Form 2-4-2

Japan-U.S. Brain Research Cooperation Program
Group Joint Study Project Program FY2011- FY2012: Report

Field: _ Cells and molecules_

1. Principal Researcher
   Name Ryuichi Shigemoto
   Title Professor
   Affiliation National Institute for Physiological Sciences
2. Project Title:

   Localization of membrane signaling proteins by SDS-FRL
3. Japanese Group
   Names, Titles and Affiliations of Principal Researcher and Collaborating Research Members
   Principal Researcher: Ryuichi Shigemoto, Professor, NIPS
   Collaborating Research Member: Katsuhiko Tabuchi, Associate professor, NIPS
   Collaborating Research Member: Ko Matsui, Assistant professor, NIPS
   Collaborating Research Member: Laxmi Kumar Parajuli, PhD student
   Collaborating Research Member: Dwi Wahyu Indriati, PhD student
4. U.S. Group
   Names, Titles and Affiliations of Principal Researcher and Collaborating Research Members
   Principal Researcher James Trimmer, Professor, University of California
   Collaborating Research Member Hannah Bishop, PhD student
   Collaborating Research Member Danielle Mandikian, PhD student
5. Research Period, from/to (mm/dd/yyyy) and total number of years.
   From 04/01/2011 to 03/31/2013, 2 years
6. Abstract, Results, and Research Significance (300 words):

The overall goal of the program is to combine the techniques of SDS-FRL in Shigemoto laboratory, with the collection of specific anti-Kv2.1 and Cav polyclonal and monoclonal antibodies generated in Trimmer laboratory, to define the localization of clustered and diffuse ion channels relative to the plasma membrane, and the intracellular Ca^{2+} signaling membranes. Trimmer's group has shown in studies at the light microscope level that neurons exhibit Kv2.1 with either clustered or diffuse localizations. Applying SDS-FRL to these samples allowed us to define the precise relationship of the clustered or diffuse ion channels to other neuronal membrane structures and associated molecules. Danielle visited Shigemoto lab for 4 months and found that Kv2.1 is strongly clustered in pyramidal cell and striatal medial spiny neuron somatic plasma membrane domains above subsurface cisitern, where Ryanodine receptors are clustered. AMIGO, an associated protein to Kv2.1 also showed similar localization. These results indicated an intriguing potential of Ca ions released from internal stores can modulate Kv2.1 through Ca dependent mechanisms such as kinase activation changing phosphorylation states of Kv2.1. Laxmi visited Trimmer lab for 3 months to screen useful antibodies for Cav for electron microscopy and found some useful Cav beta subunits antibodies. We found that Cav2.1 makes small clusters within presynaptic active zone in parallel fiber terminals in the cerebellum and Cav beta4 subunits make co-clusters with Cav2.1. We also found that Cav2.3 is diffusely distributed in medial habenular terminals in the interpeduncular nucleus and associated with beta3 subunits. In Purkinje cells, clustered but not scattered Cav2.1 were colocalized with Ca activated potassium channels, BK and SK2. There results suggest differential colocalization of alpha and beta subunits of Cav channels and that excitability of Purkinje cells is finely tuned by nanodomain interaction of Cav2.1 and calcium activated potassium channels. We further aim at determining the molecular mechanisms underlying regulating of Kv2.1 and Cav2.1 clustering in neurons exhibiting different levels of activity-dependent phosphorylation in our future studies.

7. Other (Research-related concerns, particular points of note):
   Nothing in particular.

*Please attach any reference materials as necessary.