新学術領域研究３領域合同国際シンポジウム

“INTERNATIONAL SYMPOSIUM ON ORGANIZATION AND FUNCTION OF THE NERVOUS SYSTEM”

Abstracts

Dates: November 27 (Tue) - 28 (Wed), 2012

Venue: Koshiba Hall, The University of Tokyo

Session 1

“Molecular Ethology and Operating Principles of the Nervous System”

November 27, 2012

13:00-18:00

Odor-evoked innate and learned fear responses are mediated by distinct neuronal mechanism

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We previously reported that aversive and fearful responses to odorants are innately regulated by olfactory neural pathways originating from the dorsal zone of the olfactory epithelium. Natural predator compounds such as 2,4,5-trimehylthiazoline (TMT), a component of fox feces, are thought to induce innate fear responses in rodents. However, whether TMT can really induce fear is debatable, because TMT-induced fear-related responses are rather weak. We screened synthetic artificial chemical libraries and identified a series of "fear odorants" that can induce extremely strong fear responses. The level of fear responses varied with the type of odorant. Innate fear was evoked by fear odorants, and conditioned fear was elicited by association of a neutral odorant with an electrical foot shock; both innate and conditioned fear responses induced similar levels of freezing behavior and secretion of stress hormones. However, only innate fear was accompanied by specific physiological responses, e.g., rapid and as much as 50% reduction of heart rate and simultaneous reduction of cutaneous and core body temperature by more than 3°. Moreover, extinction of fear responses was not observed upon repeated presentation of fear odors. Further, we found that in addition to the central nucleus of the amygdala–periaqueductal gray system, a wide spectrum of neural networks, including the striatal system, regulate the innate fear responses evoked by fear odors. "Cold fear" evoked by fear odorants provides a new framework to understand indelible and strong fear.

Unravelling the neuronal mechanism of the spinal locomotor network -what we learn from hopping mice-

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Coordinated rhythmic limb movement is essential for walking in terrestrial mammals. Neuronal circuits located in the spinal cord generate the basic output patterns underlying such movements. The circuit controlling hindlimb muscles is mainly located in the lumbar spinal cord and consists of reciprocal connections between excitatory and inhibitory interneurons. However, little is known about the actual organization and function of these interneurons. In the mammalian spinal cord, the receptor tyrosine kinase EphA4 is expressed in a subpopulation of neurons in the spinal cord and has been suggested to play a role in the configuration of the locomotor circuit in mammals. Ephrin B3, expressed at the midline of the spinal cord is its ligand and acts as a repellent for the ipsilaterally-projecting axons expressing EphA4. It has been shown that alpha-chimerin (Chn) plays a crucial role in axon guidance of EphA4-positve neurons. In the global Chn knockout (Chn-KO) mouse, the downstream signaling of EphA4 is disrupted, resulting in aberrant midline-crossing of ipsilateral-projecting axons in the spinal cord. These axons are suggested to be responsible for the abnormal hopping gait of this mouse (Iwasato et al. 2007). In this study we examined if aberrant crossing excitatory axons may be responsible for the hopping gait by crossing BAC-vesicular glutamate transporter type 2 (VGLUT2)-Cre Tg mice (Borgius et al. 2010) with Chn flox mice to generate a mouse that lacks Chn only in VGLUT2-positive excitatory neurons (VGLUT2-Chn-KO mouse). Interestingly, the VGLUT2-Chn-KO mice showed both hopping and normal alternating gaits. Electrophysiological studies using isolated spinal cord preparations in vitro revealed that the spinal network of these mice is capable of generating hopping and walking patterns, indicating that excitatory neurons expressing EphA4 during development have an important role in the assembly of the spinal locomotor circuit.

Temporal-spatial regulation of singing-induced genes and epigenetic dynamics in the critical period for vocal learning in songbirds

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A complex animal behavior is not only genetically developed but also is affected with influence of environmental factors. Regulation of gene expression in the brain is controlled by species-specific genome information modified under epigenetic influences. Epigenetic molecular mechanisms, such as DNA methylation and histone modification, involve in regulation of gene expression in neuronal cells. Songbirds learn species-specific song pattern from a tutor during the critical period of vocal learning. For vocal learning and production, songbirds possess specific neural pathways called song system including vocal motor pathway (VMP) and anterior forebrain pathway (AFP). VMP is critical for vocal production and AFP is necessary for vocal learning. These pathways consist of a set of brain areas called song nuclei. In these song nuclei, many genes are known to be regulated by singing. It has been shown that some of these singing-induced genes are differentially regulated during the critical period of vocal learning.

