◎ Form 2-4-2

Japan-U.S. Brain Research Cooperation Program
Group Joint Study Project Program FY2012 - FY2014: Report

Field: ③

1. Principal Researcher
   Name: Akihiro Yamanaka
   Title: Professor
   Affiliation: Research Institute of Environmental Medicine

2. Project Title: The analysis of neural mechanism which regulates instinctive behaviors using functional connectome

3. Japanese Group
   Names, Titles and Affiliations of Principal Researcher and Collaborating Research Members

   Principal Researcher:
   Akihiro Yamanaka, Professor, Research Institute of Environmental Medicine, Nagoya University
   Collaborating Research Members
   Kenji F. Tanaka, Associate Professor, Keio University
   Tomomi Tsunematsu, Ayumu Inutsuka, Postdoctoral Fellow, Research Institute of Environmental Medicine, Nagoya University
   Sawako Tabuchi, Graduate student, The Graduate University for Advanced Studies
   Arata Sakuramoto, Graduate student, Nagoya University

4. U.S. Group
   Names, Titles and Affiliations of Principal Researcher and Collaborating Research Members
   Principal Researcher:
   Edward S. Boyden, Associate Professor, Department of Biological Engineering, Massachusetts Institute of Technology
   Collaborating Research Members:
   Thomas S. Kilduff, Director, Biosciences division, Stanford Research Institute International
   Nao Chuhma, Assistant Professor, Department of Psychiatry and Pharmacology, Columbia University

5. Research Period : From Apr. 1, 2012 To Mar. 31, 2015 (3 Years)

6. Abstract, Results, and Research Significance (300 words):
Optogenetics is recently developed experimental technique to control the activity of specific type of neurons by illuminating specific wavelength of light. Although optogenetics enables control the activity of neurons with high time accuracy, it was difficult to control the activity of neurons for long period. Thus the aim of this project is to develop new protein for optogenetics and to generate new transgenic animal which expressing that protein to control the activity of neurons for longer period. Dr. Boyden developed new protein named Archaerhodopsin (ArchT). We generated new transgenic mice which express ArchT under control of tetracycline trans activator using originally developed KENGE-tet system (TetO ArchT mice). Orexin-tTA mice (generated by collaboration with Dr. Kilduff) were bread with TetO ArchT mice. Innunohistochemical study confirmed that ArchT was exclusively expressed in orexin neurons. Slice patch clamp confirmed that green light illumination inhibited generation of action potential up to 10 min. The next, the activity of orexin neurons

was controlled by in vivo. Fiber optics were bilaterally inserted into the hypothalamus. To monitor sleep/wakefulness state, electroencephalogram (EEG) and electromyogram (EMG) was monitored. Illumination of green light into hypothalamus through fiber optics for 1 hr in active period inhibited expression of c-Fos in orexin neurons suggesting that green light inhibited the activity of orexin neurons for long period. In this period, wakefulness was fragmented. This is specific symptom of narcolepsy which is caused by specific loss of orexin neurons. Inhibition of orexin neurons using optogenetics reproduced narcolepsy-like symptom in mice. We shipped these TetO ArchT mice to Dr. Chuma to collaborate further functional conectome experiments. Collaboration with Dr. Kilduff will be lasted for the next two years since he was selected for NIH US-Japan Brain Research program in 2015.

7. Other (Research-related concerns, particular points of note):

*Please attach any reference materials as necessary.

Papers related to this program.

◎ Tsunematsu T, Ueno T, Tabuchi S, Inutsuka A, Tanaka KF, Hasuwa H, Kilduff TS, Terao A, Yamanaka A (2014) Optogenetic manipulation of activity and temporally controlled cell-specific ablation reveal a role for MCH neurons in sleep/wake regulation. **J Neurosci** 34:6896-6909.

◎ Beppu K, Sasaki T, Tanaka KF, Yamanaka A, Fukazawa Y, Shigemoto R, Matsui K (2014) Optogenetic countering of glial acidosis suppresses glial glutamate release and ischemic brain damage. **Neuron** 81:314-320.

◎ Black SW, Morairty SR, Chen TM, Leung AK, Wisor JP, Yamanaka A, Kilduff TS (2014) GABAB agonism promotes sleep and reduces cataplexy in murine narcolepsy. **J Neurosci** 34:6485-6494.

◎ Tabuchi S, Tsunematsu T, Black SW, Tominaga M, Maruyama M, Takagi K, Minokoshi Y, Sakurai T, Kilduff TS, Yamanaka A (2014) Conditional ablation of orexin/hypocretin neurons: a new mouse model for the study of narcolepsy and orexin system function. **J Neurosci** 34:6495-6509.

◎ Tabuchi S, Tsunematsu T, Kilduff TS, Sugio S, Xu M, Tanaka KF, Takahashi S, Tominaga M, Yamanaka A (2013) Influence of inhibitory serotonergic inputs to orexin/hypocretin neurons on the diurnal rhythm of sleep and wakefulness. **Sleep** 36:1391-1404.

◎ Tsunematsu T, Tabuchi S, Tanaka KF, Boyden ES, Tominaga M, Yamanaka A (2013) Long-lasting silencing of orexin/hypocretin neurons using archaerhodopsin induces slow-wave sleep in mice. **Behav Brain Res** 255:64-74.

◎ Tanaka KF, Matsui K, Sasaki T, Sano H, Sugio S, Fan K, Hen R, Nakai J, Yanagawa Y, Hasuwa H, Okabe M, Deisseroth K, Ikenaka K, Yamanaka A (2012) Expanding the repertoire of optogenetically targeted cells with an enhanced gene expression system. **Cell Rep** 2:397-406.