ of male adult Wistar rats. Analyzing the expression of galectin-1in specific subpopulations of untreated rats we found 55% of the total DRG population to be positive, mainly the small diameter nociceptive cells. The expression study in other neuronal populations is currently under way. These results suggest that galectin-1 has a role in axonal regeneration within the CNS environment.

S30-4 Galectin-1 facilitates neuropathic pain and muscle regeneration

Emiko Senba[1], Hiroki Imbe[1], Katsuya Kami[2]
[1]Dept Anat & Neurobiol, Wakayama Medical Univ, Wakayama, Japan, [2]Dept Health Sci, Osaka Univ. Health & Sport Sci, Osaka, Japan

Galectin(Gal)-1 is abundant in sensory ganglia, spinal cord and muscle. We tried to elucidate the roles of Gal-1 in the mechanisms of neuropathic pain and muscle regeneration. Gal-1-IR in the dorsal horn was increased 1-2 wks after axotomy. In order to block functions of Gal-1 in the dorsal horn, we injected anti-rhGal-1 antibody (Ab) intrathecally for 2 wks in spared nerve injury model. This procedure attenuated mechanical hypersensitivity, suggesting that endogenous Gal-1 facilitates neuropathic pain. One day after muscle contusion, injured intramuscular nerves showed intense Gal-1-IR. These IR structures were NF-200(+) and DAPI(-), and considered as nerve fibers. Gal-1-IR was seen in plasma membrane of intact muscle fibers. After crush injury, some MyoD(+), putative activated satellite cells showed Gal-1-IR. Myotubes formed after 5 days were also Gal-1-IR. Since injection of Gal-1 Ab into damaged muscle hampered its regeneration, Gal-1 may play a role in muscle regeneration.

S30-5 Regulation of galectin-1 expression by *fosB* gene products

Yusaku Nakabeppu[1,2], Yoshinori Ohnishi[1,2], Tomofumi Miura[1,2], Hideaki Kurushima[1,2], Ping Xu[1,2], Kosuke Kajitani[1,2], Hidenori Horie[3], Toshihiko Kadoya[4], Kunihiko Sakumi[1,2]
[1]Div Neurofunc Genomics, Med Inst Bioreg, Kyushu Univ, Fukuoka, Japan, [2]CREST, JST, Japan, [3]Adv Res Cent Biol Sci, Waseda Univ, Tokyo , [4]Kirin Brewery Co Ltd, Maebashi, Japan

Expression of galectin-1 is induced in rat hippocampus after ischemia-reperfusion, following expression of *fosB* gene encoding splicing variants, FosB and ΔFosB. We established mouse *fosB*-null embryonic stem (ES) cell line and ES cell lines expressing only either one of FosB or ΔFosB. Expression of galectin-1 is induced after serum stimulation in both wild type and mutant ES cells expressing only ΔFosB. Such expression of galectin-1 was completely abolished in mutant ES cells expressing only FosB. However, in the *fosB*-null ES cells, galectin-1 expression was restored, indicating that both FosB and ΔFosB are involved in the regulation of galectin-1 expression. Furthermore, level of galectin-1 mRNA was not altered among all ES cell lines, indicating that expression of galectin-1 is regulated in the post-transcriptional manner.

S31-1 Functional and structural plasticity in premotor cortex after cortical ischemia

Randolph J. Nudo[1]
[1]Univ Kansas Med Sch, Kansas City, Kansas, USA

It has been suggested that after a stroke affecting the primary motor cortex (M1), premotor areas may be involved in functional recovery. In a squirrel monkey ischemia model, the hand representations in M1 and the ventral premotor cortex (PMv) were located using microelectrode stimulation techniques. Then an ischemic infarct was made over the entire M1 hand representation. At the end of three months, significant spontaneous recovery of motor skill was demonstrated in the impaired hand, though residual deficits persisted. Subsequent neurophysiological mapping experiments indicated that the PMv hand area increased by as much as 50%. A neuroanatomical tract tracer, biotinylated dextran amine (BDA), was injected into the reorganized PMv. BDA terminal labeling was found in cortical areas,not normally connected to PMv. These included dense BDA terminal labeling in somatosensory cortical regions. These results suggest that compensatory changes in PMv may underlie motor recovery after stroke.

