Current Trends and Future Directions of Synapse-Circuit Plasticity Research

September 3-6, 2019 at Shizuoka, Japan
(Toki No Sumika conference hall)

Organizers:
Scott Soderling (Department of Cell Biology, Duke University)
Masanori Murayama (RIKEN Center for Brain Science)

Contributors:

*N This Symposium is a part of the RIKEN Symposium Series.*
Current Trends and Future Directions of Synapse-Circuit Plasticity Research

Japan-U.S. Brain Research Cooperation Program

September 3 – 6, 2019
Gotemba Kogen Resort : TOKI NO SUMIKA
Shizuoka-ken, Japan

Organizers:
Scott Soderling (Department of Cell Biology, Duke University)
Masanori Murayama (RIKEN Center for Brain Science)
# Meeting Program

## Tuesday (9/3)

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<tr>
<td>15:00</td>
<td>Arrival and Check-In</td>
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<tr>
<td>18:30</td>
<td>Welcome Dinner @ Fuji</td>
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## Wednesday (9/4)

### Welcome and Introduction

- **9:00** - 9:10 | Masanori Murayama, on behalf of meeting organizers  
  Scott Soderling, on behalf of meeting organizers

### Keynote Lecture

- **9:15** - 10:00 | Kaoru Inokuchi (Introduction: Masanori Murayama)

### New Molecules in Synaptic plasticity (Chair: Elly Nedivi)

- **10:00** - 10:30 | Takeshi Imai (J)
- **10:30** - 11:00 | Susumu Tomita (US)
- **11:00** - 11:20 | Coffee/Tea break
- **11:20** - 11:50 | Mineko Kengaku (J)
- **11:50** - 12:20 | Scott Soderling (US)

### Luncheon seminar (Sponsor: NIKON INSTECH CO., LTD.)

- **12:40** - 13:40 | Keisuke Ota (J)  
  Masako Ino (NIKON INSTECH CO., LTD.)

### New concepts of synaptic plasticity in neural circuit function (Chair: Takeshi Imai)

- **14:00** - 14:30 | Schuichi Koizumi (J)
- **14:30** - 15:00 | Tetsuya Takano (US)
- **15:00** - 15:30 | Haruhiko Bito (J)
- **15:30** - 15:50 | Coffee/Tea break
- **15:50** - 16:20 | Thomas Blanpied (US)
- **16:20** - 16:50 | Masanori Murayama (J)

- **17:00** - 18:20 | Poster Session and Discussion @ Kaede  
  Group Photo
- **18:40** - 20:00 | Dinner @ Mugibatak e restaurant
- **20:10** - 22:00 | Poster Session and Discussion (Optional)
### Thursday (9/5)

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<thead>
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<th>Time</th>
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<tr>
<td>8:45</td>
<td>Welcome and solicitation of feedback for next day</td>
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<tr>
<td></td>
<td>Masanori Murayama and Scott Soderling</td>
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<tr>
<td>9:00</td>
<td>Keynote Lecture</td>
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<td></td>
<td>Richard Mooney (Introduction: Scott Soderling)</td>
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<tr>
<td>9:45</td>
<td>New concepts of synaptic plasticity in disease (Chair: Matthew Kennedy)</td>
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<tr>
<td>10:15</td>
<td>Akiko Hayashi-Takagi (J)</td>
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<tr>
<td>10:45</td>
<td>Gavin Rumbaugh (US)</td>
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<tr>
<td>10:45</td>
<td>Coffee/Tea break</td>
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<tr>
<td>11:05</td>
<td>Novel and emerging imaging methods for synaptic analysis (Chair: Akiko Hayashi-Takagi)</td>
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<tr>
<td>11:05</td>
<td>Hideji Murakoshi (J)</td>
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<tr>
<td>11:35</td>
<td>Makoto Higuchi (J)</td>
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<tr>
<td>12:05</td>
<td>Takuya Takahashi (J)</td>
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<td>12:55</td>
<td>Luncheon seminar (Sponsor: Olympus Corporation)</td>
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<tr>
<td>13:55</td>
<td>Shinichiro Tsutsumi (J)</td>
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<td></td>
<td>Kazuhiko Hosono (Olympus Corporation)</td>
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<td>14:00</td>
<td>New faces in synaptic neuroscience: Trainee talks (Chair: Masanori Murayama)</td>
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<tr>
<td>14:00</td>
<td>Kareem Abdou (J)</td>
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<td>14:15</td>
<td>Dalila Ordonez (US)</td>
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<td>14:30</td>
<td>PinWu Liu (J)</td>
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<td>14:45</td>
<td>Austin M Ramsey (US)</td>
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<tr>
<td>15:00</td>
<td>Daichi Kawaguchi (J)</td>
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<td>15:15</td>
<td>Nerea Llamosas (US)</td>
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<td>15:30</td>
<td>Coffee/Tea break</td>
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<tr>
<td>15:50</td>
<td>New ideas in dynamics of synaptic signaling and structure (Chair: Hideji Murakoshi)</td>
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<tr>
<td>15:50</td>
<td>Elly Nedivi (US)</td>
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<td>16:20</td>
<td>Shigeo Okabe (J)</td>
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<tr>
<td>16:50</td>
<td>Lin Tian (US)</td>
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<tr>
<td>17:20</td>
<td>Yukiko Gotoh (J)</td>
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<tr>
<td>Time</td>
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<tr>
<td>18:00</td>
<td><strong>Poster Session and Discussion @ Kaede</strong></td>
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<td>19:00</td>
<td><strong>Dinner @ Fuji (PIs vote for poster awards)</strong></td>
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<tr>
<td>21:10</td>
<td><strong>Poster Session and Discussion (Optional)</strong></td>
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**Friday (9/6)**

**New ideas in pre and postsynaptic function (Chair: Lin Tian)**

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<thead>
<tr>
<th>Time</th>
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<tr>
<td>9:00</td>
<td>Yasunori Hayashi (J)</td>
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<tr>
<td>9:30</td>
<td>Jason Shepherd (US)</td>
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<tr>
<td>10:00</td>
<td>Takeshi Sakaba (J)</td>
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<tr>
<td>10:30</td>
<td><strong>Coffee/Tea break</strong></td>
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<tr>
<td>10:50</td>
<td>Michisuke Yuzaki (J)</td>
</tr>
<tr>
<td>11:20</td>
<td>Matthew Kennedy (US)</td>
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**Luncheon seminar (Sponsor: Thorlabs Japan Inc.)**

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<tbody>
<tr>
<td>12:00</td>
<td>Henry Haeberle (Thorlabs, Inc.)</td>
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<tr>
<td>13:10</td>
<td><strong>Poster Award</strong> (3 persons)</td>
</tr>
<tr>
<td>13:25</td>
<td><strong>General discussion</strong> and organization of collaborations <strong>Departure of participants</strong> Organizer meeting to draft summary document</td>
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# Poster List

Display your poster at the board # indicated below.

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<th>#</th>
<th>Name</th>
<th>Affiliation</th>
<th>Poster Title</th>
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<tbody>
<tr>
<td>1</td>
<td>Abdou, Kareem</td>
<td>University of Toyama</td>
<td>Synapse-specific plasticity governs the identity of overlapping memory traces</td>
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<tr>
<td>2</td>
<td>Aimi, Takahiro</td>
<td>Keio University</td>
<td>Molecular Mechanisms Underlying Dynamic Changes of Synapses in Adult Cerebellum</td>
</tr>
<tr>
<td>3</td>
<td>Fujishima, Kazuto</td>
<td>Kyoto University</td>
<td>Mechanisms of dendrite growth guided by axonal bundles in cerebellar circuits</td>
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<tr>
<td>4</td>
<td>Hatano, Mai</td>
<td>Yokohama City University</td>
<td>Positron Emission Tomography Tracer for AMPA receptors Characterizes Psychiatric Disorders in Human</td>
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<tr>
<td>5</td>
<td>Hatsuda, Akane</td>
<td>Kyoto University</td>
<td>Analyzation of mitochondrial dynamics in developing dendrites</td>
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<tr>
<td>6</td>
<td>Hosokawa, Tomohisa</td>
<td>Kyoto University</td>
<td>Mechanism of subsynaptic segregation of AMPAR during LTP</td>
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<tr>
<td>7</td>
<td>Inagaki, Shigenori</td>
<td>Kyushu University</td>
<td>Inhibitory Odor Responses in the Mouse Olfactory Sensory Neurons</td>
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<td>8</td>
<td>Jitsuki, Susumu</td>
<td>Yokohama City University</td>
<td>CRMP2 Binding Compound Accelerates Recovery from Central Nervous System damage</td>
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<tr>
<td>9</td>
<td>Kawaguchi, Daichi</td>
<td>The University of Tokyo</td>
<td>A mechanism of region-specific neocortical overgrowth relevant to autism spectrum disorder</td>
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<tr>
<td>10</td>
<td>Kyrke-Smith, Madeleine</td>
<td>University of Utah</td>
<td>The immediate early gene Arc is required for the persistence of NMDAR-dependent LTD but not LTP</td>
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<tr>
<td>11</td>
<td>Leiwe Nathanael, Marcus</td>
<td>Kyushu University</td>
<td>Spontaneous activity generated within the olfactory bulb establishes the discrete wiring of mitral cell dendrites</td>
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<td>12</td>
<td>Liu, PinWu</td>
<td>Kyoto University</td>
<td>Reconstitution of Synaptic Plasticity In Vitro</td>
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<tr>
<td>13</td>
<td>Llamosas, Nerea</td>
<td>The Scripps Research Institute</td>
<td>Syngap1 Dynamically Regulates Experience-Dependent Cortical Circuit Reorganization</td>
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<tr>
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<td>Affiliation</td>
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<tr>
<td>14</td>
<td>Miki, Takafumi</td>
<td>Doshisha University</td>
<td>Fast Recruitment Of Synaptic Vesicles at Cerebellar Mossy Fiber Terminals</td>
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<td>15</td>
<td>Miyazaki, Taisuke</td>
<td>Hokkaido University</td>
<td>Principal rule of neurotransmitter receptor localization at synapse</td>
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<td>Mizuno, O. Grace</td>
<td>University of California, Davis</td>
<td>Tools To Visualize Where And How Neuropeptides Modulate Neural Circuits</td>
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<td>Nozawa, Kauzya</td>
<td>Keio University</td>
<td>Epitope-tag knock-in mice revealed the localization and the competition of synaptic organizers <em>in vivo</em></td>
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<tr>
<td>18</td>
<td>Ordonez, Dalila</td>
<td>Massachusetts Institute of Technology</td>
<td>CPG15 mediates activity-dependent synaptic plasticity in the rodent visual cortex</td>
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<td>Ota, Keisuke</td>
<td>RIKEN Center for Brain Science</td>
<td>In vivo calcium imaging with a single cell resolution using &quot;cosmoscope&quot;, a new wide-field two-photon microscope</td>
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<td>Ramsey, M. Austin</td>
<td>University of Maryland School of Medicine</td>
<td>Subsynaptic positioning of AMPA-type glutamate receptors determines receptor activation</td>
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<td>21</td>
<td>Saneyoshi, Takeo</td>
<td>Kyoto University</td>
<td>Reciprocal activation within a kinase-effector complex underlying persistence of structural LTP</td>
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<td>22</td>
<td>Schwartz, Samantha</td>
<td>University of Colorado Denver</td>
<td>An optogenetic tool for acute modulation of inhibitory synapse function</td>
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<td>Suzuki, Akinobu</td>
<td>University of Toyama</td>
<td>Manipulation of fear memory association by posterior parietal cortex</td>
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<td>Takuwa, Hiroyuki</td>
<td>National Institutes for Quantum and Radiological Science and Technology</td>
<td>Neuronal dysfunction due to hyperexcitation under mild chronic cerebral hypoperfusion</td>
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<td>25</td>
<td>Tsutsumi, Shinichiro</td>
<td>The University of Tokyo</td>
<td>Enhancement of climbing fiber synchrony drives timed sensorimotor associations in the cerebellar cortex</td>
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<td>Zhou, Chuying</td>
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<td>The dynamic interplay between microtubule-based motors and nuclear movement during neuronal migration</td>
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Abstracts

Oral Presentation
Cell ensemble mechanisms underlying memory formation

K. Inokuchi

Memories are not stored in isolation from other memories but are integrated into associative networks. At the same time, each memory has its own identity. Because association of related memories, with keeping the identity of each memory, is the fundamentals of knowledge formation, it is important to understand the underlying mechanisms. In this seminar, I will show that sharing memory engram cells underlies the linkage between memories (1), while synapse-specific plasticity guarantees the identity and storage of individual memories (2). In addition, I will suggest that engram cells in the hippocampus are organized into sub-ensembles representing distinct pieces of information, which are then orchestrated to constitute an entire memory (3).