We found an epigenetic change of one of these genes, activity-regulated cytoskeleton-associated (Arc) gene. The DNA methylation in the upstream genome region of Arc was differentially regulated through the critical period in zebra finch, a closed-ended learner. This data suggests that epigenetic mechanisms involve in regulation of singing-induced gene expressions. To further clarify the relationship between gene expression and epigenetics, we examined expression patterns of epigenetics-related genes, histone H3 variants (H3.3) and Growth arrest and DNA-damage inducible gene 45 (Gadd45) families. Both H3.3B and Gadd45b genes were up-regulated in song nuclei of both VMP and AFP by singing. The induction of gene expressions was differentially regulated during the critical period of song learning in zebra finch. To clarify whether these regulations depend on age or sensorimotor learning itself, we examined gene expression of these two genes after seasonally singing in canary, an open-ended learner, which has ability to re-learn new songs even after sexually matured as an adult. As the result, gene induction of H3.3B and Gadd45b was higher in birds singing variable and plastic songs than in the birds singing crystallized songs. These data suggest that histone H3.3B and Gadd45b may contribute the critical period by epigenetic regulation for several gene expressions that are related with vocal learning.

Acceleration of forgetting by neuronal communication in *C. elegans*

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Animals acquire a tremendous quantity of information from their environment that is partially retained in their nervous systems. These memories lead to behavioral plasticity, in which experiences induce changes of behavioral responses to environmental stimuli. Most memories considered as short-term memories are vulnerable to disruption and are forgotten within hours if not consolidated into stable long-term memories. However, the molecular and neuronal mechanisms of forgetting remain largely unknown.

In *C. elegans*, animals preexposing to an odorant showed weaker response to the odorant, and this olfactory adaptation is sustained for a few hours. We use this phenomenon as a simple model for behavioral plasticity, by our genetic screening, we found that *tir-1* showed the prolonged retention of the olfactory adaptation to diacetyl, which is sensed by AWA neurons. Since TIR-1 is an adaptor protein for p38 MAP kinase, we tested whether the downstream molecules regulate the forgetting, and found that p38 MAPKKK, NSY-1, p38 MAPKK SEK-1, and JNK-1 are required for the proper regulation of forgetting. We also found that the signaling pathway functions in a pair of sensory neurons, AWC, at the adult stage. In addition, we found that the neurosecretion from AWC sensory neurons is important for the acceleration of forgetting. Next, we analyzed the sensory responses to diacetyl in AWA neurons by Ca^{2+} imaging. In olfactory adaptation, conditioning induces attenuation of odor-evoked Ca^{2+} responses in AWA neurons and this attenuation is prolonged in the TIR-1/JNK-1 pathway mutant animals. These results suggested that AWC sensory neurons accelerate the forgetting of olfactory adaptation in AWA sensory neurons by neuronal communication.

Sensory transduction in *C. elegans*: What can't a worm sense?

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The ability to sense and react to sensory cues is essential for animal survival. Although the morphology of sensory organs is highly diverse among different organisms, the cellular and molecular mechanisms underlying sensory transduction show similarities across phylogeny. As such, invertebrate organisms, such as *C. elegans* and *Drosophila*, have been widely used as a model for the study of sensory transduction. Among the three major sensory stimuli are chemicals, mechanical forces, and light. Worms rely on olfactory neurons (e.g. AWA and AWC) and gustatory neurons (e.g. ASE) to respond to chemical stimuli, while reacting to mechanical forces (e.g. gentle touch) via touch receptor neurons (e.g. ALM, AVM and PLM). Over the years, we have been characterizing sensory transduction using *C. elegans* as a model. We have found that worms possess the sense of proprioception, a type of mechanical sense that they rely on to control body posture during movement. We have systematically characterized harsh touch sensation by identifying the neural circuitry and sensory channels mediating this mechanosensory behavior. We have also shown that though long thought to light-insensitive due to the lack of eyes, worms do have the sense of light and engage in phototaxis behavior that is mediated by a group of photosensory neurons. This behavior is also critical for their survival and may help keep worms in the dark soil. In addition to behavior, sensory cues regulate other types of physiological processes, for example, aging and longevity. Our data reveals that temperature sensation and transduction play an important role in regulating lifespan in *C. elegans*. These results and those from many others together demonstrate that the nematode *C. elegans* possesses a remarkably rich repertoire of sensory modalities that are critical for their life.