S31-2 Role of the premotor cortex in locomotor recovery after stroke

Ichiro Miyai[1]
[1]Neurorehabilitation Research Institute , Bobath Memorial Hospital, Osaka, Japan

An optical imaging system using near-infrared spectroscopy enabled real-time monitoring of cortical activation during human gait (Miyai et al. Neuroimage 2001;14:1186-92). In healthy subjects, walking activated the medial sensorimotor cortices and supplementary motor areas. In patients with hemiparetic stroke, cortical activation patterns during hemiparetic gait were characterized by asymmetrical activation in the sensorimotor cortices and recruitment of other motor-related areas such as the premotor cortices and prefrontal regions. Activation patterns could be modified by rehabilitative intervention (Miyai et al. Ann Neurol 2002;52:188-194). Facilitation technique, by which therapists assisted patients to walk by handling the pelvis to ensure stable stance and swing of the paretic leg, induced enhanced activation in the motor related areas, particularly that in the premotor cortex. A serial optical imaging study revealed that improved asymmetry in the sensorimotor activation and enhanced premotor activation were associated with locomotor recovery.

S31-3 Short-term compensation for behavioral deficits following premotor cortex inactivation

Kiyoshi Kurata[1]
[1]Dept Physiol, Hirosaki Univ Sch Med, Hirosaki, Japan

The premotor cortex (PMv) of human and non-human primates consists of several subregions, and each of them plays an important role in a specific aspect of hand movements. For example, when muscimol was injected in a focal region, the dorsal, but not ventral, PM inactivation resulted in deficits in a conditional motor behavior. By contrast, inactivation of the ventral, but not dorsal, PM led to reacquisition deficits in prism adaptation of reaching. However, effects of muscimol was short-lasting, and the deficits were completely compensated within 30 min. Furthermore, when the monkeys performed reaching movements by their left or right hand, they did not show any deficit in prism adaptation after the ventral PM inactivation of a hemisphere. Because we have a growing evidence that each of the regions has adaptive elements (or a hidden layer) in their neuronal circuits, it can be postulated that the elements in the remaining cortex contributes to the quick compensation for the behavioral deficits.

S31-4 Motor areas active during somatic perception of limb movements

Eiichi Naito[1]
[1]Kyoto Univ.

When the tendon of the wrist extensor muscles is vibrated at around 80 Hz, blindfolded healthy human subjects experience that their vibrated wrist is flexing despite the wrist being absolutely immobile. This kinesthetic illusion is elicited because tendon vibration excites the muscle spindles in a manner similar to when the muscle actually stretches. We have shown by positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) that the primary motor cortex that is contralateral to the vibrated limb is one of the cortical targets of the muscle spindles afferents and primarily responsible for the somatic perception of limb movements. We also demonstrated that the rostral part of dorsal premotor cortex, cytoarchitectonic area 2, supramarginal gyrus and inferior frontal cortex (areas 44/45) in the right hemisphere are commonly active no matter whether the subjects experience the illusion of right hand or left hand. These results suggest that the somatic perception of limb movements requires the activity of several motor and sensory areas activated in a resonant way.

S32-1 Brain research-historical background and its future prospect

Yasuhiro Okada[1]
[1]Dept Physiol, Kobe Univ, Kobe, Japan

Brain research from molecular level to higher brain function has made a glorious progress during the past half century with rapid development of molecular biology and with physiological techniques such as intracellular recording, patch clamp method and system and image analysis of brain. In this symposium, on the basis of fruitful progress mentioned above and taking account of historical background of brain research including philosophical ideas such as Cartesian mechanics and phenomenology, and autopoietic system theory, we are discussing consciousness and memory mechanisms to explore the future prospect of brain science.