References
Dendritic Compartment-Specific Regulation of Spine Density during Development

M-T. Ke, R. Egashira, and T. Imai

Dendrites receive most of their excitatory synaptic inputs from dendritic spines. It is generally believed that the number of dendritic spines increases during childhood, and then declines during adolescence. However, their distribution on a whole-neuron scale has not been fully established, due to limited throughput of electron microscopy-based connectomics and limited spatial resolution in conventional light microscopy. Here we performed comprehensive high-resolution mapping of dendritic spines in cortical pyramidal neurons in mice using an index-optimized tissue clearing agent, SeeDB2, and volumetric super-resolution imaging. In this study, we focused on thick-tufted layer 5 neurons (also known as subcerebral projection neurons) in the somatosensory cortex (S1). Spine density was relatively uniform in basal and oblique dendrites in these neurons. However, we found that the spine density is highly biased along the middle compartment of long apical dendrites (a spine density “hotspot”), demonstrating ~10-fold accumulations. We next examined developmental changes of spine density in thick-tufted layer 5 neurons. The number of dendritic spines increased during the first two postnatal weeks. In basal dendrites, spine density moderately declined during the subsequent adolescence stage. In apical dendrites, however, the spine density continued to increase specifically at the middle compartment, forming the spine density hotspot. Accumulation of dendritic spines to the hotspot was specifically impaired by single-cell knockout of NMDA receptor. Together, our results demonstrate that the spine “accumulation” to the hotspot is a hallmark of cortical circuit maturation during adolescence.
Molecular machinery for synaptic transmission

S. Tomita

A goal of my laboratory is to understand how synaptic strength is determined by abundance and channel properties of ionotropic neurotransmitter receptors. We have found that ionoropic receptors in the brain form a complex with their unique auxiliary subunits that determine receptor localization or properties. In this talk I will discuss recent data that have established the molecular machinery for excitatory synaptic plasticity via the identification of auxiliary receptor subunits. Our findings may shift the field of molecular neuroscience of synaptic transmission and their functional analysis push the field forward in terms of basic understanding of machinery and drug discovery.
Cell-extrinsic control of dendritic tree patterns of the cerebellar Purkinje cell

K. Fujishima, K. Kawabata-Galbraith, and M. Kengaku

The positions and shapes of neurons are critical determinants of the structural organization of neural circuits in the brain. In the cerebellar cortex, the functional subdomains are stringently determined by geometric arrangement of the fan-shaped planar dendrites of Purkinje cells. During postnatal development, Purkinje cells extend their dendrites in a single parasagittal plane perpendicular to the trajectories of parallel fiber axons. Such organization has shown to maximize potential synaptic connections, yet the mechanism and dynamics underlying how neurons acquire their unique shape facilitating this organization is unknown.

A major effort of my lab has been devoted to live-imaging of growing neurons for a mechanistic understanding of brain formation, with special focus on the clear and simple architecture of the cerebellar cortex. Our observation of the growth dynamics of Purkinje cell dendrites unveiled the simple parametric rules and underlying cytoskeletal dynamics that enable each neuron, with fine-tuning, to achieve their various shapes. We have also found that the geometry of the parallel fiber-Purkinje dendrite contacts relies on intrinsic properties of Purkinje dendrites, which selectively extend their branches perpendicular to the parallel fiber arrays. We now seek to determine the mechanisms and molecular bases of how chemical signals and physical contacts sculpt dendritic arbors to achieve an efficient axonal-dendritic wiring in the cerebellar cortex.
Over the past three decades, purification and analysis of protein complexes at the mature excitatory postsynapse has led to fundamental insights in neurobiology. These insights include how receptor trafficking, synaptic adhesion, cytoskeletal remodeling, and protein phosphorylation contribute to the synaptic plasticity underlying learning and memory. Moreover, genetic perturbations of excitatory postsynaptic proteins are now known to contribute to developmental brain disorders and psychiatric conditions.

In contrast to the well-studied excitatory synapse, biochemical purification and analysis of other neuronal structures, such as immature synapses or the inhibitory postsynaptic specialization, has remained largely intractable. We have developed novel proteomic and CRISPR-based approaches to resolve the molecular composition of diverse synapses and image their proteins as they exist \textit{in vivo}. These results and the analysis of several novel synaptic proteins will be discussed.
In vivo calcium imaging with a single cell resolution using “cosmoscope”, a new wide-field two-photon microscope


In neuroscience, in vivo functional imaging with a single-cell resolution over a wide field of view (FOV) is challenging. To accomplish this, we developed a wide-field two-photon microscope, which we named “cosmoscope”, that equips a very large objective lens with low magnification and a high numerical aperture. We evaluated the optical resolution and aberrations of this microscope by means of simulation. Eighty percent of the energy was contained within a radius of 1.1 µm in all FOV. This result was almost equivalent to the performance at the diffraction limit, indicating that this microscope possesses the high efficacy of two-photon excitation and high spatial resolution in all three axes across the entire field of view. To further evaluate this microscope, we performed in vivo calcium imaging of L2/3 and L5 cortical neurons expressing GCaMP in an awake mouse. GCaMP fluorescence was observed in the cytoplasm, not in the nucleus, providing confirmation that this microscope possesses single-cell resolution. We also developed a low computational cost cell detection (LCCD) algorithm (Ito et al., bioRxiv 502153), because the data size acquired by our microscope was so large that the previously reported algorithms could not detect neurons within a practical period of time. LCCD enabled us to extract more than 16,000 neural activities of L2/3 cortical neurons. Finally, we identified the functional map of the neurons. Whereas sensory stimulus-evoked activity was localized to the responsible region, movement-related activity was globally distributed. Cosmoscope will open the door to monitor a great multitude of single cortical neurons.
Network remodeling by glial cells

S. Koizumi

Glial cells are very sensitive to environmental changes, and then, change their phenotypes into very different ones. As for astrocytes, they become “reactive astrocytes” and contribute to both beneficial and hazardous brain functions. Here, I show reactive astrocyte-mediated synapse remodeling and its physiological and pathophysiological consequences in the somatosensory cortex (S1) and the striatum. Mechanical allodynia is caused by peripheral nerve injury, and its pathology remains unknown. After peripheral nerve injury, we found that S1 cortical astrocytes change neuronal circuits by excess synaptogenesis. Using in vivo two-photon imaging with genetic and pharmacological manipulations, we show that peripheral nerve injury induces a re-emergence of immature mGluR5 signaling in S1 reactive astrocytes, which elicits spontaneous somatic Ca\(^{2+}\) transients, release of synaptogenic molecules such as TSP-1 and synapse formation. Such S1 astrocyte reactivation was evident only during the first week post-injury, correlating with the temporal changes in S1 extracellular glutamate levels and dendritic spine turnover. Blocking this astrocytic signaling pathway suppressed mechanical allodynia, while activating this pathway in the absence of any peripheral injury induced long-lasting (>1 month) allodynia. We conclude that reactive astrocytes are a key trigger for S1 circuit rewiring and contribute to neuropathic mechanical allodynia. In addition to these, reactive astrocytes cause remodeling of the neuronal network of the ischemic penumbra in the striatum. In this case, astrocytes become rather phagocytic and contribute to elimination of synapses, thereby leading to remodeling of the penumbra networks. I will also talk the beneficial roles of reactive astrocytes.
Chemico-genetic discovery of molecules underlying tripartite-synaptic function in vivo

T. Takano

Neuronal synapses are intimately ensheathed by abundant astrocytic perisynaptic processes, which is critical for synapse formation and function. In contrast to well-studied neuronal synaptic compartments, however, the molecular mechanisms of how astrocytic perisynaptic structures govern neuronal synapses remain ill-defined. Here, we develop a new in vivo chemico-genetic approach, Split-TurboID GRAPHIC, that uses a cell surface fragment complementation strategy combined with informatics to identify the molecules at astrocyte-synapse junctions in vivo. We identify more than 100 proteins enriched at astrocyte-neuronal junctions. We find novel adhesion molecules highly expressed in cortical astrocytes whose deletion dramatically alters excitatory/inhibitory synaptic balance and also impairs spatial learning. Using Split-TurboID GRAPHIC we thus establish a new mechanism by which astrocytes coordinate inhibitory synaptic balance with excitation via a chemo-affinity code of the tripartite synapse.
Multiplex imaging of neural activity and signaling dynamics

H. Bito

A central goal of neuroscience is to elucidate how information is encoded and processed at the circuit, neuronal ensemble, single neuron, or subcellular structure resolution in vivo. Ca2+ imaging allows the tracking of population dynamics of neuronal activity at the cell soma levels as well as within neuronal compartments. We here rationally engineered a next-generation of linear and quadricolor, genetically encoded calcium indicator suite, XCaMPs. Using green XCaMP-Gf, single AP detection was achieved within 3–10 msec of spike onset, enabling measurements of fast-spike trains in parvalbumin-positive interneurons in the barrel cortex in vivo. A non-invasive, subcortical, imaging using red XCaMP-R uncovered somatosensation-evoked persistent activity in hippocampal CA1 neurons. A combinatorial use of XCaMP-Gf, XCaMP-R and a blue XCaMP-B enabled fiber photometric recording of three distinct (two inhibitory and one excitatory) ensembles during pre-motion activity in freely moving mice. Finally, two-photon co-imaging of yellow XCaMP-Y and XCaMP-R allowed in vivo paired recording of pre- and postsynaptic firing in vivo, revealing spatiotemporal constraints of dendritic inhibition in layer 1 in vivo, between axons of somatostatin (SST)-positive interneurons and apical tufts dendrites of excitatory pyramidal neurons. Thus, XCaMPs represent new multiplexable GECIs, with previously unattained high SNR, linear property, and high frequency spike resolution and offer a critical enhancement of solution space in studies of complex neuronal circuit dynamics. In combination with previous studies on activity-dependent synthetic promoters such as E-SARE, our findings collectively provide a novel toolkit to investigate key molecular, cellular and circuit machineries that are essential for coordinating the formation and maintenance of long-term information processing and regulate cognitive behavior in vivo.
Molecular nanopositioning and alignment control function of single synapses

T. Blanpied

Mechanisms that create, maintain, and modulate synapses are essential building blocks of human behavior. Our long-term goal is to pursue a deep understanding of the molecular organization underlying synaptic transmission and plasticity. At glutamatergic synapses, key proteins in the active zone and the postsynaptic density are enriched in subsynaptic nanodomains (<100 nm). Most surprisingly, nanodomains of the critical fusion-regulatory proteins RIM and Munc13 in the presynaptic active zone align with high precision across the synaptic cleft from nanodomains enriched in postsynaptic glutamate receptors. Using single-vesicle fusion mapping, we determined that the local density of RIM within active zone subregions predicts the probability of action potential-evoked vesicle fusion. This striking architectural arrangement has important implications for how synapses function, and nano-alignment between release sites and receptors can modulate synaptic transmission and potentially influence intracellular signaling. I will discuss new work regarding the mechanisms establishing and controlling this organization, including adhesion complexes, receptors, and cytoskeleton, and solicit feedback on our strategies for determining more systematically the characteristics of synaptic protein architecture. The outcomes of these experiments will answer core questions about the genesis of an important new aspect of synaptic architecture and enable test the physiological role of synaptic nanoalignment in brain circuits.
Emotional arousal enhances memory through the basolateral amygdala (BLA). We previously reported that the top-down inputs from the secondary motor cortex (M2) to the primary somatosensory cortex (S1) play crucial roles in somatosensory perceptual memory consolidation. However, it is unclear whether emotional arousal enhances perceptual memory, and whether it is dependent on BLA-cortical projections. By using a novel perceptual-emotional associative learning task and chemogenetic manipulation of the BLA-cortical circuit, we report that BLA neurons not only store emotional memory, but also enhance perceptual memory. In the associative learning task, male mice learned the floor texture paired with a presented female mouse in a cup on the floor. Male mice that had learned associatively texture and female, showed longer retention of memory of the texture than male mice that had not been presented a female mouse. In anatomical studies, we found that the BLA projects strongly to M2, but only weakly to S1. In male mice, BLA neurons were activated by exposure to a female mouse. Chemogenetic inactivation of M2-projecting BLA neurons during the post-learning period inhibited consolidation of memories of exposure to female mice. In addition, inactivation of this circuit did not impair consolidation of memories of the texture, but did reduce the enhancement of memory of the texture by exposure to female mouse. Our results suggest that the BLA-M2 circuit enhances perceptual memory by means of emotional associations.
Vocalizations are an essential medium for social communication in most vertebrates. Whereas many types of vocalizations are innate, humans and a few other animal groups culturally transmit their species-typical vocalizations. How does the brain enable the learning of complex, culturally transmitted vocal behaviors? The presentation will cover fundamental discoveries concerning how the brain enables the learning of birdsong, a behavior with many parallels to human speech. Similar to speech learning, birdsong learning requires that the pupil first memorize and then imitate the song of a tutor. Experiments in songbirds have begun to identify the synaptic mechanisms that underlie this remarkable form of learning. Indeed, in vivo multiphoton imaging has revealed that hearing a tutor song rapidly changes the size and strength of synapses in the pupil’s brain. Gain and loss of function methods, including genetic perturbations of neural activity in freely singing birds, have established the synaptic mechanisms that are both necessary and sufficient for forming an auditory memory of the tutor song and converting this memory into a vocal pattern. In fact, the neuromodulator dopamine has been shown to play an important role both in the formation of the auditory memory of the tutor song and the subsequent reinforcement of vocal patterns that match this auditory memory. This latter process involves midbrain-basal ganglia circuitry that is highly conserved across all vertebrates, suggesting that a circuit that initially evolved to reinforce relatively simple behaviors has been harnessed to enable virtuosic feats of vocal learning.
Multi-scale understanding of synaptic pathology of schizophrenia