Anatomical and functional organization of the *Drosophila* auditory system

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Decoding and controlling Brain Information, PRESTO, JST

Many animals utilize acoustic signals to transmit information regarding species identity during courtship, from insects and fish to birds and primates. An understanding of the sensory processing of such acoustic signals requires detailed knowledge of the underlying auditory neural circuits in the brain. The fruit fly *Drosophila melanogaster* serves an attractive model system in the field of sensory neurobiology, because of its small brain and the abundant genetic tools to identify and study individual neurons in a living animal. Through a combination of neurogenetics, calcium imaging, and molecular neuroethology, we studied the anatomical and functional organization of the *Drosophila* auditory system, which includes the fly ear, its brain targets, and their downstream neural circuits. In the fly ear, the internal sensory neurons are comprised of specialized clusters that are each required for sound and gravity sensing. These two neuronal groups terminate in different areas of the fly brain. Identification of higher-order neurons that feed into the primary auditory and gravity centres revealed their characteristics, which is reminiscent of the cochlear and vestibular pathways in our brain. The FLP-FRT recombination technique further revealed the neuroanatomy of single neurons in the downstream circuits for the auditory pathway: distinct types of neurons were found, each of which connects high- and/or low- frequency zones in the primary auditory center and various regions in the higher brain region(s). The secondary auditory centers would thus distributed in various regions in the fly brain, which might be an anatomical substrate for a parallel processing of various aspects of acoustic information.

Long-term enhancement in the mushroom body in cultured *Drosophila* brain-cellular substrate for olfactory learning

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During olfactory aversive conditioning in *Drosophila*, odor and shock information are delivered to the mushroom bodies (MBs) through projection neurons in the antennal lobes (ALs) and ascending fibers of the ventral nerve cord (AFV), respectively. Using an isolated cultured brain expressing a Ca^{2+} indicator in the MBs, we demonstrated that the simultaneous stimulation of the AL and AFV establishes the long-term enhancement (LTE) in AL-induced Ca^{2+} responses. The physiological properties of LTE, including associativity, input specificity and persistence, are highly reminiscent of those of olfactory memory. Similar to olfactory aversive memory, LTE requires the activation of nicotinic acetylcholine receptors that mediate the AL-evoked Ca^{2+} response, NMDA receptors that mediate the AFV-induced Ca^{2+} response, and D1 dopamine receptors during the simultaneous stimulation of the AL and AFV. Considering the physiological and genetic analogies, we propose that LTE at the AL-MB synapse can be a relevant cellular model for olfactory memory.

Bending the not so simple mind of the fruit fly

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Directed behaviour emerges from neural integration of sensory stimuli, memory of prior experience and internal states. We seek an understanding of these conserved neural mechanisms using genetically-encoded tools and the relatively small brain of *Drosophila*. By temporally controlling neural function memories can be implanted and internal states altered so that most flies behave according to our direction. Such recent studies have revealed a role for distinct subsets of dopaminergic neurons that innervate the mushroom bodies in reward learning and the control of motivated fly behaviour. Therefore, the positive reinforcement system of flies is more similar to that of mammals than previously envisaged.

One might interpret the relative ease of altering behaviour to indicate that everything is simple in the fly brain. However, complexity arises in unexpected ways. Cell-type specific gene expression profiling revealed transposable element expression in long-term memory relevant neurons of the mushroom body. Importantly, brain-specific transposon mobilization is prevalent and likely to be heterogeneous within and between fly brains. Since neural expression and retrotransposition of LINE-1 transposable elements has been observed in mammals, it appears that genomic heterogeneity is a conserved feature of the brain. We propose that it may prove beneficial to specific cell-types and neural processes and could plausibly contribute to behavioural individuality.

Session 2

“Optogenetics and Neural Imaging”

November 28, 2012

10:00-12:00

Functional analysis of locomotor circuits in the spinal cord and brainstem in zebrafish

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Neuronal circuits in the spinal cord and brainstem play an important role for producing locomotion in vertebrates. Investigation of locomotor circuits in amniotes, however, is not trivial due to enormous complexity of their neuronal circuits. Zebrafish locomotor circuits are much simpler with less number of distinct classes of neurons, making it more feasible to address this issue. We have set to define the morphology and functional properties of neurons that express a particular transcription factor, hoping that the results obtained will provide insights into the properties of the corresponding cells in higher vertebrates. In this symposium, we focus on our studies in chx10 positive neurons in the brainstem. We first analyzed the function of Chx10 neurons in locomotion by using of channelrhodopsin (ChR). ChR was expressed in Chx10 neurons using a Gal4-UAS system. Photo-stimulation of the hindbrain of Chx10:Gal4; UAS:ChR transgenic zebrafish reliably elicited swimming, indicating that activation of Chx10 neurons are sufficient to evoke swimming. Then, we performed calcium imaging and electrophysiological recordings to ask whether hindbrain Chx10 neurons were active during fictive swimming. We found that some of the Chx10 neurons were active during fictive swimming. Finally, to confirm necessity of the activity of Chx10 neurons for swimming, we used halorhodopsin (Halo) and archaerhodopsin-3 (Arch) for optogenetic inhibition. Both Chx10:Gal4/UAS:Halo and Chx10:Gal4/UAS:Arch fish stopped spontaneous swimming upon green light-application to the hindbrain. These results indicate Chx10 neurons in the hindbrain play indispensable roles for producing swimming.