S32-2 The prefrontal cortex as intra- and inter-operating systems

Toshiyuki Sawaguchi[1]
[1]Lab Cognitive Neurobiolgy, Hokkaido Univ Grad Sch Med

Higher brain functions, including active consciousness, have been implicated in the prefrontal cortex (PFC). Indeed, working memory, which is the basis of various cognitive functions, is well known to be a central function of the PFC. However, the conventional concept of working memory is relatively narrow and not so suitable for understanding actual functions of the PFC. Based on recent findings, the PFC should be an "intra-brain operating system" to operate various brain areas ("modules") for flexible control of behavior in a given situation. Further, the PFC appears to be also an "inter-brain operating system" to operate other brains of other individuals; e.g., communication and the "Theory of Mind". The major driving force for the PFC evolution appears to be the social interaction, and intra- and inter- brain operations are critical for this evolutionary factor. The concept of "intra- and inter- brain operating system" should be useful for further studies for higher brain functions as well as for understanding the nature of our mind.

S32-3 Is it valid for molecular research to clarify the mechanism of memory?

Masahiko Nomura[1]
[1]Dept Physiology, Saitama Medical School, Moro, Saitama, Japan

Molecular mechanisms of memory are getting most interesting events and facts in this decade. One of reason was getting much interest concerning the mechanisms of memory, and also especially the technical and theoretical development of this research field. These researches showed what kinds of the molecularly treated animals indicated poorer score in learning and memory tasks. And how kinds of the substances or chemical properties show to degrade the performances of tasks. The memory is the one of the highest function of brains. So we have to select and decide what kinds of learning tasks is the best ways.There are many tasks of negative reinforcement learning , instead of positive one. But negative tasks are far from the examination to know the genuine memory mechanisms. Are there real satisfied methods to clear and to know the genuine memory? We also have to make the phenomena of memory-acquisition, consolidation and retrieve. I hope to present at my talk on my real idea what are the true evaluation methods of memory there are.

S32-4 First-person phenomenology of mind-brain correlation

Bin Kimura[1], Kenjirou Fukao[2]
[1]Kawai Institute for Culture and Education, [2]Dept Neuropsych, Grad Sch Med Kyoto Univ

Daily psychiatric practice confirms that psychological behaviors of patients obviously depend on the actual interpersonal relationship with surroundings. It attests that current discussions about correlations between individual conscious mind and individual brain must be essentially supplemented by serious investigations into a possible connection of an individual mind with collective behavior in toto of the group the individual actually belongs in, a connection which can be ubiquitously observed in non-human creatures. The first-person subjectivity of the individual consciousness should be deeply embedded in the phylogenetically older layer of mental structure, i.e. unconscious collective subjectivity. The well known findings of B. Libet about the timing gap between unconscious and conscious intentions of bodily movement as well as that between perceptions induced by cortical and skin stimuli may deliver an interesting cue for this issue.

S32-5 The brain as an autopoietic system

Hideo Kawamoto[1]
[1]Dept Philosophy, Toyo Univ. Tokyo, Japan

Consciousness itself is a kind of action. Consciousness includes many kind of activities such as sensation, perception, and thinking, but it continuously becomes the consciousness itself through its own activities of the action. The action is the essence of consciousness, but consciousness does contain actions as its function. How is the field of the action determined? The field of this action does not correspond to the field which is indicated by the anatomy about the brain. The self-organizing system theories show the mechanism of the action. The autopoietic system theory in the forefront of these theories integrates the science of movements and the material science only in the point of the continuance of the action. As a result, many new problems and finding of the brain science are sure to be found with the system theory.

S33-1 Effects of repetitive TMS (rTMS) at the premotor area on electroencephalographic (EEG) oscillatory activities

Tatsuya Mima[1], Wei-Hung Chen[1], Hidenao Fukuyama[1], Hiroshi Shibasaki[1]
[1]Human Brain Research Center, Kyoto Univ Grad Sch Med

It is reported that low-frequency subthreshold rTMS at 1Hz given over the premotor area can strongly suppress the motor cortical activity which may outlast the period of stimulation. To clarify the effect of rTMS over premotor area, we measured the EEG and EMG oscillatory activities before and after rTMS in 8 normal volunteers (Chen et al., in press). Carrying-over-effects on the motor cortical activation was assessd by movement-related EEG power and cortico-cortical (EEG-EEG) coherence change, and cortical-muscular (EEG-EMG) coherence. We found the decrease of the movement-related EEG power change and EEG-EMG coherence after rTMS which may be in line with the reduced motor cortical activity. In addition, EEG-EEG coherence after rTMS increased selectively in the upper alpha band, which suggests the transient reorganization of the movement-related neuronal network caused by rTMS.