A. Hayashi-Takagi

Various lines of evidence, including human genetics, brain imaging, and postmortem brain studies, have repeatedly suggested that disturbances in neuronal connectivity, synaptopathy, underlie a variety of psychiatric disorders. However, it is not yet known whether synaptopathy is an underlying mechanism of disease or a secondary consequence. Thus, we performed a longitudinal in vivo 2-photon imaging analysis of the brain of a schizophrenia model (DISC1 knockdown mice). Our previous work demonstrated that Disc1 knockdown mice exhibited a decrease in the density of dendritic spines, where the majority of excitatory synapses are formed. Furthermore, we found a significantly greater number of large dendritic spines in the model mice compared to wild-type mice. The presence of the large spines in the schizophrenia mice model mirrors findings from another schizophrenia mice model, calcineurin knockout mice. It is well-known that there is a strong correlation between spine head size and its synaptic efficacy, whereby the large spines can generate a larger synaptic current. This led us to hypothesize that large spines can affect the dendritic computation, causally resulting in subsequent behavioral alterations. To test this hypothesis, in our current project, we use a multiscale analysis that consists of an electrophysiological method and Ca2+ imaging to visualize the synaptic input (synaptic level), dendritic event (dendritic level), action potential (cell level), and behavioral manifestations (individual level). In addition, with use of in vivo optical and in silico manipulation of the spines in the model animal, we show the data showing what kind of synaptic pathology would causally underlie the pathology of disorders at the multiscale level.
Synapse development is crucial to the formation of functional brain circuitry. Pathogenic variants in genes that encode proteins targeted to the postsynaptic density are enriched in patients with childhood cognitive disorders. This suggests that childhood cognitive disorders arise, at least in part, through altered development of synapse structure and function. The SYNGAP1 gene encodes the protein, SynGAP, which is a major constituent of the postsynaptic density. This protein restricts the level of AMPA receptor incorporation in the synapse during postnatal development, while also dynamically regulating Ras-dependent signaling that controls LTP. Pathological SYNGAP1 variants are a frequent cause of sporadic intellectual disability with epilepsy and autistic features. This has led to the hypothesis that SYNGAP1 pathogenesis leads to cognitive dysfunction by disrupting the structural and functional maturation of synapses during developmental critical periods. A major isoform of SynGAP contains a motif that enables direct binding to PDZ domains of PSD95 and SAP102, and this interaction is thought to be critical for the structural development of the postsynaptic density. We have constructed an animal model that selectively disrupts the ability of SynGAP to interact with PDZ proteins. Using this model, we are investigating how SynGAP-PDZ binding directly contributes to synapse structural and functional development. We are also assessing how loss of this protein-protein interaction impacts circuit functions linked to cognitive processes and behavioral decision making.
Optical manipulation and imaging of signaling molecules in dendritic spines of neurons

H. Murakoshi

A Ca$^{2+}$/Calmodulin-dependent kinase II (CaMKII) is one of the most important signaling molecules for long-term potentiation and associated spine enlargement underlying learning and memory. Here, to understand the function of CaMKII for synaptic plasticity, we developed genetically encoded light-inducible CaMKII inhibitor by using LOV2 derived from phototropin1. We applied this newly developed optogenetic tool for the study of structural plasticity of single dendritic spines by using 2-photon fluorescence microscope and glutamate uncaging, and found that ~60 s of CaMKII activation is sufficient for inducing transient and sustained spine enlargement. In addition, we developed a photo-activatable CaMKII using LOV2 domain. Using this new genetically-encoded tool, we tested if CaMKII activation alone is sufficient for triggering structural plasticity or not. Interestingly, optogenetic CaMKII activation in single dendritic spines induced the spine enlargement and AMPA receptor recruitment, suggesting that CaMKII activation alone is sufficient for triggering synaptic plasticity. Furthermore, using 2-photon fluorescence lifetime imaging microscopy, we visualized the activity of potential CaMKII downstream molecules (Cdc42 and RhoA) to understand the spatiotemporal dynamics of these molecules during structural plasticity induced by CaMKII activation. Whereas glutamate activates both Cdc42 and RhoA, paCaMKII preferentially activated Cdc42 in a spine-specific manner. Furthermore, paCaMKII activation at multiple individual dendritic spines induced enhanced Cdc42 activation and sLTP, suggesting that CaMKII carries out neuronal computation for converting spatial input patterns to robust and clustered synaptic plasticity. Taken together, paCaMKII should be a useful tool for dissecting CaMKII signaling and functions as well as for the optogenetic induction of synaptic plasticity in vitro and in vivo.
Imaging homeostatic versus detrimental glial actions on neuronal and synaptic integrities

M. Higuchi, H. Takuwa, M. Shimojo, Y. Takado, and N. Sahara

A progressive loss of neurons following synaptic abnormalities is a core neurodegenerative process in diverse neuropsychiatric disorders represented by Alzheimer's disease (AD) and Parkinson's disease (PD). This deleterious pathway is known to be triggered by the accumulation of fibrillar protein aggregates, such as intraneuronal tau and alpha-synuclein deposits in AD and PD, respectively. We have recently constructed an imaging platform enabling visualization of protein aggregation, activation of detrimental astrocytes and microglia, and altered neurotransmissions in the brains of living animal models and humans with positron emission tomography and MR spectroscopy. Mechanistic involvements of glial cells with specific phenotypes in the pathophysiology of degenerative conditions have been further pursued by in-vivo two-photon laser microscopy and mesoscopy of the mouse brains. With the aid of these assaying modalities, it has been demonstrated that astrocytic volumes change in a manner coupled with neuronal excitation in a homeostatic condition, which could be mediated by astrocytic uptake and release of water and taurine, an inhibitory gliotransmitter. In a mouse model of neurodegenerative tau pathologies, loss of a water channel, aquaporin 4, and taurine occurs in concurrence with upregulation of monoamine oxidase B and consequent overproduction of GABA in astrocytes, leading to tonic suppression of neuronal activities. Meanwhile, a subset of neurons bearing highly mature tau fibrils exhibit hyperexcitability, and these neurons are preferentially phagocytosed and eliminated from the brain by proinflammatory microglia. Notably, pharmacological potentiation of interneurons has profoundly attenuated tau accumulation and neuronal loss, implying pivotal roles of the excitatory-inhibitory balance in neurodegenerative proteinopathies.
Glutamatergic synapses play central roles in almost all of neuronal functions such as learning, motor and sensory functions. Among glutamate receptors, AMPARs are the "actual mediator" at glutamatergic synapses. Since the cloning of AMPARs approximately two decades ago, enormous number of papers have reported the importance of AMPARs on neuronal functions including diseases. Despite the accumulation of knowledge of physiological roles of AMPARs, its clinical translation is limited. Main reason for this is that we are not currently able to visualize AMPARs in living human brain. Although rodent neuronal disease models are elegant and well characterized, it remains unclear if these animal models fully mimic human disease. Characterization of these diseases with AMPARs in living human brain should provide us biological basis of neuropsychiatric disorders.

We developed novel PET probe for AMPARs, named $^{[11]}$C-K-2. We detected $^{[11]}$C-K-2 signals reflecting specific binding to AMPARs in rat, non-human primate and human. We detected elevated $^{[11]}$C-K-2 signals in the temporal lobe of the hemisphere with epileptic foci where the $^{99m}$Tc-ECD-SPECT blood flow signal was lower compared to the contralateral hemisphere. Further, we detected significant positive correlation between $^{[11]}$C-K-2-signals and protein amount of AMPARs with surgically removed tissue from epileptic patients. Thus, our PET probe for AMPARs specifically detects AMPARs and the first PET probe to visualize AMPAR in living human brain. We are currently imaging patients with neuropsychiatric disorders.

Further, we have recently identified CRMP2-binding compound, edonerpic maleate, facilitates synaptic AMPAR delivery and results in the acceleration of motor function recovery after brain damage in rodent and non-human primate.

These small compounds will be promising tools to translate the knowledge of synaptic physiology to the elucidation of human neuropsychiatric disorders and brand-new clinical settings.
Enhancement of climbing fiber synchrony drives timed sensorimotor associations in the cerebellar cortex

S. Tsutsumi, O. Chadney, T-W. Yiu, E. Bäumler, L. Farragiana, and M. Häusser

The cerebellum has a well-known role in precise motor control, supported by climbing fiber inputs. At the same time, climbing fibers have been shown to robustly respond to sensory stimuli in the absence of motor outputs. However, how climbing fiber inputs coordinate sensory processing and motor timing remains unknown.

Here we probed how cerebellar cortex contributes to the rapid sensorimotor associations by performing two-photon calcium imaging to monitor population complex spikes and optogenetics to manipulate cortical activity in Crus I in head-fixed mice. Head-fixed mice were trained in a multisensory go/no-go task to lick during 0.5 s of paired somatosensory and auditory stimuli and withhold licking when presented with these stimuli individually.

Optogenetic activation of Purkinje cells showed that cerebellar cortical activity in Crus I during the sensory cue is crucial for temporally precise motor initiation in response to sensory stimuli, but not for the motor performance per se. Two photon calcium imaging from Crus I revealed that behaviorally relevant complex spike signals were organized into alternating parasagittal bands. The probability, latency and magnitude of synchronous complex spike signals within these bands discriminated sensory modality. Synchrony level of complex spikes within the salient bands was specifically enhanced during sensory-evoked motor actions, but not for voluntary movement. Synchronous complex spike signals in response to sensory stimuli were correlated with temporally precise motor initiation. These results suggest that enhancement of synchrony in sensory-evoked complex spikes in spatially organized Purkinje cells is critical for timed sensorimotor associations.
Memories are encoded and stored in specific neuronal ensemble, called engram cells. Some of these memories are associated and stored in shared ensemble. However, brain machinery that underlies memory storage and defines certain memory identity amidst numerous number of memories stored in the same ensemble is poorly understood.

Here we show that when two associative memories are encoded in shared ensemble, engram-specific synaptic plasticity delineates specific memory entity and that specific plasticity is both sufficient and crucial for information storage. Using auditory fear conditioning and c-fos-TetTag system, optogenetic stimulation of the activated ensemble terminals of auditory cortex (AC) and medial geniculate nucleus (MGm) in lateral amygdala (LA) after complete retrograde amnesia -accomplished by autophagy induction with protein synthesis inhibition- failed to induce memory recall at recent and remote time points, indicating that memory engram no longer exists in that circuit. This result was correlated with the resetting of plasticity and functional connectivity between the engram assemblies. Furthermore, potentiating or depotentiating the plasticity at synapses specific to a given memory did not affect the linked memory that is encoded in the same ensemble, suggesting that memories are stored in specific synapses.

