DIVISION OF BRAIN CIRCUITS †

Professor
MATSUZAKI, Masanori

Assistant Professor:	*HIRA, Riichiro*
	MASAMIZU, Yoshito
Technical Staff:	*OHSAWA, Sonoko*
NIBB Research Fellow:	*EBINA, Teppei*
Postdoctoral Fellow:	*TANAKA, Yasuhiro*
	TANAKA, Yasuyo
	KONDO, Masashi
	SHINOTSUKA, Takanori
	TAKEDA, Yuta
SOKENDAI Graduate Student:	*OHKUBO, Fuki*
	HASEGAWA, Ryota
	SUZUKI, Ayami
Visiting Graduate Student:	*TERADA, Shin-Ichiro*
Visiting Scientist:	*HIRAKAWA, Reiko*
Technical Assistant:	*ATSUMI, Urumi*
	OHARA, Kaori
	KOTANI, Keiko
	TAKAHASHI, Yoichi
	IWASE, Etsuko
	IMOTO, Eiko
Secretary:	*SUGIYAMA, Tomomi*

Various firing patterns of many cortical neurons represent information processing in the brain. The microarchitecture of synaptic connections control information processing in cortical circuits. The structure and location of synapses determine and modify the strength of this information processing. The aim of our laboratory is to reveal how information is formed, maintained, selected, and decoded in the brain at the levels of single cells and of single synapses. To do so, we mainly use two-photon microscopy that allows us to see fluorescence signals from deep within living tissue, developing novel photostimulation methods and animal behavioral tasks. The goals of our recent studies are to reveal how voluntary movement is memorized and represented in cortical circuits. In addition, we are working to apply two-photon microscopy to a non-human primate, the common marmoset, in order to understand information processing in the brain, which is relevant to high cognitive functions.

I. Measurement of thalamocortical activities in the primary motor cortex during voluntary movements.

The primary motor cortex (M1) integrates a variety of information from other brain regions and transmits motor commands to the spinal cord. Information from the basal ganglia and cerebellum, which is thought to be critical for motor learning and motor execution, is signaled to the M1 through the thalamus. However, little is known about the pattern of thalamocortical activities in the M1 during self-initiated voluntary movements. Thus, we have examined activity patterns in layer 1 (L1) and 3 (L3) thalamocortical axons in the M1, which presumably receive information from the basal ganglia and cerebellum, respectively. We conducted two-photon calcium imaging of L1 and L3 thalamocortical axons in the mouse forelimb M1 during a lever pull task (Hira et al., 2013; Masamizu et al., 2014). We found that the timing of peak activity in L1 thalamocortical axons was sequentially distributed as a population during lever-pull movement. By contrast, the peak and onset timings of the activity in L3 thalamocortical axons were mainly seen at lever pull initiation. During learning, the population of L1 TC axonal boutons obtained sparse lever-relevant neural code, while the population of L3 TC axonal boutons evolved to be robust. Optogenetic experiments showed that the thalamocortical axons have the ability to induce a forelimb movement and modify lever pull movement. Our results indicate that L1 and L3 thalamocortical activities convey distinct information required for learning of voluntary movements.

The axon imaging described above is much more vulnerable to the motion of the imaging plane than imaging of cell bodies because of the small diameters (less than 1 μm) of axons. Especially, motion in the normal direction to the imaging plane (z-motion) often causes artefactual changes in fluorescence intensity of axons. These changes can be confounding in interpreting fluorescence transients observed in axons of awake active animals. To solve this problem, we developed a novel registration method with z-motion correction for axon imaging. In the mouse motor cortex, four-dimensional images of axons expressing fluorescent protein were obtained with two-photon microscopy equipped with a piezoelectric objective lens positioning system. Then, the imaging data were analyzed off-line. At every time point, two of three z-planes were blended at an appropriate ratio to obtain a composite plane similar to the target, the temporal average image of second z-planes. The x-y displacements of two blended images and their blending ratio were determined using a particle filter. In the particle filter, each of three parameters decomposed to two system variables, one obeying a second-order trend model and another a third-order auto-regressive model and system noise was generated from Gaussian or Cauchy distribution. The resample weight for each particle was calculated with the correlation between each composite image and the target. The fluorescence intensity of axons in obtained time series of composite planes was significantly less deviated than that in original images. By using this method, we were able to analyze the axonal imaging data.