S33-2 0.2 Hz repetitive TMS (rTMS) has no add-on effects as compared with a realistic sham stimulation in Parkinson disease (PD)

Shingo Okabe[1], Yoshikazu Ugawa[1], Ichiro Kanazawa[1]
[1]Dept Neurol, Tokyo Univ Hos

To study the efficacy of 0.2 Hz rTMS on PD, 85 patients were enrolled into three groups: motor cortical, occipital and sham stimulation. In one session, 100 stimuli of 0.2 Hz rTMS at an intensity of 1.1 times active motor threshold (AMT) were given. In sham stimulation, electric currents were given with electrodes fixed on the head to mimic the sensation in real stimulation. Each session was performed once a week for the first 8 weeks. The Unified Parkinson Disease Rating Scale (UPDRS), Hamilton Rating Scale for Depression (HRSD) and subjective score were assessed. Total and motor score of UPDRS were improved to the same extent by rTMS over Cz, inion and sham stimulation. HRSD was improved by rTMS over Cz and sham stimulation in the same manner. Subjective score was not significantly improved by any methods of stimulation. The present rTMS has only a placebo effect on PD. Our realistic sham stimulation maneuver must produce powerful placebo effects as a real stimulation.

S33-3 rCBF changes during prefrontal rTMS

Takashi Ohnishi[1]
[1]Dept Radiology NCNP

Recently, rTMS has been used as a potential treatment for neuropsychiatric disorders. It has several methodological issues to be resolved. Further, the precise mechanisms of action of rTMS over the DLPFC for an antidepressant are still unknown. We measured rCBF during sham, during rTMS and after stimulation using repeated 15O-labeled H2O PET scanning in healthy subjects. The slow rTMS over the right DLPFC could produce significant rCBF increase in the anterior cingulate cortex and neighboring medial prefrontal cortex during stimulation as compared with sham stimulation. The lasting activation was noted in the medial prefrontal cortex, ventrolateral PFC, and the ventral striatum including the NAc. These data indicate that slow rTMS is able to produce rCBF changes in the distant brain areas. We conclude that such rCBF changes should explain antidepressant effects of rTMS. Particularly, the lasting effect on the ventral striatum should reflect modulatory effects of rTMS over the DLPFC on meso-limbic dopaminergic system, which must play critical role in antidepressant effects.

S33-4 Neuroimaging of rTMS - A primate PET study.

Takuya Hayashi[1]
[1]Dept. of Investigative Radiology, Research Institute, National Cardiocvascular Center, Osaka, Japan

Repetitive transcranial magnetic stimulation (rTMS) has a potent therapeutic effect on neuropsychiatric diseases. We studied the effect of rTMS on neuronal activity in terms of distribution and temporal profile, as well as neurotransmitter: dopamine (DA) function in anaesthetized primates. Total of 2000 pulses of 5Hz-rTMS was delivered on the unilateral primary motor cortex using eight-figured coil optimized for monkey cranium. Neuronal activity was sequentially estimated using 18F-fluorodeoxyglucose and positron emission tomography (PET), as well as DA release using 11C-raclopride. rTMS induced changes in neuronal activity in the ipsilateral sensorimotor and other motor-related areas, as well as in the reward-related limbic-striatum loops. Interestingly, long-lasting effect was also observed at least several-day post rTMS. DA release was induced in the ipsilateral ventral striatum. These results suggest that rTMS may have several kinds of long-lasting modulatory effects on the local and remote neuronal circuitry, besides DA function.