These findings unravel how the brain organizes and stores multiple associative memories in shared ensemble, underpinning a causal relationship between synaptic input-specific plasticity and memory identity and storage. Moreover, our study sheds light on the capability of selective and integral erasure of memory trace from the engram network, suggesting a potential way to treat post-traumatic stress disorder (PTSD).
CPG15 Mediates Activity-Dependent Synaptic Plasticity In The Rodent Visual Cortex

D. Ordonez, S. Manole, M. Benoit, J. Subramanian and E. Nedivi

During developmental critical periods, patterned activity driven by experience refines neuronal circuits to form highly efficient networks. CPG15, the product of an activity-regulated gene, is a small glycosylphosphatidylinositol (GPI)-linked extracellular protein that has been implicated in synapse stabilization, axonal and dendritic arborization, as well as synaptic maturation. Recently, \textit{in vivo} imaging of spine and synapse formation in CPG15 KO mice has revealed that while spine formation can occur normally in the absence of activity or CPG15, postsynaptic density protein 95 (PSD95) recruitment to nascent spines is deficient. Expression of CPG15 in the absence of activity is sufficient to restore normal PSD95 recruitment and spine stabilization, suggesting that PSD95 recruitment is the critical step in spine and synapse stabilization, and implicating CPG15 as the molecular mediator of activity-dependent synapse selection. Yet, the mechanism of action remains unclear. Using molecular and biochemical approaches, we probed the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor proteome and identified CPG15 as part of the protein complex that co-precipitates with AMPA receptor subunits. Preliminary structure-function analysis of CPG15 binding domain on AMPARs suggests that CPG15 binds the S1-S2 extracellular domains of AMPARs. \textit{In vitro} imaging of AMPARs in cortical cultures of CPG15 KO mice suggest that CPG15 aids AMPARs stabilization and delays receptor turnover at postsynaptic density sites. Thus, we propose that in response to salient activity CPG15 binds AMPARs extracellularly and stabilizes them to promote intracellular recruitment of PSD95 to nascent spines and synapse maturation.
Reconstitution of Synaptic Plasticity In Vitro

PW. Liu, T. Hosokawa, and Y. Hayashi

Synaptic plasticity, such as long-term potentiation (LTP), is known to be the basis for learning and memory. The major mechanism to induce and maintain LTP is an increase of neurotransmitter receptors, like AMPA receptor, at postsynaptic site and postsynaptic density (PSD). Previously, our group revealed that not only AMPA receptor, but also various synaptic proteins translocate into postsynaptic structure in the response to LTP stimulation, calcium influx, and the enlargement of dendritic spine (structural LTP). However, how synaptic proteins translocate and accumulate into postsynaptic structure remains unclear.

It has been reported that mixing several purified PSD proteins results in the formation of PSD proteins condensate (Zeng et al., 2018). This condensate is in phase transition of free-diffusion and condensate, and it’s highly reversible. Using this system as in vitro reconstitution of PSD structure, we can demonstrate the reconstitution of protein translocation and accumulation into PSD during LTP.

We found that Calcium/calmodulin-dependent protein kinase II (CaMKII) plays crucial role for protein translocation and accumulation under calcium-dependent manner. Conformational change of CaMKII results in the exposure of its interacting site and enables CaMKII to interact and cross-link PSD proteins by using its dodecamer-structure. Adding CaMKII and calcium ion to the PSD condensates resulted in the enlargement and stabilization of PSD condensates. This result suggests that CaMKII plays important role during induction of LTP not only as a kinase but also as a structural protein. Furthermore, this mechanism would be related with synaptic dysfunction and the basal maintenance of synapse.
Subsynaptic positioning of AMPA-type glutamate receptors determines receptor activation

A. M. Ramsey, A. Tang, T. LeGates, S. Thompson, T. Biederer and T. A. Blanpied

Previously, our lab discovered that a protein called Rab3 Interacting Molecule (RIM), which is essential for neurotransmitter release, is clustered into ~100 nm subdomains within the active zone, and that vesicle exocytosis preferentially occurs where there is a higher subsynaptic density of RIM. Furthermore, these presynaptic sites of neurotransmitter exocytosis are aligned with postsynaptic nanoclusters of receptors. There are many potential mechanisms, however, a particularly attractive model is that synaptic cell adhesion molecules mediate alignment through trans-synaptic protein binding. Leucine Rich Repeat Transmembrane neuronal (LRRTM2) participates in trans-synaptic protein binding and is critical for basal AMPAR-mediated transmission. In order to test the ongoing role of LRRTM2 in established synapses, we adapted an approach to acutely disrupt LRRTM2 binding interactions in the cleft. Using dSTORM, we found that acute disruption of LRRTM2 results in a rapid reduction in the nanoscale alignment of proteins at synapses. Furthermore, we performed whole-cell patch clamp of cultured hippocampal neurons to test how acute disruption of the LRRTM2 extracellular binding interaction impacts synaptic transmission. We find that acute cleavage of LRRTM2 results in a substantial decrease in the evoked AMPAR-mediated EPSC amplitude. Together, these findings provide experimental support for the idea that trans-synaptic nanoscale organization plays an important role in maintaining synaptic strength. A structural role played by one or more specific cleft proteins provides further evidence for a molecularly guided “nanocolumn” architecture within the synapse. Broadly, these results also indicate that synaptic cell adhesion molecules can play specific and unexpected roles in regulating function at established synapses well after synaptogenesis.
A mechanism of region-specific neocortical overgrowth relevant to autism spectrum disorder

D. Kawaguchi, N. Watanabe, K. Nagahama, T. Watanabe, M. Kano, and Y. Gotoh

The neocortex regulates complex cognitive tasks such as sensory perception and higher cognition. Defects in cortical development are believed to cause neurological disorders including autism spectrum disorder (ASD). ASD children often have an overgrowth of the frontal cortex, but the underlying molecular mechanisms responsible for this region-specific overgrowth as well as its pathological relevance remain elusive. Recently, ASD has genetically been linked to members of fibroblast growth factor morphogens and their signaling pathways. Here we show that manipulation of this signaling pathway in mice from early embryonic stage (around E9.0) results in overgrowth of the frontal cortex at early postnatal stages. Interestingly, manipulation of the same signaling pathway from later embryonic stage (around E11.0) did not cause overgrowth phenotype, suggesting that early embryonic stage before E11.0 is critical for the regional specification of neocortex at postnatal stages. Furthermore, the mutant mice defective in fibroblast growth factor signaling also display autism-like behavioral defects and disorganization of neuronal activity in the frontal cortex. Our data together suggest that fibroblast growth factor signaling might have a causal role in brain morphological and behavioral alternations relevant to ASD.
Syngap1 Dynamically Regulates Experience-Dependent Cortical Circuit Reorganization

N. Llamosas, T. Vaissiere, S. D. Michaelson, C. Rojas and G. Rumbaugh

A substantial portion of neurodevelopmental disorders (NDDs) are caused by genetic variants that disrupt brain function. However, the neurobiological processes that connect genetic pathogenicity to brain dysfunction remain unclear. Experience is known to reorganize cortical circuits through the refinement of synaptic inputs, which is believed to promote cortical functions relevant to cognition and behavior. Therefore, we hypothesized that genetic variants causally-linked to NDDs disrupt experience-dependent reorganization of synaptic inputs that comprise disease-relevant cortical circuits. To test this, we paired a whisker-trimming paradigm with serial in vivo two-photon imaging in somatosensory cortex (S1) to visualize experience-dependent changes in synaptic inputs and circuit activity in mouse models. First, we studied how Syngap1 heterozygosity, which is a leading cause of sporadic NDDs, impacts the dynamic reorganization of axonal inputs onto S1 neurons. Serial imaging of axons in WT mice revealed an increase in the formation rate of presynaptic boutons in response to altered whisker experience. This form of experience-dependent structural plasticity was completely absent in Syngap1 heterozygous mice. Second, we imaged whisker-evoked circuit activity before and after whisker trimming. Syngap1 heterozygosity prevented both the scaling up of network activity and the remapping of discrete whisker inputs in S1. Remarkably, re-expression of the Syngap1 gene in adult mice reactivated experience-dependent cortical circuit plasticity (scaling up and remapping of inputs). These findings demonstrate that an NDD risk gene dynamically regulates forms of cortical plasticity required for experience-dependent circuit reorganization. Genetic disruption of these forms of plasticity may contribute to cortical dysfunction and cognitive impairments observed in NDDs.
Visualizing Molecular Events in Synapse Formation \textit{in vivo}

E. Nedivi

The introduction of two-photon microscopy for \textit{in vivo} imaging has opened the door to chronic monitoring of individual neurons in the adult brain, and the study of structural plasticity mechanisms at a very fine scale. Perhaps the biggest contribution of this modern anatomical method has been the discovery that even across the stable excitatory dendritic scaffold there is significant capacity for synaptic remodeling, and that adjusting synapse strength and number are key mechanisms mediating neural circuit adaptation and behavioral plasticity in the adult. The activity-regulated gene \textit{cpg15/neuritin} has been previously implicated in stabilization and maturation of excitatory synapses. We combined multicolor two-photon microscopy with genetic and sensory manipulations to delineate, \textit{in vivo}, steps in synapse formation and maturation, and examine sufficiency and requirement for activity and CPG15 at these defined steps.
Dendritic spines are submicron-scale structures protruding from neuronal dendrites that receive excitatory synaptic inputs from afferent axons. Precise measurement of spine morphology and objective analysis of large numbers of spines are required to understand both the physiological regulation of synaptic transmission and pathological changes in this process. Dendritic spines exhibit a high degree of structural variability: their sizes vary over more than one order of magnitude and their shapes range from thin elongated filopodia-like protrusions to round mushroom-like structures. We developed an accurate method for measurement and analysis of spine morphology based on structured illumination microscopy and computational geometry. Surface mesh data converted from SIM images were comparable to data reconstructed from electron microscopic images. Dimensional reduction and machine learning applied to large data sets enabled identification of spine phenotypes caused by genetic mutations in key signal transduction molecules. This method, combined with timelapse live imaging and glutamate uncaging, could detect plasticity-related changes in spine head curvature. The results suggested that the concave surfaces of spines are important for the long-term structural stabilization of spines possibly via interaction of cell adhesion molecules across the synaptic cleft.
To study the neural circuitry, the action of one cells under the context of others, one would precisely measure and perturb specific neuronal populations and molecules in behaving animals who are specifically engaged in performing the computation or function of interest. The dataset of millions of neurons firing together underlying a behavior are required to develop and refine theories (hypotheses) explaining animal behavior in terms of brain physiology. The focus of lab is to develop novel genetically encoded indicators based on fluorescence proteins, especially focusing on direct and specific measurement of myriad input signals with needed spatial and temporal resolutions. In this talk, I will discuss our recent progress into develop and apply a new suite of genetically encoded indicators to enable 1) In vivo measurement of afferent activity of any length with axon-specific calcium imaging in previously inaccessible deep brain area; 2) ultrafast neuronal imaging of dopamine dynamics in vivo. I will discuss the design, characterization and applications of these genetically encoded indicators. We also validate our sensor design platform, which could also be applied to developing sensors for a broad range of neuromodulators, including norepinephrine, serotonin, melatonin, and opioid neuropeptides. In combination with calcium imaging and optogenetics, our sensors are well poised to permit direct functional analysis of how the spatiotemporal coding of neural input signaling mediates the plasticity and function of target circuits.
Embryonic origin of neural stem cells in the adult mouse subependymal zone

Sotokawa, R., Yuizumi, N., Harada, Y., Kawaguchi, D. and Gotoh, Y.

Throughout life, neural stem cells (NSCs) in the adult brain continuously generate new neurons that modify neural circuitry. Adult NSCs constitute a relatively quiescent cell population, but they can be activated by extrinsic neurogenic stimuli. We have previously shown that the cyclin-dependent kinase inhibitor p57kip2 (p57) is required for NSC quiescence. Our results indicated that in NSCs deletion of p57 initially results in increased neurogenesis but later leads to NSC exhaustion and impaired neurogenesis in aged mice. The role of p57 appears to be key to understanding the rate of neurogenesis and life-long maintenance of adult NSCs both in the subgranular zone of the hippocampal dentate gyrus and in the subependymal zone (SEZ) of the lateral ventricles.

