

Japan-U.S. Brain Research Cooperation Program
Information Exchange Seminar FY2013: Report

Field: _____ 2 _____

1. Seminar title: Current Trends and Future Directions of Synaptic Plasticity Research

2. Dates: 07/18/2013 – 07/20/2013

3. Location: University of Washington, Foege Building, Dept. of Genome Science,
Auditorium (S060)

4. Coordinators

Japanese Coordinator

Name: Yasunori Hayashi
Title: Senior Team Leader
Affiliation: RIKEN BSI

U.S. Coordinator

Name: Andres Barria
Title: Associate Professor
Affiliation: University of Washington

Name: Zheng Li
Title: Investigator
Affiliation: NIMH-NIH

Name: Karen Zito
Title: Associate Professor
Affiliation: UC Davis

5. Participants:

Japan: Invited participants 9 people Others 9 people

- RIKEN BSI • Senior Team Leader • Yukiko Goda
- University of Tokyo • Assistant Professor • Takashi Hayashi
- RIKEN BSI • Senior Team Leader • Yasunori Hayashi
- University of Tokyo • Professor • Haruo Kasai
- University of Tokyo • Professor • Shigeo Okabe
- Kyushu University • Associate Professor • Michiko Shirane
- Shinshu University • Professor • Tatsuo Suzuki
- Yokohama City University • Professor • Takuya Takahashi
- Keio University • Professor • Michisuke Yuzaki

U.S.: Invited participants 14 people Others 15 people

- University of Washington • Associate Professor • Andres Barria
- University of Maryland • Associate Professor • Thomas Blanpied

- MIT • Postdoctoral Fellow • Miquel Bosch
- Albert Einstein College of Medicine • Professor • Pablo Castillo
- Max Planck Florida Institute for Neuroscience • Research Group Leader • Hyungbae Kwon
- Johns Hopkins University • Associate Professor • Hey Kyoung Lee
- Cold Spring Harbor Laboratory • Associate Professor • Bo Li
- NIMH-NIH • Investigator • Zheng Li
- Brandeis University • Professor • John Lisman
- Georgia Regents University • Professor • Lin Mei
- Northwestern University • Associate Professor • Peter Penzes
- Duke University • Assistant Professor • Sridhar Raghavachari
- Max Planck Florida Institute for Neuroscience • Scientific Director • Ryohei Yasuda
- UC Davis • Associate Professor • Karen Zito

6. Seminar Outline and Significance:

The overall aim of this meeting is to bring together neuroscientists in the synaptic plasticity field from the US and Japan to identify outstanding major questions in the field and to discuss how to tackle them in the next 10 years. We will focus our discussions on: 1) New molecules and molecular signaling pathways important for synaptic plasticity; 2) Dynamics of molecules within micro-compartments of dendritic spines during synaptic plasticity; 3) New technologies that will contribute to the study of synaptic plasticity; and 4) Relevance of synaptic plasticity to neurological diseases and drug addiction.

1) New molecules and molecular signaling pathways

Mass spectrometric studies have identified hundreds of molecules in the postsynaptic density (PSD), a proteinous structure beneath the synapse. While many of them are specific to neurons, others are ubiquitously expressed. Obviously it is not possible to study all of these molecules individually, and therefore, we need to inspect the lengthy list of proteins with a clear idea of pathways involved in synaptic plasticity. Given the emerging importance of membrane trafficking, structural change of dendritic spines, pre- and postsynaptic communication, and protein synthesis and degradation in synaptic plasticity, we will discuss about both old and new molecules and signaling pathways involved in these processes.

Covalent modification of proteins, such as phosphorylation, glycosylation and lipid modification are also important for understanding the function of synaptic proteins. Such modifications can either directly modify protein functions or indirectly affect protein activities by changing protein-protein interaction or subcellular localizations (such as membrane tethering induced by lipid modification). In addition to various molecules, spines and dendrites contain various cellular organelles like proteosomes, polyribosomes, Golgi apparatus and mitochondria, which have been shown to play important roles in synaptic plasticity. We will discuss the significance of different signaling cascades regulating synaptic protein modifications and organelles involved in synaptic plasticity.

2) Dynamics of molecules within micro-compartments of dendritic spines during synaptic plasticity
The current central dogma of LTP/LTD is that AMPAR insertion into the synapse mediates LTP while its removal mediates LTD³⁵. However, AMPAR does not exist by itself; rather it is a part of large proteinous structures in synapses, termed postsynaptic density or PSD. When AMPARs are trafficked to synapses, what happens to other proteins associated with them at the PSD? Do these proteins also translocate? If they do, do they translocate in a particular order and how this translocation is regulated and/or modulated? Recent development of fluorescent proteins, such as GFP and related molecules, and the

super-resolution imaging technique, such as STED and FRET, allow us to visualize protein distribution and interactions in real time at nano-scales, well below the detection limit of conventional optical microscopy. Studies using these techniques have shown that AMPARs trafficking to synapses is just the tip of the iceberg in the molecular reorganization of the PSD. In fact, activity-dependent changes in the protein composition of synapses occur in large scale and involve many proteins with clear orderly rules of change. We will discuss about the recent findings of protein dynamics within micro domains of dendritic spines as well as the responsible signaling events.

