

DANDRITE Topical Seminar by visitor Nami Ohmura

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Aud. D2, 2nd floor, building 1531, room 119 Dept. Mathematics, Aarhus University



Nami Ohmura

Division of Integrative Bioscience Tottori University Graduate School of Medical Sciences Japan

A novel electroporation technique to analyze fine neuronal structures in postnatal mammalian brain

Mammalian brain can alter its function and neural circuits in response to sensory experience during early postnatal life. For example, monocular deprivation (MD) causes physiological loss of cortical responses to a deprived eye and anatomical retraction of geniculocortical afferents serving the deprived eye. On the other hand, geniculocortical afferents serving an open eye show significant retraction when cortical neurons are pharmacologically inhibited. Thus, an uncorrelated activity between pre and post synaptic neurons leads to the pruning of afferent axons. To investigate the mechanisms of axonal pruning, which had been found in cats, mouse model is useful because genetic manipulation is easily available. Therefore, I determined whether pruning of thalamocortical axons in the inhibited visual cortex takes place in mice. I injected an anterograde neural tracer, biocytin into the lateral geniculate nucleus (LGN) and measured the density of labeled afferent axons in the visual cortex. The amount of thalamocortical axons in the pharmacologically inhibited cortex of mice showed a decrease in visual input dependent manner as observed in cats.

In these experiments, I was faced with a difficulty to analyze single axonal morphology of mouse thalamocortical afferents using conventional tracer labeling for their thinness and high density. To solve this problem, I have established a novel electroporation method to transfer genes into a few neurons in the target area which is identified electrophysiologically in *in vivo* postnatal animals (Ohmura et al., *Brain Struct Funct.* 2014). I recorded the neuronal activity to identify the location of the LGN using a glass-pipette electrode which contains the plasmid DNA encoding green fluorescent protein (GFP). After confirming the location of the LGN by monitoring visual responses, I pressure-injected the plasmid solution into the recording site and applied voltage pulses through the glass-pipette electrode. I found a few labeled somata and dendrites in the thalamus after several days, and labeled axons in the cortex after 1-2 weeks. This technique allows visualization of the fine structure of neuronal processes of single-neuron to explore the neuronal circuit morphologically in various animals.

Host: Group Leader Keisuke Yonehara, DANDRITE