Neural circuits characterizing the posterior parietal cortex in mice

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Mice navigate nearby space by vision and whiskers, and young mice with growing body parts need to adjust visual perceptual space to somatosensory perceptual space. We found that intermodal spatial mismatch between visual and whisker inputs were detected in the posterior parietal cortex (PPC) of young mice wearing a monocular prism goggle. The mismatch produced cortical depression in the primary visual cortex (V1) during the critical period of V1. Clustered protocadherins (cPcdh) are neuro-specific cell adhesion molecules, and the extraordinarily diverse structural repertoire of cPcdh molecules may provide the molecular basis for the formation of specific neural circuits. In cPcdh-α knockout mice, the prism-induced depression in V1 was impaired, while ocular dominance plasticity after monocular deprivation and orientation selectivity in V1 neurons were apparently normal. These results suggest that PPC functions might be specifically depended on cPcdh. Recently, it has been demonstrated that the mouse PPC is required for the spatial working memory measured in a T-maze experiment (Harvey et al., Nature 484: 62-68, 2012). We found that the spatial working memory in a T-maze experiment was impaired in mice with genetically-manipulated cPcdh-α. These results suggest that cPcdh-dependent neural circuits may play essential roles in the two different PPC functions, spatial working memory and detection of intermodal spatial mismatch.

Structure and structure-based variant design of channelrhodopsin

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Channelrhodopsins (ChRs) are light-gated cation channels derived from algae that have been found to provide experimental utility in the technology of optogenetics; for example, neurons expressing ChRs can be optically controlled with high temporal precision within systems as complex as freely moving mammals. Although ChRs have been broadly applied to neuroscience research, little is known about the molecular mechanism by which these remarkable and powerful proteins operate. Here we present the crystal structure of a ChR (C1C2 chimera between ChR1 and ChR2 from *Chlamydomonas reinhardtii*) at 2.3 Å resolution. The structure reveals the essential molecular architecture of ChR, including the retinal-binding pocket and the cation conducting pathway. Our MD simulation of 13-cis-retonal bound ChR revealed channel opening involving sequential movement of transmembrane helices. Integration of structural and electrophysiological analyses provide insight into the molecular basis for the unusual operation of ChR, and pave the way for precise and principled design of ChR variants with novel properties.

Session 3

“Neural Progenitor and Stem Cells, and Synapse Formation”

November 28, 2012

13:00-17:00

Regulation of neural stem cell fate in the developing mouse neocortex

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NPCs undergo progressive neuronal fate restriction during development of the brain. In earlier stages NPCs are highly proliferative, self-renewing cells that produce various types of neurons. Later in development, NPCs become less proliferative, lose the neurogenic potential and produce only glial cell types. The mechanisms underlying this stage-dependent fate restriction are fundamental to our understanding of brain organization, although they are not fully understood. In this study, we show that HMGA proteins, whose expression levels decline over time during brain development, play an essential role in conferring neurogenic potential on NPCs in early stages. We now show that chromatin of NPCs gradually becomes more condensed and less dynamic on a global scale during neocortical development. Furthermore, we found that high mobility group A (HMGA) proteins are essential for the open chromatin state of NPCs at early developmental stages. Importantly, knockdown of HMGA proteins in early-stage NPCs reduced their neurogenic potential. Conversely, overexpression of HMGA proteins conferred the neurogenic potential on late-stage NPCs, an effect that was antagonized by coexpression of a mutant form of histone H1 that inhibits chromatin opening. HMGA proteins thus contribute to the neurogenic potential of NPCs in the early stages of neocortical development, possibly through induction of an open chromatin state.