One of the long-sought questions about adult NSCs is their embryonic origin. We have previously discovered that during the early stage of neural development (embryonic day 13 to 15) a subpopulation of embryonic neural precursor cells (NPCs) are set aside by slowing down their cell cycle, and some of these cells later become adult NSCs in the SEZ (Furutachi et al. Nat Neurosci 2015). Importantly, p57 is highly expressed in this slowly-dividing embryonic subpopulation and its deletion impairs the emergence of adult NSCs. We also found that overexpression of p57 promotes the maintenance of NPCs in an undifferentiated state. I will discuss the mechanism underlying this function of p57 and how this slow-dividing cell population emerges during development.
A new role of CaMKII as a postsynaptic crosslinker

Y. Hayashi

Despite the lack of demarcating membranous structure, a synapse can not only maintain the integrity of its components but also dynamically modifies it in response to the neuronal activity. The mechanism by which the synapse maintains and modifies its components to meet its demand has not been totally understood. Here we report that CaMKII, a well-known kinase implicated in the synaptic plasticity, serves as a postsynaptic structural element that captures various proteins. This is mediated by a binding site on CaMKII normally occupied by the autoinhibitory domain called T-site. Many proteins including synaptic scaffolding, structural, and signaling proteins binds with T-site and are cross-linked through the dodecameric structure of CaMKII. The activation of the kinase opens up T-site to facilitate the binding and one bound, the binding persists even after Ca$^{2+}$ wanes. In this way, CaMKII serves as a universal mechanism to capture synaptic proteins activity dependently.
Viral-like mechanisms of intercellular plasticity

J. D. Shepherd

My lab recently discovered a novel mechanism of neuronal communication that resembles the life-cycle of retroviruses. The neuronal gene Arc, a master regulator of synaptic plasticity and memory, contains a Gag retroviral homology domain that has conserved secondary structure with HIV-1 that is derived from a distinct family of retrotransposons. Arc protein self-assembles into viral-like capsids that are released from cells and carry RNA/proteins to neighboring cells. Our findings open up a new area of investigation in the cell biology of intercellular communication and mechanisms of synaptic plasticity, by revealing that some retrotransposon-derived genes, like Arc, retain the ability to form capsids that shuttle RNAs and proteins between cells.
Hippocampal mossy fiber (hMF) –CA3 synapses exhibit various forms of short- and long-term presynaptic plasticity. The presynaptic mechanisms of transmitter release and synaptic plasticity still remain to be studied. We have used simultaneous recordings of pre- and postsynaptic compartments at the hMF-CA3 synapse and measured presynaptic capacitance and EPSCs to measure the kinetics of exocytosis. In addition, total internal reflection fluorescence (TIRF) microscopy was used to directly visualize dynamics of single synaptic vesicles near the plasma membrane. Readily releasable synaptic vesicles mostly consisted of already-tethered vesicles in the TIRF field and their release kinetics were 10-fold slower compared with other synapses, for example, the calyx of Held in the auditory brainstem. Other than the release kinetics, the release properties were similar to other types of synapses, and the slow release was mainly determined by the loose coupling between Ca channels and synaptic vesicles. Application of cAMP, a molecule crucial for LTP, increases the vesicular release probability, possibly by changing the coupling between Ca$^{2+}$ channels and synaptic vesicles.
Casting new light on synaptic plasticity using optogenetic tools

M. Yuzaki

Synaptic connectivity is functionally strengthened or weakened for long duration by changes in neuronal activities, phenomena known as long-term potentiation (LTP) and long-term depression (LTD), respectively. LTP and LTD are thought to be mainly mediated by increase or decrease in the number of postsynaptic AMPA receptors. While molecular mechanisms underlying postsynaptic trafficking of AMPA receptors are beginning to be understood, it remains incompletely clear whether and how LTP and LTD are causally linked to brain functions mediating specific behavioral changes in vivo. To address this question, we have recently developed a new optogenetic tool PhotonSABER, which can acutely and reversibly inhibit postsynaptic AMPA receptor endocytosis and LTD both in vitro and in vivo. Using PhotonSABER, we have demonstrated that LTD at parallel fiber-Purkinje cell synapses is causally related to visual adaptation of vestibule-ocular reflex and optokinetic response, cerebellar motor learning paradigms (Kakegawa et al., Neuron 2018). In the present talk, I would like to introduce and discuss new optogenetic tools we are developing to manipulate LTP and LTD to further clarify their roles in various brain functions.
New approaches for controlling synaptic function and plasticity with light

M. J. Kennedy

While optogenetic tools for triggering or suppressing neuronal firing have transformed experimental neuroscience, there remains an unmet need for tools allowing precise control of more subtle, but fundamentally important neural functions. We are developing new approaches for rapidly and locally manipulating cellular and biochemical mechanisms important for synaptic connectivity and plasticity in the central nervous system. We have engineered new approaches for conditionally tuning the number and position of important postsynaptic signaling molecules and receptors within excitatory synapses. This has led to new and surprising insights into the importance of nanoscale spatial organization of neurotransmitter receptors for synaptic transmission. We are also engineering new tools for manipulating presynaptic function, including first-in-class tools for persistent disruption of vesicular release of neurotransmitters, neuromodulators and hormones.
Abstracts

Poster Presentation
Memories are encoded and stored in specific neuronal ensemble, called engram cells. Some of these memories are associated and stored in shared ensemble. However, brain machinery that underlies memory storage and defines certain memory identity amidst numerous number of memories stored in the same ensemble is poorly understood.

Here we show that when two associative memories are encoded in shared ensemble, engram-specific synaptic plasticity delineates specific memory entity and that specific plasticity is both sufficient and crucial for information storage. Using auditory fear conditioning and c-fos-TetTag system, optogenetic stimulation of the activated ensemble terminals of auditory cortex (AC) and medial geniculate nucleus (MGm) in lateral amygdala (LA) after complete retrograde amnesia -accomplished by autophagy induction with protein synthesis inhibition- failed to induce memory recall at recent and remote time points, indicating that memory engram no longer exists in that circuit. This result was correlated with the resetting of plasticity and functional connectivity between the engram assemblies. Furthermore, potentiating or depotentiating the plasticity at synapses specific to a given memory did not affect the linked memory that is encoded in the same ensemble, suggesting that memories are stored in specific synapses.

These findings unravel how the brain organizes and stores multiple associative memories in shared ensemble, underpinning a causal relationship between synaptic input-specific plasticity and memory identity and storage. Moreover, our study sheds light on the capability of selective and integral erasure of memory trace from the engram network, suggesting a potential way to treat post-traumatic stress disorder (PTSD).
Activity-dependent synapse formation and elimination are thought to serve as fundamental processes underlying learning and memory. Synapse formation between cerebellar Purkinje cells (PCs) and climbing fibers (CFs) have served as a good model to study activity-dependent synapse regulation in the CNS. Immature PCs are initially innervated by multiple CFs, but during several postnatal weeks in rodents weak “looser” CFs are eventually eliminated, leaving a single, strong “winner” CF input. We have previously demonstrated that C1ql1, a C1q-family protein secreted from CFs, bind to brain-specific angiogenesis inhibitor 3 (Bai3), a cell-adhesion G protein-coupled receptor expressed in PCs, not only facilitate elimination of loser CFs, but also strengthen a winner CF during development (Kakegawa et al., Neuron, 2015). Although C1ql1 and Bai3 remain highly expressed in adult cerebellum, it remains unclear whether and how they function in mature CF synapses. Here, we show that appropriate levels of Bai3 are essential to maintain 1:1 innervation pattern at CF-PC synapses throughout life by knocking out or overexpressing Bai3 in adult PCs. The amplitude of CF-evoked synaptic currents was weakened by knockout of Bai3 in adult PCs. Conversely, PCs become re-innervated by multiple CFs by overexpression of Bai3 in adult PCs. These findings indicate that CFs possess synaptogenic capacity throughout life. Since family proteins closely related to C1ql1 and Bai3 are expressed in various brain regions, similar mechanisms likely mediate certain aspects of activity-dependent synaptic changes.
Mechanisms of dendrite growth guided by axonal bundles in cerebellar circuits

K. Fujishima, M. Yamada, J. Kurisu and M. Kengaku

The wiring pattern of a neural circuit determines its function and properties. However, the mechanisms underlying how the geometric patterns of axons and dendrites may affect each other remain elusive.

In the cerebellar cortex, Purkinje cells develop mono-planar dendrites that make perpendicular contacts with parallel fibers, the axons of the cerebellar granule cells. Several lines of evidence suggest that the growth direction of Purkinje cell dendrites is confined perpendicularly to parallel fiber bundles, which ensures flat dendrite formation of Purkinje cells. However, the cellular and molecular mechanisms of how dendrite growth orientation is determined by axonal bundles are unknown.

We utilize an artificial nanofiber substrate to align parallel fiber axons two dimensionally to recapitulate a simplified model of the perpendicular contact between Purkinje cells and parallel fibers in vitro. Time-lapse observation of growing Purkinje dendrites using the nanofiber culture system revealed that the dendrites perpendicular with respect to the parallel fibers were preferentially formed, mainly by biased extension of perpendicular branches, rather than selective elimination of mis-oriented branches.

We found that dendritic membrane skeletal protein, $\beta$ III Spectrin, is required for the axonal-bundle guided dendrite growth. $\beta$ III Spectrin displayed periodic membrane skeleton patterns underneath the plasma membrane of Purkinje cell dendrites and modulates microtubule dynamics. $\beta$ III Spectrin deficiency causes abnormal invasion of microtubules into dendritic filopodia, resulting in the ectopic growth of dendrite deflected from the precise orientation. We provide the evidence that $\beta$ III Spectrin organize cytoskeletal components in dendrites to steer the dendrite growth.
The glutamate $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) plays central roles in neuronal functions. However, clinical translation of AMPAR knowledge is limited due to the inability to visualize AMPAR in the living human brain. Here we developed a positron emission tomography (PET) tracer for AMPARs, named $[^{11}\text{C}]\text{K}-2$, and showed its specific binding to AMPARs. Logan graphical analysis in first-in-human PET study with healthy participants revealed reversible binding of $[^{11}\text{C}]\text{K}-2$. Further, $[^{11}\text{C}]\text{K}-2$ revealed systemic reduction of AMPARs in patients with depression, while patients with schizophrenia exhibited focal decrease of AMPARs in parahippocampal and cingulate gyrus. These decreases were significantly correlated with their symptomatology scores in both disorders. Thus, $[^{11}\text{C}]\text{K}-2$ could be a useful tool to study biological base of psychiatric disease, and expected to be a novel diagnostic drug in the clinical setting.
Analyzation of mitochondrial dynamics in developing dendrites

A. Hatsuda, K. Fujishima, and M. Kengaku

Neurons consume a large amount of ATP for the construction and maintenance of their complex structures and for the regulation of neuronal activities. In order to meet these requirements, mitochondria, a source of ATP, need to be transported throughout neuronal processes. Previous papers have shown that defects in mitochondrial function or trafficking induce abnormal dendritic morphology and precocious neurodegeneration, indicating that mitochondrial trafficking is important for neuronal development and survival.

In hippocampal neurons, dendrites dynamically change their shape during development (postnatal 1-2 weeks) before they construct complicated dendritic arbors which receive afferent neuronal signals on mature spines. We hypothesize that mitochondria may change their dynamics in response to the rapid increase in ATP demands during dendritic growth. In order to visualize mitochondrial dynamics during neuronal development, we transfected mitochondrial matrix-targeted GFP (mito-GFP) in CA1 pyramidal neurons and in primary hippocampal neural cultures. Immunofluorescence revealed that mitochondrial density in distal dendrites was very low in young neurons and gradually increased during dendritic growth until mitochondria were regularly arranged along the dendritic shaft in later stages. Time-lapse imaging showed that mobile mitochondria decreased as the dendrite matured. We currently seek for a possible feedback regulation of mitochondria mobility by local ATP concentrations.
Mechanism of subsynaptic segregation of AMPAR during LTP

T. Hosokawa, PW. Liu and Y. Hayashi

Within synaptic contact, the constituent proteins segregate into distinct subdomains. Furthermore, the pre- and postsynaptic subdomains are aligned with each other. Such alignment is modulated by neuronal activity thereby potentially serving as a mechanism to regulate the efficacy of synaptic transmission. This is accomplished without any demarcating structure which can limits the diffusion of constituents and how it is accomplished remain largely elusive.

Recently it was reported that major postsynaptic density (PSD) proteins such as PSD95, NR2B, Shank and Homer undergo liquid-liquid phase separation to form PSD-mimetic droplet. Using this system, we observed the behavior of PSD proteins during LTP-mimetic Calcium stimulation. We found that Stargazin, as a proxy of AMPAR, forms segregated cluster with PSD95 as phase-in-phase. CaMKII and NR2B plays central role for that segregation by forming surrounding phase in Calcium-dependent manner. Neuroligin segregates together with Stargazin and PSD-95. This phenomenon explains the formation of trans-synaptic nanocolumn during LTP. We will report this as another presentation in this meeting.

However, precise mechanism of this segregation as phase-in-phase remains unclear. In this presentation, we will introduce recent results with different conditions, mutants and interaction competitors. As tentative conclusion, we found that CaMKII-NR2B interaction would weaken the interaction between PSD95-NR2B so that CaMKII-NR2B and PSD95-Stargazin can be separated as immiscible droplets.
Inhibitory Odor Responses in the Mouse Olfactory Sensory Neurons

S. Inagaki, R. Iwata, and T. Imai

It is generally believed that odorants “activate” odorant receptors (ORs) and odor information is spatiotemporally represented by the “activation” patterns of glomeruli in the olfactory bulb (OB). Here we performed in vivo two-photon Ca$^{2+}$ imaging of OSN axon terminals in the OB, and found that > 5% of glomeruli show robust inhibitory responses to odors, while ~25% demonstrated excitation. As OSNs are known to show basal spontaneous activity without odors, this is most likely due to the reduction of spontaneous activity in OSNs.