S33-5 Effects of transcranial magnetic stimulation on the brain function: PET study with monkeys

Hideo Tsukada[1]
[1]Centr Res Lab, Hamamatsu Photonics, Hamakita, Japan

Parkinson's disease (PD) is a degenerative disorder with a loss of midbrain dopamine (DA) neurons. Treatment with L-DOPA, a precursor of DA, works initially, but reduced efficacy of L-DOPA requires alterative treatments such as deep brain stimulation and DA neuron transplantation. Transcranial magnetic stimulation (TMS) is also expected to be an alterative, by which neuronal activity can be controlled from outside of head. In order to monitor the efficacy of TMS treatment, PET can provide the useful information regarding neurochemical and neurophysiological information in the living brains. We have developed a high-resolution animal PET, and applied many kinds of labeled compounds specific for dopaminergic, serotonergic and cholinergic neuronal systems. In this symposium, several preliminary data about the effects of TMS on the monkey brain functions, mainly in dopaminergic neuronal system using [^{11}C]L-DOPA, [^{11}C] β-CFT and [^{11}C]raclopride, will be discussed to give some insights for the possible alterative treatment for PD.

S34-1 PET studies on the histaminergic neuron system: its role in arousal mechanism in humans.

Kazuhiko Yanai[1], Hideki Mochizuki[1], Manabu Tashiro[1]
[1]Depart Pharmacol, Tohoku Univ Sch Med, Sendai, Japan

The histaminergic neuron system cause general cortical activation through the activation of H1 receptors (H1R). Classical antihistamines impair various cognitive functions such as psychomotor speed and verbal learning due to H1R blockage. Studying the brain mechanism of sedation by sedative antihistamines is an aid to understand how natural arousal can be maintained. The mechanism of impaired cognitive performance and subjective feeling of sleepiness and tiredness was studied using [^{11}C]doxepin and $H_2{}^{15}O$. We reported here that these drugs induced sleepiness and impaired performance in humans in proportion to H1R occupancy. Our PET studies also demonstrated that impaired performances and sleepiness were associated with the regional decreased brain activities relevant to the task performances, and that several other areas were rather activated to compensate the impairment. The interaction between the cortical and subcortical areas would be also altered by our conscious levels during natural sleep-wake cycles.

S34-2 Molecular Imaging of mental illness

Tetsuya Suhara[1]
[1]Brain Imaging, Nat Inst Radiological Sci, Chiba , Japan

There have been a number of discussions about the molecular mechanism of mental illness. Molecular imaging with positron emission tomography can investigate several components of nerotransmission. Regarding dopaminergic transmission, an increasing body of evidence favors a crucial role of extrastriatal regions in the pathophysiology of positive symptoms, and the extrastriatal D2 receptor (D2R) is expected to be the common site of action of antipsychotics. Using [^{11}C]FLB 457, we found reduced D2R binding in the anterior cingulate cortex of patients with schizophrenia, and a significant negative correlation was observed between D2R binding and the positive symptom score on BPRS. On the other hand, serotonergic transmission has been discussed in relation to mood disorders. We used [^{11}C]McN5652 to measure the serotonin transporter (5HTT). The 5HTT is one of the target sites of antidepressants and we have evaluated the 5HTT occupancy by two different types of antidepressants. We found increased binding of [^{11}C]McN5652 in the thalamus of patients with mood disorders.

S34-3 Activation of limbic GABAergic system by sleep deprivation

Hirotaka Onoe[1], Kayo Onoe[2], Hideo Tsukada[3], Yasuyoshi Watanabe[2]
[1]Dept. Psychol, Tokyo Metropol Inst Neurosci, Fuchu,, [2]Dept Physiol, Osaka City Univ Grad Sch Med, Osaka,, [3]Central Lab, Hamamatsu Photonics, Hamakita, Japan

Gamma-aminobutyric acid (GABA)ergic system is considered to play a critical role in regulation of sleep as a major inhibitory system in the CNS. Recently, we demonstrated that a presumable alpha 5 subunit containing $GABA_A$ receptor is dominantly localized in the limbic regions of the macaque monkey brain by positron emission tomography (PET) with 11C-labeled benzodiazepine analogue, [11C]Ro15-4513. To assess the functional role of GABA in sleep and wakefulness, we examined the effects of sleep deprivation (SD) on $GABA_A$ receptors by PET of the monkey. We also measured the reaction time of a simple reaction task to estimate the change in behavioral performance by SD. The results showed that the binding activity of [11C]Ro15-4513 was significantly increased by SD indicating that the increase in sleepiness and/or fatigue by prolonged wakefulness is associated with the activation of GABAergic neurotransmission in the limbic structure.