3) New technologies that will contribute to the study of synaptic plasticity

Before modern microscopy technologies became available in the last ten years, electrophysiology was the only tool available to measure synaptic plasticity. It gives definite readouts of synaptic strength, but has limited power in the analysis of detailed molecular mechanisms at individual synapses. In particular, it measures only the end product in the modification of synaptic transmission; therefore it is ineffective as a tool to analyze the multiple signal transduction cascades working in parallel during the process of change. Recently, with the development of new optical imaging techniques, multiple new tools have become available to visualize synaptic plasticity as it occurs. Two-photon microscopy enables us to image the synapse with minimal photodamage in the brain slice 38, which is the same preparation used in electrophysiology. GFP allows us to depict the structure of synapse as well as localization of synaptic proteins 39. Photoactivatable-GFP has been used to visualize protein trafficking, and been combined with high-sensitivity camera for super-resolution imaging of single molecule dynamics within dendritic spines 36. This imaging method has revealed tread-milling of actin filaments within dendritic spines. Color variants of GFP allow not only dual labeling but also FRET for detection of biochemical changes or protein-protein interactions within the nano-domains of dendritic spines. The spatial resolution imaging has also been improved by photouncaging to stimulate the exact spine being visualized. In addition, technologies to control the activity of molecules, such as the use of LOV domain, are emerging. These technologies are being used, improved, and developed by many of the speakers invited and will be discussed in the workshop.

4) Relevance of synaptic plasticity to neurological diseases and drug addiction.

Recent studies have linked alterations in synaptic transmission and synaptic plasticity to neurocognitive disorders. First, molecules involved in synaptic plasticity have been identified as risk genes for neurocognitive disorders. These molecules include Shank, a group of large synaptic scaffolding proteins that along with Homer form a mesh-like structure serving as a framework for PSD, the postsynaptic cell adhesion molecule Neuroligin and its presynaptic counterpart Neurexin. Furthermore, genetically modification of Shank and other risk genes in animals recapitulates some of the symptoms of autistic human patients.

Second, synaptic transmission and plasticity are clearly altered during certain neuropathologies and drug addiction. For instance, it has been recently shown that in animal models of depression, excitatory synapses onto LHB neurons projecting to the VTA are potentiated. Synaptic potentiation linked to an enhancement of presynaptic probability of release correlates with the animal's helplessness behavior. Depleting transmitter release by repeated electrical stimulation of LHB afferents, a protocol effective for some depression patients markedly suppresses synaptic drive onto VTA-projecting LHB neurons in brain slices and significantly reduces learned helplessness behavior. Also, drug abuse can hijack synaptic plasticity mechanisms in the mesolimbic dopamine system, which is central to reward processing in the brain.

Many animal models for neurocognitive diseases exhibit altered synaptic plasticity. For example, knockout of Shank2, a gene implicated in autism and mental retardation, reduces both LTP and LTD in mice. FMRP knockout mice, a model for Fragile X-syndrome, have enhanced group I mGluR-dependent LTD. This finding has led to the idea of using an antagonist of group I mGluR in the treatment of Fragile X-syndrome. Hence, investigation of the molecular mechanisms underlying synaptic plasticity will be important for understanding the pathophysiology of neurological diseases and drug addiction.

Goal of the Workshop

7. Seminar Results and Future Implications:

We intend to stimulate discussions among US and Japanese scientists regarding the key questions in the field of synaptic plasticity. Specific Goals: to (1) exchange ideas and approaches; (2) build working hypotheses regarding the molecular mechanisms underlying synaptic plasticity and its significance for neuropathologies; (3) foster future collaborations; (4) determine priority areas of research; (5) establish areas of focus of US-Japan collaborative research.

As an organizer, we paid utmost attention to invite not only established researchers but also young researchers at assistant and associate professor class. Also we tried to invite researchers who shares common interest but with different technological approach. We asked a keynote speaker to cover not only about his own research but also about global view of the field.

We covered various experimental modalities to study synaptic plasticity from single molecule imaging, analyses of isolated molecule and cellular fractions, to imaging of live animal and as well as approaches in the slice to animal behavior. Examples of specific research collaboration include those between Yasuda lab (Max Planck Florida) and Hayashi lab (RIKEN BSI). Also, Hayashi could deepen existing collaboration with Blanpied (Univ. Maryland) by discussing over raw data face-to-face by scientists involving in the project.

I would like to conclude the report with my personal view of goal for research in next 10 years, based on the discussion during this meeting. We counted multiple presentations on visualization of molecular mechanisms underlying synaptic plasticity during this meeting. It is a great advance compared with past studies relied on mostly electrophysiological recordings of synaptic transmission. On the other hand, there were multiple studies focusing on neuronal assembly and synaptic plasticity in the live animal. Optical technique to suppress or potentiate synaptic transmission at single, identified synapse was also introduced. Furthermore, a technique to simultaneously visualize pre- and postsynaptic formations was also reported. It has not been proven whether synaptic plasticity can indeed explains memory. By combining these techniques, it would be possible to associate memory and plastic changes in particular synapse.

We counted 24 posters by graduate students and postdoctoral fellows, which provided opportunity not only to convey scientific message but to promote collaboration and postdoc opportunities.

8. Other (implementation issues, feedback, etc.)

We thank Japan-U.S. Brain Research Cooperation Program for support of this seminar. However, the amount of 1,700,000 JPY is simply not sufficient. Flight and hotel cost about 200,000 JPY per person. We had to look into additional funds and fortunately we could obtain fund from Integrative Brain Science Network (900,000 JPY), RIKEN (200,000 JPY), and various

companies (about 700,000 JPY). We still had to ask each laboratory to cover some of the cost. In future, more generous funding would be necessary to alleviate burden of applicant PIs.