To examine a possible role for presynaptic lateral inhibition, we generated an OSN-specific knockout of GABAB receptors and dopamine D2 receptors. However, inhibitory responses were still seen at OSN axon terminals. We also tested OSN-specific tetanus toxin light chain (TeNT) transgenic animals, in which synaptic transmission from all OSNs was blocked; however, robust inhibitory responses remained in OSN axons. These results suggested that at least some fractions of the inhibition occur non-synaptically. We therefore performed in vivo two-photon Ca$^{2+}$ imaging of OSN somata in the olfactory epithelium (OE), and found that the inhibitory responses are already happening at the OSN somata.

In *Drosophila*, reduction of basal OR current upon odorant binding and non-synaptic lateral inhibition via ephaptic coupling have been known to produce inhibitory responses in some OSNs. Therefore, similar mechanisms may underlie the widespread inhibition in the mammalian OSNs. Similarly to the visual system, both excitation and inhibition of OSNs may contribute to the efficient coding of odors.
CRMP2 Binding Compound Accelerates Recovery from Central Nervous System damage


Damage to the central nervous system (CNS) causes severe neurological condition, such as sustained sensory, motor, cognitive dysfunction and compromise work capacity and self-care. No pharmacological intervention that could foster recovery and complement current rehabilitation has yet been established as effective. Restoration of motor impairment after CNS damage is considered to be the result of compensative neural plasticity in spared neural circuit, and the Experience-dependent synaptic AMPA (\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid) receptor (AMPAR) delivery underlies behaviors that require neural plasticity such as learning. We found that a small compound, edonerpic-maleate (also known as T-817MA), facilitated experience-driven synaptic glutamate AMPA receptor delivery and resulted in the acceleration of motor function recovery after cortical or spinal cord cryoinjury. Edonerpic bound to collapsin-response-mediator-protein 2 (CRMP2), a downstream molecule of semaphorin, and is thought to be related to synaptic plasticity and learning. Furthermore, we detected CRMP2-dependent activation of ADF/cofilin by edonerpic maleate in the plasticity-inducing condition. Indicating edonerpic could facilitate synaptic AMPAR delivery through the regulation of actin dynamics. Thus, edonerpic-maleate, a neural plasticity enhancer, could be a clinically potent small compound to accelerate rehabilitation after damage of CNS.
The neocortex regulates complex cognitive tasks such as sensory perception and higher cognition. Defects in cortical development are believed to cause neurological disorders including autism spectrum disorder (ASD). ASD children often have an overgrowth of the frontal cortex, but the underlying molecular mechanisms responsible for this region-specific overgrowth as well as its pathological relevance remain elusive. Recently, ASD has genetically been linked to members of fibroblast growth factor morphogens and their signaling pathways. Here we show that manipulation of this signaling pathway in mice from early embryonic stage (around E9.0) results in overgrowth of the frontal cortex at early postnatal stages. Interestingly, manipulation of the same signaling pathway from later embryonic stage (around E11.0) did not cause overgrowth phenotype, suggesting that early embryonic stage before E11.0 is critical for the regional specification of neocortex at postnatal stages. Furthermore, the mutant mice defective in fibroblast growth factor signaling also display autism-like behavioral defects and disorganization of neuronal activity in the frontal cortex. Our data together suggest that fibroblast growth factor signaling might have a causal role in brain morphological and behavioral alternations relevant to ASD.
The immediate early gene Arc is required for the persistence of NMDAR-dependent LTD but not LTP

M. Kyrke-Smith and J.D. Shepherd

The immediate early gene Arc is believed to be a master regulator of plasticity mechanisms that underlie long-term memory, such as long-term potentiation (LTP) and depression (LTD). Arc interacts with endocytic proteins to reduce surface AMPAR, leading to LTD. Additionally, Arc has been implicated in the maintenance of LTP, suggesting that Arc could play a role in both LTP and LTD. To elucidate the requirement of Arc in LTD and LTP, we compared plasticity at CA3-CA1 synapses in slices from Arc knockout animals (ArcKO) and wild-type littermates. NMDA-dependent LTD was attenuated in ArcKO animals but there was no difference in LTP between the groups when using a high frequency stimulation protocol. However, LTP was enhanced in ArcKO slices when using a theta burst stimulation protocol. To determine whether this enhancement was due to compensatory mechanisms initiated during development, we used conditional ArcKO animals where the gene was only knocked out upon viral deliver and expression of cre-recombinase in excitatory neurons. Theta burst stimulation LTP was normal in slices from animals injected with cre-recombinase, suggesting this phenotype is due to secondary processes in the full ArcKO mice. Our data are consistent with the idea that Arc may mediate a reduction of synaptic strength at nonpotentiated synapses after LTP induction and learning. Additionally, Arc may mediate non-cell autonomous plasticity by forming virus-like capsids that mediate intercellular transport of RNA, potentially regulating circuit-level plasticity.
Spontaneous activity generated within the olfactory bulb establishes the discrete wiring of mitral cell dendrites


*Authors contributed equally

In the mouse olfactory bulb, sensory information detected by ~1,000 types of olfactory sensory neurons (OSNs) is represented by the glomerular map. The second-order neurons, mitral and tufted cells, connect a single primary dendrite to one glomerulus. This forms discrete connectivity between the ~1,000 types of input and output neurons. It has remained unknown how this discrete dendrite wiring is established during development. We found that genetically silencing neuronal activity in mitral cells, but not from OSNs, perturbs the dendrite pruning of mitral cells. In vivo calcium imaging of awake neonatal animals revealed two types of spontaneous neuronal activity in mitral/tufted cells, but not in OSNs. Pharmacological and knockout experiments revealed a role for glutamate and NMDARs. The genetic blockade of neurotransmission among mitral/tufted cells reduced spontaneous activity and perturbed dendrite wiring. Thus, spontaneous network activity generated within the olfactory bulb self-organizes the parallel discrete connections in the mouse olfactory system.
Reconstitution of Synaptic Plasticity In Vitro

PW. Liu, T. Hosokawa, and Y. Hayashi

Synaptic plasticity, such as long-term potentiation (LTP), is known to be the basis for learning and memory. The major mechanism to induce and maintain LTP is an increase of neurotransmitter receptors, like AMPA receptor, at postsynaptic site and postsynaptic density (PSD). Previously, our group revealed that not only AMPA receptor, but also various synaptic proteins translocate into postsynaptic structure in the response to LTP stimulation, calcium influx, and the enlargement of dendritic spine (structural LTP). However, how synaptic proteins translocate and accumulate into post-synaptic structure remains unclear.

It has been reported that mixing several purified PSD proteins results in the formation of PSD proteins condensate (Zeng et al., 2018). This condensate is in phase transition of free-diffusion and condensate, and it’s highly reversible. Using this system as in vitro reconstitution of PSD structure, we can demonstrate the reconstitution of protein translocation and accumulation into PSD during LTP.

We found that Calcium/calmodulin-dependent protein kinase II (CaMKII) plays crucial role for protein translocation and accumulation under calcium-dependent manner. Conformational change of CaMKII results in the exposure of its interacting site and enables CaMKII to interact and cross-link PSD proteins by using its dodecamer-structure. Adding CaMKII and calcium ion to the PSD condensates resulted in the enlargement and stabilization of PSD condensates. This result suggests that CaMKII plays important role during induction of LTP not only as a kinase but also as a structural protein. Furthermore, this mechanism would be related with synaptic dysfunction and the basal maintenance of synapse.
**Syngap1 Dynamically Regulates Experience-Dependent Cortical Circuit Reorganization**

N. Llamosas, T. Vaissiere, S. D. Michaelson, C. Rojas and G. Rumbaugh

A substantial portion of neurodevelopmental disorders (NDDs) are caused by genetic variants that disrupt brain function. However, the neurobiological processes that connect genetic pathogenicity to brain dysfunction remain unclear. Experience is known to reorganize cortical circuits through the refinement of synaptic inputs, which is believed to promote cortical functions relevant to cognition and behavior. Therefore, we hypothesized that genetic variants causally-linked to NDDs disrupt experience-dependent reorganization of synaptic inputs that comprise disease-relevant cortical circuits. To test this, we paired a whisker-trimming paradigm with serial *in vivo* two-photon imaging in somatosensory cortex (S1) to visualize experience-dependent changes in synaptic inputs and circuit activity in mouse models. First, we studied how *Syngap1* heterozygosity, which is a leading cause of sporadic NDDs, impacts the dynamic reorganization of axonal inputs onto S1 neurons. Serial imaging of axons in WT mice revealed an increase in the formation rate of presynaptic boutons in response to altered whisker experience. This form of experience-dependent structural plasticity was completely absent in *Syngap1* heterozygous mice. Second, we imaged whisker-evoked circuit activity before and after whisker trimming. *Syngap1* heterozygosity prevented both the scaling up of network activity and the remapping of discrete whisker inputs in S1. Remarkably, re-expression of the *Syngap1* gene in adult mice reactivated experience-dependent cortical circuit plasticity (scaling up and remapping of inputs). These findings demonstrate that an NDD risk gene dynamically regulates forms of cortical plasticity required for experience-dependent circuit reorganization. Genetic disruption of these forms of plasticity may contribute to cortical dysfunction and cognitive impairments observed in NDDs.
Fast Recruitment of Synaptic Vesicles at Cerebellar Mossy Fiber Terminals

T. Miki, and T. Sakaba

During neuronal activity, continuous synaptic vesicle release is required at presynaptic terminals firing at high frequency. As limited size of active zone (AZ) in presynaptic terminals and limited number of release site in AZ, vesicle recruitment is thought to be crucial for sustained vesicle release. However, it has been little known how fast the recruitment occur in such synapses. In this study, we investigated synaptic vesicle dynamics near presynaptic membrane in cerebellar mossy fiber (cMF) terminals, which can fire up to 1-kHz. We labeled synaptic vesicle by loading FM-dye in dissociated cMF terminals, and observed individual synaptic vesicle movement upon electrical stimulations using total internal reflection fluorescence (TIRF) imaging with simultaneous electrophysiological recording. FM dye-labeled vesicles appeared rapidly near plasma membrane upon 100-ms depolarization pulse with a time constant of 100-200 ms, showing that vesicle recruitment occur as fast as vesicle release represented by disappeared events in TIRF image. The rapid recruitment was abolished by applying actin polymerization inhibitor, LatrunculinA. Electrophysiological recording revealed that LatrunculinA also suppressed sustained release upon a depolarization pulse. These results suggest that fast recruitment occur synchronously with vesicle release, and contribute on sustained release at cMF terminals.
Principal rule of neurotransmitter receptor localization at synapse

T. Miyazaki, M. Morimoto-Tomita, K. Konno, Y. Noam, M. Verhage, M. Watanabe and S. Tomita

In the central nervous system, neurotransmitter receptors localize at postsynaptic sites, and mediate synaptic transmission upon binding to the transmitters released from presynaptic terminals. As a mechanism to localize neurotransmitter receptors at postsynapses, several categories of molecules have been proposed including scaffolding, cell adhesion, and secreted molecules. Despite several models proposed, a principle to maintain neurotransmitter receptors at postsynaptic sites remains unclear. Here we ask a relationship between synaptic formation and receptor localization. To address this, we have developed a new approach to ablate excitatory and inhibitory neurons precisely. Using these tools, we investigate a contribution of presynaptic terminals to localization of both excitatory and inhibitory receptors. We would like to present our recent findings.
Tools To Visualize Where And How Neuropeptides Modulate Neural Circuits

Mizuno, G. O., Davis, C.M.O., Patriarchi, T., Wong, S. J., Bouladian, S. and Tian, L.