II. Wide-field two-photon microscopy for simultaneous imaging of multiple cortical areas at cellular resolution

Understanding the dynamics of cortico-cortical communications is essential because information processing in the brain is not only performed in intra-areal circuits but also through inter-areal interactions. To execute voluntary movements, the interactions between M1 and the secondary motor area (M2) and between M1 and sensory-associated areas are crucial. Recent advances in two-photon microscopy have allowed us to image a relatively large area (up to 1 mm) at cellular resolution. However, it is still difficult to image a continuous large field (>2 mm) and two distant (>3 mm) brain areas at cellular resolution using a single two-photon microscope. We developed super-wide-field two-photon microscopy with

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a single objective, which allows the imaging of two distant (up to 6 mm apart) cortical areas and a large continuous area (up to 3 mm) at cellular resolution. The method depends on placing a novel optical device under a high-NA objective with a long working distance in a standard two-photon microscope. The device is composed of a pair of mirrors and a holder to rotate the mirrors perpendicular to the optical axis. By controlling the rotation timing and angle using custom-made software, the field of view can be rapidly switched without moving either the objective or the sample.

By rotating the mirror pair back and forth between two angles, we conducted sequential two-photon calcium imaging of neuronal activities in two distant areas up to 6 mm apart and at a depth of up to 800 μm from the cortical surface. Furthermore, by stitching the fields of view, we succeeded in imaging a 3 mm × 1 mm continuous area. We applied it to concurrent calcium imaging of layer 2/3 and layer 5 neurons in rostral (M2) and caudal (M1) motor cortical areas while the mice performed a lever-pull task. We are analyzing how the neural activities in these fields are coordinated during motor execution. Importantly, the optical device together with the controller can be easily installed on a standard two-photon microscope. Its adaptation by neuroscientists should open the door to the study of information processing in brain networks at cellular resolution.

III. Marmoset forelimb-movement tasks for two-photon Ca^{2+} imaging of the motor cortex

Recent advances in calcium imaging have revealed cellular and subcellular mechanisms underlying a variety of brain functions in rodents, fishes, and invertebrates. However, the calcium imaging technique is still difficult to apply to awake non-human primates, especially during forelimb movement tasks, which are very useful for investigating the mechanisms underlying cognitive behaviors, decision making, motor planning/execution and motor skill learning. We have extended the technique of two-photon calcium imaging, which we established in a non-human primate, the common marmoset, in an anesthetized state (Sadakane et al., 2015), to record neuronal activity in the cerebral cortex of behaving marmosets.

To do so, we developed a novel lever-manipulation device with a chair that restrains the head and body, but not forelimbs. Three marmosets were trained with the device 10–60 min per day for 2–3 weeks. The marmosets successfully learned a self-initiated lever-pull task, in which the animals have to pull the lever to get a reward. The marmosets also learned a visual cued lever-pull task, in which the reward is delivered only when the cue is presented on the monitor and the lever is pulled, with additional 1–2 weeks of the training. We conducted two-photon calcium imaging in M1 of the marmosets during the task, using novel marmoset-specific two-photon microscopy, which allows us to observe any dorsal cortical area in marmosets sitting on the chair. We detected calcium transients responding to forelimb movements from somata and dendrites.

Overall, these results indicate that forelimb movement tasks for head-restrained marmosets are feasible and that neural activities can be monitored in the neocortex of behaving marmosets with cellular and subcellular resolution over days by two-photon calcium imaging.

Publication List:

〔Original paper〕

- Terada, S., Matsubara, D., Onodera, K., Matsuzaki, M., Uemura, T., and Usui, T. (2016). Neuronal processing of noxious thermal stimuli mediated by dendritic Ca^{2+} influx in *Drosophila* sensory neurons. eLife *5*, e12959.