Human stem cell models of cerebral cortex development and disease

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Methods to model human cortical development in a controlled, defined manner from embryonic and induced pluripotent stem cells have considerable potential to enable functional studies of human cortical development, evolution, circuit formation and function, and for constructing in vitro models of cortical diseases. Given that many of the major diseases of the cerebral cortex are diseases of synaptic function, including epilepsy, dementia and schizophrenia, a goal of the field is to generate cortical networks in vitro that closely resemble those found in vivo. Effective cellular models of cerebral cortex disease would use the appropriate neural circuits, in this case human cerebral cortex, develop relevant pathology, and do so in a reproducible manner over a timescale short enough for practical use. To explore the potential of this in vitro system for modelling cortical disease, we have focused on the pathogenesis of Alzheimer's disease (AD). In this presentation I will describe how we have used our mechanistic understanding of the cell and molecular biology of neural development to develop an efficient process for directing differentiation of human pluripotent stem cells to neurons and networks of the cerebral cortex, and the use of this system to study developmental and neurodegenerative diseases of the cerebral cortex.

Migrating transient neurons: organizing activity in patterning of the cerebral cortex

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The neocortex represents the brain structure that has been subjected to a major expansion in its relative size during the course of mammalian evolution. An exquisite coordination of appropriate growth of competent territories along multiple axes and their spatial patterning is required for regionalization of the cortical primordium and the formation of functional areas.

During development, progenitors expressing the Dbx1 homeodomain transcription factor are strategically positioned at boundaries between compartments, including the pallial-subpallial borders in mice, and their location is coinciding with signaling centers. Using genetic tracing and ablation in mice we have shown that at the earliest stages of cerebral cortex development $Dbx1^+$ progenitors give rise to subsequent waves of glutamatergic neurons which have the unique characteristics to migrate tangentially at long distance from their generation site and to be transiently present during development. Cortical patterning and the fine tuning of neuronal numbers leading to the formation of functional areas depends on the migration of Dbx1-derived transient neurons. By signaling to cortical progenitors in the mitotic compartment these neurons serve as organizers during development, therefore acting as "mobile signaling units".

Our work points towards a novel general strategy for long-range patterning in large structures whereby morphogens at signaling centers induce the generation of migrating cells which by producing themselves morphogens deliver them at distant locations.

We will discuss how the acquisition of new progenitor domain(s) at patterning centers and of migrating transient signaling neurons in mammals might represent one of the evolutionary steps leading to increase vertebrate brain complexity.

Spatial and temporal roles of the homeobox gene Gsx2 in the specification of telencephalic cell fates

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The molecular mechanisms that underlie the generation of cellular diversity in the CNS remain largely unknown. We have chosen to study this issue in the mouse telencephalon both at embryonic andadult stages. At embryonic stages, progenitors in the lateral ganglionic eminence (LGE) give rise to striatal projection neurons and olfactory bulb interneurons. Our work has shown that the homeobox gene Gsx2 is required for the specification of these LGE-derived neuronal subtypes, at distinct embryonic time points. The role of Gsx2 in the subsequent generation of oligodendrocytes from LGE progenitors, however, was unclear. We have found that in order for LGE progenitors to progress from neurogenesis to gliogenesis (oligodendrogenesis), Gsx2 must be down-regulated at late stages of embryogenesis. Finally, progenitors in the LGE are thought to contribute to the subventricular zone (SVZ) at postnatal and adult time points and Gsx2 remains expressed in a subset of SVZ progenitors. Our studies have found that Gsx2 is required for neural stem cell activation and subsequent neurogenesis of olfactory bulb interneurons in the adult SVZ.

Synapse formation in the brain

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Synapse formation is the key step in the development of neuronal networks. Precise synaptic connections between nerve cells in the brain provide the basis of perception, learning, memory, and cognition. A number of trans-synaptic cell adhesion molecules have been identified that play roles in pre- and postsynaptic differentiation of cultured hippocampal neurons. However, the precise roles of these molecules in synapse formation in vivo remain elusive. We recently provided evidence that the trans-synaptic interaction of postsynaptic glutamate receptor δ2 (GluRδ2) and presynaptic neurexins through Cbln1 mediates parallel fiber-Purkinje cell synapse formation in vivo in the cerebellum. Furthermore, the stoichiometry of synaptogenic GluRδ2-Cbln1-neurexin triad suggests that GluRδ2 triggers presynaptic differentiation by clustering four neurexins. We also found that IL1-receptor accessory protein-like 1 (IL1RAPL1) responsible for nonsyndromic mental retardation and autism mediates synapse formation of cortical and hippocampal neurons through trans-synaptic interaction with protein tyrosine phosphatase δ (PTPδ). These results imply the impairment of synapse formation as a common pathogenic pathway of mental disorders.