(Form 3)

Summary of Doctoral Thesis

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Title: Structural dynamics and stability of corticocortical and thalamocortical axon terminals during motor learning

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Synapses consist of presynaptic and postsynaptic sites, and are indispensable prerequisites for communication between neurons. Functionally, synapses can change the strength of their chemical transmission by processes such as modification of the probability of transmitter release and varying the number of postsynaptic glutamate receptors. Anatomically, synapses can be newly formed, eliminated, or undergo a change in size. Such functional and/or structural changes are termed synaptic plasticity, and form the cellular basis of learning and memory. In the majority of excitatory synapses in the mammalian brain, axonal boutons contact with dendritic spines, and the size of the dendritic spines correlates strongly with the excitatory postsynaptic response. Many spines are maintained for a long period of time and probably play critical roles in lifelong memory storage and the stability of neural circuits. By contrast, a subset of spines are dynamic; they can be newly formed, eliminated, or changed in size, and such structural plasticity probably plays critical roles in memory formation and extinction, homeostasis, and reorganization of neural circuits. In fact, motor learning induces synaptic plasticity in M1. *In vivo* two-photon imaging studies in rodents revealed that dendritic spines in M1 dynamically change during motor learning, and that the stabilization of a subset of layer 1 (L1) spines of L5 pyramidal neurons, which are newly formed in the early stage of learning, is relevant to improvement in motor performance.

To understand motor-learning-induced circuit plasticity, it is important to describe the bouton plasticity of long-range projection axons; however, it is poorly understood how presynaptic axonal boutons are formed, eliminated, and maintained during motor learning, and whether long-range corticocortical and thalamocortical axonal boutons show distinct structural changes during

learning. Therefore, the present study aims to unravel the axonal bouton dynamics occurring during motor learning. In particular, because previous studies have revealed that secondary motor cortex (M2) and motor thalamus are essential for motor learning, the current study focused on M2–M1, and thalamocortical connections and their plasticity.

Initially, I anatomically confirmed that L1 in M1 receives axonal projections from M2 and the thalamus. By using retrograde tracer, M2 and motor thalamus were identified as the major input sources. Next, long-range axons originating from M2 and the thalamus were separately labeled with adeno-associated virus (AAV) encoding different colors of fluorescent protein, and two-photon microscopy was used to pursue the structural plasticity of these boutons in M1 during learning of an accelerating rotarod task. Over seven consecutive days of motor training, M2 and thalamocortical boutons showed distinct dynamics: M2 boutons showed an increasing formation rate, whereas thalamocortical boutons showed a decreasing elimination rate and increasing survival fraction during learning. In addition, pre-existing thalamic boutons showed a slight increase in their sizes in the early stage of learning. This may reflect that a small subset of the pre-existing thalamic boutons, including the enlarged boutons, may make contact with newly formed spines during the early stage of learning. Taken together, these results suggest that the late stabilization of thalamic boutons in M1 contributes to motor skill learning.

Although the current study has described learning-related axonal bouton plasticity, there are some difficulties in interpreting the results of the experiment. One obstacle is that presynaptic bouton plasticity and postsynaptic dendritic spine plasticity do not occur in a one-to-one manner; for instance, previous electron microscopy studies have revealed that newly formed dendritic spines frequently make synapses with pre-existing axonal boutons. Therefore, to understand pathway-specific structural plasticity, presynaptic and postsynaptic sites should be simultaneously labeled. To achieve this goal, the present study took advantage of the mammalian green fluorescence protein reconstitution across synaptic partners (mGRASP) technique, which is known to label precisely the actual synaptic pairs. In this method, non-fluorescent split-GFP fragments are inserted into the synaptic membranes, and reconstituted GFP fluorescence can be detected in

synaptic pairs. Optimization of the vector constructs allowed mGRASP signals to be detected with *in vivo* two-photon microscopy. Although, this study is a preliminary attempt at the methodology, the technique may be useful to describe learning-induced synaptic remodeling in specific synaptic pairs *in vivo*.