Much of the focus of neuroscience has been on the rapid inputs and outputs of neurons, however the immense importance of neuropeptides in profoundly influencing neuronal and astrocytic function is now coming to light. The functional significance of their ability to modulate global brain processes such as arousal, attention, or emotion are now well recognized. Yet how they control the activity of local neurons in the brain—how they alter circuit dynamics or modulate behavioral states—is largely unknown. Therefore, we have developed a toolbox of genetically encoded indicators that enable direct, specific and long-term imaging of neuropeptide signals in target circuits during natural behavior. In combination with calcium/voltage imaging and optogenetics, the fast-growing toolbox of sensors for chemical transmission is well poised to permit direct functional analysis of how the spatiotemporal coding of chemical input signaling mediates the plasticity and function of brain circuits.
Epitope-tag knock-in mice revealed the localization and the competition of synaptic organizers in vivo


Synapses are precisely formed and regulated through the transsynaptic signaling mediated by synaptic organizers, such as neurexins (Nrxn) and neuroligins (Nlgn). Various synaptic organizers likely play specific roles by interacting with each other in a temporal and spatial manner. For example, we previously showed that Nlgn1 and Cbln1, another synaptic organizer released from cerebellar parallel fibers (PFs), compete for the binding to Nrxn containing splice site 4 (Nrxn(+S4)) in vitro (Matsuda et al., Eur J Neurosci, 2011). However, it remains largely unclear whether and how such interactions occur in vivo, partly because of the paucity of specific antibodies for the immunohistochemical detection of synaptic organizers in vivo. To overcome this problem, instead of raising specific antibodies, we generated knock-in mice, in which a hemagglutinin (HA) epitope was inserted in the Nlgn1 gene, to detect subcellular localization of endogenous Nlgn1 proteins in vivo (Nozawa et al., Cerebellum, 2018). We found that endogenous HA-Nlgn1 were highly enriched at synapses between cerebellar PFs and molecular-layer interneurons (MLI) where Nrxn(+S4) and Cbln1 are also enriched. Interestingly, while Nrxn(+S4) expressed in heterologous cells induced postsynaptic differentiation including the accumulation of Nlgn1 on dendrites of co-cultured neurons, the addition of recombinant Cbln1 to the medium inhibited this effect. We are currently addressing the functional significance of this competitive mechanism in vivo. Our study is expected to lead to better understanding how multiple synaptic molecules interact with each other to establish subcellular organization of mature synapses.
CPG15 Mediates Activity-Dependent Synaptic Plasticity In The Rodent Visual Cortex

D. Ordonez, S. Manole, M. Benoit, J. Subramanian and E. Nedivi

During developmental critical periods, patterned activity driven by experience refines neuronal circuits to form highly efficient networks. CPG15, the product of an activity-regulated gene, is a small glycosylphosphatidylinositol (GPI)-linked extracellular protein that has been implicated in synapse stabilization, axonal and dendritic arborization, as well as synaptic maturation. Recently, in vivo imaging of spine and synapse formation in CPG15 KO mice has revealed that while spine formation can occur normally in the absence of activity or CPG15, postsynaptic density protein 95 (PSD95) recruitment to nascent spines is deficient. Expression of CPG15 in the absence of activity is sufficient to restore normal PSD95 recruitment and spine stabilization, suggesting that PSD95 recruitment is the critical step in spine and synapse stabilization, and implicating CPG15 as the molecular mediator of activity-dependent synapse selection. Yet, the mechanism of action remains unclear. Using molecular and biochemical approaches, we probed the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor proteome and identified CPG15 as part of the protein complex that co-precipitates with AMPA receptor subunits. Preliminary structure-function analysis of CPG15 binding domain on AMPARs suggests that CPG15 binds the S1-S2 extracellular domains of AMPARs. In vitro imaging of AMPARs in cortical cultures of CPG15 KO mice suggest that CPG15 aids AMPARs stabilization and delays receptor turnover at postsynaptic density sites. Thus, we propose that in response to salient activity CPG15 binds AMPARs extracellularly and stabilizes them to promote intracellular recruitment of PSD95 to nascent spines and synapse maturation.
In vivo calcium imaging with a single cell resolution using “cosmoscope”, a new wide-field two-photon microscope


In neuroscience, in vivo functional imaging with a single-cell resolution over a wide field of view (FOV) is challenging. To accomplish this, we developed a wide-field two-photon microscope, which we named “cosmoscope”, that equips a very large objective lens with low magnification and a high numerical aperture. We evaluated the optical resolution and aberrations of this microscope by means of simulation. Eighty percent of the energy was contained within a radius of 1.1 µm in all FOV. This result was almost equivalent to the performance at the diffraction limit, indicating that this microscope possesses the high efficacy of two-photon excitation and high spatial resolution in all three axes across the entire field of view. To further evaluate this microscope, we performed in vivo calcium imaging of L2/3 and L5 cortical neurons expressing GCaMP in an awake mouse. GCaMP fluorescence was observed in the cytoplasm, not in the nucleus, providing confirmation that this microscope possesses single-cell resolution. We also developed a low computational cost cell detection (LCCD) algorithm (Ito et al., bioRxiv 502153), because the data size acquired by our microscope was so large that the previously reported algorithms could not detect neurons within a practical period of time. LCCD enabled us to extract more than 16,000 neural activities of L2/3 cortical neurons. Finally, we identified the functional map of the neurons. Whereas sensory stimulus-evoked activity was localized to the responsible region, movement-related activity was globally distributed. Cosmoscope will open the door to monitor a great multitude of single cortical neurons.
Subsynaptic positioning of AMPA-type glutamate receptors determines receptor activation

A. M. Ramsey, A. Tang, T. LeGates, S. Thompson, T. Biederer and T. A. Blanpied

Previously, our lab discovered that a protein called Rab3 Interacting Molecule (RIM), which is essential for neurotransmitter release, is clustered into ~100 nm subdomains within the active zone, and that vesicle exocytosis preferentially occurs where there is a higher subsynaptic density of RIM. Furthermore, these presynaptic sites of neurotransmitter exocytosis are aligned with postsynaptic nanoclusters of receptors. There are many potential mechanisms, however, a particularly attractive model is that synaptic cell adhesion molecules mediate alignment through trans-synaptic protein binding. Leucine Rich Repeat Transmembrane neuronal (LRRTM2) participates in trans-synaptic protein binding and is critical for basal AMPAR-mediated transmission. In order to test the ongoing role of LRRTM2 in established synapses, we adapted an approach to acutely disrupt LRRTM2 binding interactions in the cleft. Using dSTORM, we found that acute disruption of LRRTM2 results in a rapid reduction in the nanoscale alignment of proteins at synapses. Furthermore, we performed whole-cell patch clamp of cultured hippocampal neurons to test how acute disruption of the LRRTM2 extracellular binding interaction impacts synaptic transmission. We find that acute cleavage of LRRTM2 results in a substantial decrease in the evoked AMPAR-mediated EPSC amplitude. Together, these findings provide experimental support for the idea that trans-synaptic nanoscale organization plays an important role in maintaining synaptic strength. A structural role played by one or more specific cleft proteins provides further evidence for a molecularly guided “nanocolumn” architecture within the synapse. Broadly, these results also indicate that synaptic cell adhesion molecules can play specific and unexpected roles in regulating function at established synapses well after synaptogenesis.
Reciprocal activation within a kinase-effector complex underlying persistence of structural LTP

T. Saneyoshi

Long-term synaptic plasticity requires a mechanism that converts short Ca\(^{2+}\) pulses into persistent biochemical signaling to maintain the changes in the structure and function of synapses. Here, we present a novel mechanism of a positive feedback loop, formed by a “reciprocally activating kinase-effector complex” (RAKEC) in dendritic spines, enabling the persistence and confinement of a molecular memory. We found that stimulation of a single spine causes the rapid formation of a RAKEC consisting of CaMKII and Tiam1, a Rac-GEF. This interaction is mediated by a pseudoinhibitory domain of Tiam1, which is homologous to the CaMKII autoinhibitory domain. Therefore, Tiam1 binding results in constitutive CaMKII activation, which in turn, persistently phosphorylates Tiam1. Phosphorylated Tiam1 promotes stable actin polymerization through Rac1, thereby maintaining the structure of the spine during LTP. The RAKEC can store biochemical information in small subcellular compartments, thus potentially serving as a general mechanism for prolonged and compartmentalized signaling.
An optogenetic tool for acute modulation of inhibitory synapse function

S. L. Schwartz, B. L. Sinnen, W. C. Buchta, C. L. Tucker and M. J. Kennedy

Neuronal homeostasis is thought to be maintained through the dynamic regulation of synaptic strength and excitability. While much of the attention has focused on organization and dynamics of glutamatergic excitatory synapses, it is becoming increasingly appreciated that GABAergic inhibitory inputs serve to refine and tune cell activity in highly complex ways. To date, few genetically encoded, light activated approaches exist for directly manipulating synaptic function in ways that mimic natural plasticity mechanisms. Importantly, approaches that allow acute, local perturbation of natural circuit dynamics at the synaptic level will have high impact at both the cellular (e.g. investigating the role of inhibition in sculpting dendritic integration) and systems level (e.g. modeling neuropsychiatric disease). We use an optically-activated protein clustering system based on the Arabidopsis thaliana photoreceptor cryptochrome 2 (CRY2-Olig) fused to a FingR ‘intrabody’ against Gephyrin, Cry2-Olig GephIB, as a new approach for artificially tuning inhibitory synaptic strength that can be rapidly and locally activated with light. We find that light-induced clustering of Cry2-Olig GephIB leads to a rapid reduction in inhibitory synaptic transmission. This change in inhibitory synaptic strength disrupts neuronal E/I balance and leads to a robust increase in cell firing. We are currently investigating how clustering of gephyrin impairs inhibitory synaptic function, possibly through perturbing the nanoscale positioning of GABA\(_A\) receptors within the postsynaptic membrane.
Manipulation of fear memory association by posterior parietal cortex

A. Suzuki, S. Kosugi, E. Murayama, N. Ohkawa, M. Matsuo, H. Nishizono, and K. Inokuchi

The association of fear memory occurs when a conditioned stimulus (CS) is paired with an unconditioned stimulus (US). Although previous studies suggested that some of brain regions responded to CS and US signals, it is still unclear how the association regulates.

Here we show that the cellular ensemble in Posterior Parietal Cortex (PPC) specifically modulates CS-US association without the processing of CS and US information. In the modified context-pre-exposure facilitation effect (CPFE) paradigm, optical silencing of PPC neurons which responded to context exposure (CS), when mice received footshock (US) in the same context, failed to associate the context (CS) and the shock (US). On the other hand, optical activation of PPC neurons which responded to context exposure, when mice received footshock in a different context, generated an artificial CS-US associative memory, in which mice showed a freezing response in the initial context where mice did not received footshock, but not in the neutral context. Furthermore, 15 min optically silencing of PPC neurons that responded during reactivation of CS-US associate memory that has been once formed immediately after CS exposure 1 day after reactivation suppressed fear memory when mice were tested 1 day later without optical silencing. Thus, manipulating the PPC activity diassocate CS-US associative memory.
Neuronal synapses are intimately ensheathed by abundant astrocytic perisynaptic processes, which is critical for synapse formation and function. In contrast to well-studied neuronal synaptic compartments, however, the molecular mechanisms of how astrocytic perisynaptic structures govern neuronal synapses remain ill-defined. Here, we develop a new in vivo chemico-genetic approach, Split-TurboID-GRAPHIC, that uses a cell surface fragment complementation strategy combined with informatics to identify the molecules at astrocyte-synapse junctions in vivo. We identify more than 100 proteins enriched at astrocyte-neuronal junctions. We find novel adhesion molecules highly expressed in cortical astrocytes whose deletion dramatically alters excitatory/inhibitory synaptic balance and also impairs spatial learning. Using Split-TurboID-GRAPHIC we thus establish a new mechanism by which astrocytes coordinate inhibitory synaptic balance with excitation via a chemoa-affinity code of the tripartite synapse.
Neuronal dysfunction due to hyperexcitation under mild chronic cerebral hypoperfusion


Although behaviour analysis research has been reported that chronic cerebral hypoperfusion (CCH) causes cognitive dysfunction, the effects on actual neural activity have not been understood. Understanding these effects will provide critical information on therapeutic development of CCH. In this study, we investigated whether CCH from unilateral common carotid artery occlusion (UCCAO) directly affected spontaneous and evoked neuronal activity and oxygen metabolism using flavin fluorescence imaging and two-photon calcium imaging in awake mice. Brain glutamate (Glu) concentration was measured with magnetic resonance spectroscopy (MRS) in isoflurane-anesthetized mice. Our results revealed an increase in neural activation, oxygen metabolism, and brain Glu concentration from 2 to 7 days after UCCAO, followed by attenuation of neuronal activation from 21 to 28 days after UCCAO. Administration of the inhibitory neuromodulator taurine suppressed neuronal hyperexcitation in early stages and prevented the attenuation of neural activity in late stages. In conclusion, we demonstrate that CCH causes mild neuronal hyperexcitation, including increased neuronal activation, oxygen metabolism, and brain Glu concentration in the early stage. Further, we demonstrate the potential therapeutic effects of taurine on late neuronal dysfunction.
Enhancement of climbing fiber synchrony drives timed sensorimotor associations in the cerebellar cortex

S. Tsutsumi, O. Chadney, T-W. Yiu, E. Bäumler, L. Farragiana, and M. Häusser

The cerebellum has a well-known role in precise motor control, supported by climbing fiber inputs. At the same time, climbing fibers have been shown to robustly respond to sensory stimuli in the absence of motor outputs. However, how climbing fiber inputs coordinate sensory processing and motor timing remains unknown.