S34-4 MicroPET and its application on the mechanism of central fatigue

Masaaki Tanaka[1], Akira Matsumura[1], Shigekazu Mizokawa[1], Satoshi Nozaki[1], Yasuhiro Wada[2], Fusao Nakamura[1], Yasuyoshi Watanabe[1]
[1]Dept Physiol, Osaka City Univ Grad Sch Med, Osaka, Japan, [2]Nucl Med Dept, Siemens-Asahi Med Tech Ltd., Tokyo, Japan

MicroPET (positron emission tomography) has been developed for the experiments with smaller animals. The advantage of microPET is to permit repetitive and noninvasive studies of biological process in vivo, particularly for experimental protocols, which require a longitudinal design. Through performance study with phantom, the spatial resolution is assessed to be ca. 1.8 mm. The close correlation between the radioactivities measured directly from *ex vivo* autoradiography specimens and the ROI data from microPET has been confirmed ($r^2 = 0.99$). We here show the data from 2-[^{18}F]fluoro-2-deoxy-D-glucose uptake in the brain of rat under different anesthesia (chloral hydrate, ketamine, ketamine+Xylasine, propofol, pentobarbital) and conscious group. Then, we present the data from a newly established combined fatigued animal model.

S34-5 Neuroreceptor imaging and behavior: PET study with conscious monkeys

Norihiro Harada[1], Shingo Nishiyama[1], Takeharu Kakiuchi[1], Hideo Tsukada[1]
[1]Centr Res Lab, Hamamatsu Photonics, Hamakita, Japan

For the brain research, non-invasive imaging technologies like PET are expected to provide the useful information about neurochemical and neurophysiological functions in the living brains of experimental animals as well as humans. We have developed the total PET system with a high-resolution animal PET (Hamamatsu SHR-7700), by which the brain functional imaging of conscious behaving monkeys can be performed. Several kinds of specific labeled compounds to image dopaminergic, serotonergic and cholinergic neuronal function as well as cAMP second messenger system, are routinely synthesized to apply for PET studies regarding the cognition, aging, drug abuse, stroke, Parkinson ' s disease and dementia. In addition, we are expanding the system for bridging between the cognitive behaviors and neuronal system in molecular basis of neurotransmitter-receptor interactions in combination with microdialysis technique. Several experiences of our research using conscious monkeys and animal PET system will be discussed.

S34-7 PET imaging of CNS-type prostacyclin receptor

Yasuyoshi Watanabe[1]
[1]Dept Physiol, Osaka City Univ Grad Sch Med, Osaka, Japan

We found a novel subtype of prostacyclin receptor (IP2) in CNS neurons. (15R)-16m-Tolyl-isocarbacylin (15R-TIC) serves as a specific and stable ligand for this IP2, and its chemical structure was designed from the labelling strategy with 11C for PET. 15R-TIC exhibits excellent neuroprotective effects in primary cultured neurons, gerbil transient ischemia model, rat and monkey MCAO-reperfusion model. In PET studies using normal rhesus monkeys, methyl ester of 15R-[11C]TIC showed high uptake into the brain. Based on displacement studies and metabolite analysis, we confirm the uptake of methyl ester of 15R-[11C]TIC into the brain and the binding of 15R-[11C]TIC the receptor after rapid deesterification in brain tissue. In normal human volunteers, the uptake was high in the thalamus, caudate-putamen, cerebral cortices, and other subcortical structures. The noxious pain stimuli changed the uptake (binding) in the related brain regions. PET studies using the methyl ester of 15R-[11C]TIC might be of value to characterize prostacyclin receptors in neurological disorders.