Here we probed how cerebellar cortex contributes to the rapid sensorimotor associations by performing two-photon calcium imaging to monitor population complex spikes and optogenetics to manipulate cortical activity in Crus I in head-fixed mice. Head-fixed mice were trained in a multisensory go/no-go task to lick during 0.5 s of paired somatosensory and auditory stimuli and withhold licking when presented with these stimuli individually.

Optogenetic activation of Purkinje cells showed that cerebellar cortical activity in Crus I during the sensory cue is crucial for temporally precise motor initiation in response to sensory stimuli, but not for the motor performance per se. Two photon calcium imaging from Crus I revealed that behaviorally relevant complex spike signals were organized into alternating parasagittal bands. The probability, latency and magnitude of synchronous complex spike signals within these bands discriminated sensory modality. Synchrony level of complex spikes within the salient bands was specifically enhanced during sensory-evoked motor actions, but not for voluntary movement. Synchronous complex spike signals in response to sensory stimuli were correlated with temporally precise motor initiation. These results suggest that enhancement of synchrony in sensory-evoked complex spikes in spatially organized Purkinje cells is critical for timed sensorimotor associations.
The dynamic interplay between microtubule-based motors and nuclear movement during neuronal migration

C. Zhou, Y.K. Wu, and M. Kengaku

During brain development, pre-mature cerebellar granule cells undergo tangential and radial migration from the neurogenic site to their final destinations, eventually forming the cortex layered structure and establishing neural circuits. One of the critical steps in this process is to translocate the nucleus towards the migration direction, which is determined by cytoskeletal coordination. We have recently shown that, unlike the previous view that the nucleus is transported by the minus-end motor dynein along uni-directional microtubules, microtubules are of mixed polarities and both dynein and kinesin are involved in generating nuclear movements (Wu et al., 2018). However, many questions still remain unanswered, including how different motors could achieve one-way net migration and how the tethering between nuclear envelop and microtubule is regulated. Therefore, this study aims to further elucidate the roles of microtubule motor molecules, kinesin and dynein, in moving the nucleus during granule cell migration. We attempt to characterize nuclear deformation events and to observe phenotypes upon kinesin or dynein inhibition with fast image acquisition (1 frame/s) in a reaggregate culture of granule cells (Umeshima et al., 2007). Our results confirm the involvement of kinesin in nuclear movement and provide support for the co-dependence model of kinesin-dynein-mediated nuclear transport.
Oral Presentations

All presenters in the Current Trends and Future Directions of Synapse-Circuit Plasticity Research, can use their personal laptops or the provided laptop for their presentation. The provided laptop is supported with Windows 10 and Office (PowerPoint) 2016. We also provided adaptors for MAC user below.

**keynote speakers**
60 minutes slot (50 minutes oral presentations, 10 minutes discussion)

**Invited speakers**
30 minutes slot (20 minutes oral presentations, 10 minutes discussion)

**Trainees speakers**
15 minutes slot (10 minutes talk, 5 minutes discussion)

Poster Presentation

The provided poster boards are vertical and measure 120 cm in width and 180 cm in height. The title and authors must appear at the top of the poster. Presenter should display their poster at the board # indicated at the Poster List.

**Poster Set up and removal times:**
Posting: Sep. 4th 13:00-17:00, Removal: Sep. 6th 9:00-12:00

**Poster Session times:**
Sep. 4th 17:00-18:30, 20:10-22:00(optional)
Sep. 5th 18:00-18:55, 21:10-22:00(optional)

Breakfast

Gotemba Kogen Hotel (invited speaker) 8:00-9:00
Hotel Brush-up (poster presentator; Room upgrade to single room) 6:45-7:45
Blueberry Lodge (poster presentator) 7:30-8:30
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Photography and video shooting are prohibited.

There is no money exchange or bank ATM at the Toki no Sumika. Cash is required at small shops and vending machines, so please exchange cash at airports.
Registration Packet: Registration List

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<td>2-1 Hirosawa, Wako, 351-0198, JAPAN</td>
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<tr>
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</tr>
</tbody>
</table>
Current Trends and Future Directions of Synapse-Circuit Plasticity Research

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きれいに撮れる“顕微鏡”から
成果につながる“イメージングシステム”へ
ニコンは豊富な顕微鏡技術をベースに、幅広い実験目的に応えて顕微鏡制御から画像取得・解析・データ管理までをサポートし、研究の成果につながるイメージングソリューションをご提供します。

画像取得
次世代共焦点レーザー顕微鏡システム
A1 HD25
A1 r HD25

ライプセル超解像顕微鏡システム
N-SiMS

業界最大のFOV（視野数）25により
広視野共焦点観察を実現。

従来の顕微鏡の約2倍の超解像（115 nm）を実現し,
最速15枚/秒の高速取得が可能。

解析・データ管理
画像統合ソフトウェア
ニコンの顕微鏡システムを制御し、画像の取得から解析、データ管理までイメージングのワークフローを総合的にサポート。

制御/画像取得
研究目的に合わせたシステムで、
高品質な画像を高効率で取得。

画像解析
取得した画像をNIS-Elements上で
そのまま解析、結果をモニターしながら
画像取得を行うことも可能です。

結果表示/データ管理
画像データと結果を総合的に管理。
その後の検証、詳細画像の取得が容易
に行えます。

ニコンの顕微鏡だから、イメージングから得られる情報を最大化。
共焦点レーザー走査型顕微鏡
FV3000シリーズ

新型分光システムTruSpectralと
冷却GaAsP PMTによる圧倒的な明るさ

高感度・高精度のTruSpectral分光システム
2nmの高分解能と優れた透過率を実現する透過型回折格子を検出系に採用。
従来と比べ蛍光の検出効率が大幅に向上。

高感度冷却GaAsP PMT
高い量子効率とベルチ冷却
によるノイズ低減、
高S/N実現するGaAsP PMT
を標準2Chで搭載。

超高速イメージング**や
広視野数18の高速イメージング**を
実現するレゾナントスキャナー

マクロ1.25倍からミクロ150倍、
分解能約120nmの超解像まで
広い倍率レンジでのシームレスなイメージング

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<th>波長 (nm)</th>
<th>FV3000</th>
<th>FV1200</th>
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**1: 非線性438fps, 512×32ピクセル
**2: 30fps, 512×512ピクセル

一般的な
スキャナーの
視野数

FV3000
視野数18, 8kHz

マウス脳切片
(1.25倍, ウェッシャイトイメージ)

非線形FV-OSRで細胞構造やシナプスを
さらに高解像に可視化
In-vivo volume imaging of neuronal activity requires both submicron spatial resolution and millisecond temporal resolution. While conventional methods create 3D images by serially scanning a diffraction-limited Gaussian beam, an alternative Bessel beam-based multiphoton imaging technique relies on an axially elongated focus to capture volumetric images. The excitation beam’s extended depth of field creates a 2D projection of a 3D volume, effectively converting the traditional 2D frame rate into a 3D volumetric rate. To highlight the power of this technique, Figure 1 shows a 300 x 300 μm scan of a Thy1-GFP-M mouse brain slice imaged with Bessel (left) and Gaussian (right) scanning. 45 optical slices taken with a Gaussian focus are vertically stacked to generate a volume image, while the same structural features are visible in a single Bessel scan taken with a 45 μm-long focus. This indicates a substantial gain in volume-imaging speed, making this technique suitable for investigating sparsely labeled samples in-vivo.

In partnership with Howard Hughes Medical Institute and Prof. Na Ji (University of California at Berkeley), Thorlabs is now offering a Bessel beam module for our Bergamo® multiphoton laser-scanning microscopes. As demonstrated in Ji’s pioneering work, this rapid Bessel beam-based imaging technique has synaptic resolution, capturing the Ca²⁺ dynamics and tuning properties of dendritic spines in mouse and ferret visual cortices. The Bessel beam module has also been used to successfully measure GCaMP6s expression in the subesophageal zone of a fly brain (Figure 2), synchrony of inhibitory neuron activity in the visual cortex of a mouse, and the network dynamics of reticulospinal neurons in the hindbrains of zebrafish larvae.

Extended Focus for In-Vivo Studies

Figure 2. GCaMP6s expression in two neuron pairs arbored within the subesophageal zone of a fly brain. Ca²⁺ transients are captured by XY-scanning a 36 μm Bessel focus at a rate of 30 frames per second. A single Bessel scan corresponds to an XY-scan with no translation in the Z-direction.

To learn more, visit www.thorlabs.com/bergamo, call 1-703-651-1700, or email ImagingSales@thorlabs.com.
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A New Standard for Chronic Experiments on Mice

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Stereotaxic Instrument
(for chronic experiments on mice)

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Two-photon Ca²⁺ Imaging

Laser Microscope

Head-fix

Without angle adjustment

With angle adjustment

Illustration and image of dendrites above by courtesy of Dr. Satoshi Manita, 2013, Murayama Lab. for Behav. Neurophysiology, BSI, RIKEN, Saitama, Japan

MAG-2
Head Holding Device
(with Angle Adjuster/ for Mice)

Illustration and image of dendrites above by courtesy of Dr. Satoshi Manita, 2013, Murayama Lab. for Behav. Neurophysiology, BSI, RIKEN, Saitama, Japan

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Wireless Optogenetics...

= Teleopt

Wireless Optogenetic Stimulator

For more details... www.TELEOPTO.com
Recommended routes from Narita Airport to Toki No Sumika

**Narita Airport station**

<table>
<thead>
<tr>
<th>Route #1</th>
<th>Route #2</th>
<th>Route #3</th>
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</thead>
<tbody>
<tr>
<td>JR, Narita Express 68 min, 3,190 yen</td>
<td>JR, Narita Express 85 min, 3,190 yen</td>
<td>Odakyu, Romancecar 100 min, 2,810 yen</td>
</tr>
<tr>
<td>Shinagawa Station</td>
<td>Shinagawa Station</td>
<td>Shinagawa Station</td>
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<tr>
<td>JR, Tokaido Shinkansen 47 min, 4,000 yen</td>
<td>Shinjuku Station</td>
<td>Shinjuku &amp; Hakone Line (For Hakone Togendai) 105 min, 1,680 yen</td>
</tr>
<tr>
<td>Mishima Station</td>
<td>Gotemba Station</td>
<td>Every 30 min</td>
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<tr>
<td>Free Shuttle 40 min</td>
<td>Free Shuttle or Taxi 25 min</td>
<td>Free Shuttle or Taxi 25 min</td>
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<td>Toki No Sumika</td>
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**Route #1**

**Route #2**

**Route #3**

Romancecar Timetable on 9/3

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<td>14:40</td>
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For JR train, you can buy tickets at the JR EAST Travel Service Center in Narita Airport. [https://www.jreast.co.jp/e/customer_support/service_center.html](https://www.jreast.co.jp/e/customer_support/service_center.html)

For Odakyu train, you can buy tickets at the Narita Int’l Airport Terminal1 JTB Travel Center in Narita Airport. [https://www.tourist-information-center.jp/tokyo/narita/en/](https://www.tourist-information-center.jp/tokyo/narita/en/)
Recommended routes from Haneda Airport to Toki No Sumika

**Haneda Airport International Terminal**

**Keikyu**
- 12 min, 410 yen

**Shinagawa Station**
- JR, Tokaido Shinkansen
- 47 min, 4,000 yen

**Mishima Station**
- Free Shuttle
- 40 min

**Haneda Airport Bus Terminal**
- Odakyu Hakone Highway Bus/ Keikyu Bus (For Hakone Togendai)
- 132 min, 2,060 yen
- Every 2 hours

**Gotemba Station**
- Free Shuttle or Taxi
- 25 min

**Route #1**
- Haneda Airport International Terminal → Shinagawa Station → Mishima Station → Toki No Sumika

**Route #2**
- Haneda Airport International Terminal → Haneda Airport Bus Terminal → Odakyu Hakone Highway Bus/ Keikyu Bus (For Hakone Togendai) → Gotemba Station → Free Shuttle or Taxi → Toki No Sumika

For JR train, you can buy tickets at the JR EAST Travel Service Center in Haneda Airport.
https://www.jreast.co.jp/e/customer_support/service_center.html
Changing Trains

**Shinagawa Station**

- **Keikyu**
  - or
  - **JR, Narita Express**
  - 13,14 platform

- **Shinkansen sign**
- **Follow the signs**

**JR, Tokaido Shinkansen**
- 23 24 platform

**Shinjuku Station**

- **JR, Narita Express**
  - 5, 6 platform

- **Odakyu, Romancecar**

- **Find Odakyu Line sign**
- **Follow the signs**
- **West exit is easy to change to Odakyu train**
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**JR Mishima Station**
**JR Gotemba Station**

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**Gotemba Kogen Hotel**
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