DIVISION OF BRAIN CIRCUITS

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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 aim of our recent study is to reveal how voluntary movement is represented in cortical circuits. One of the most important problems in neuroscience is how a variety of spatio-temporally heterogeneous neural activity in the cortex emerges moment-by-moment at multiple stages of a movement.

I. Development of a novel operant task of head-restrained mice

To carry out two-photon calcium imaging while mice performed a self-initiated movement, we developed a head-restrained lever-pull task (Figure 1). Mice used their right forelimbs to pull the lever for a given amount of time and were subsequently rewarded with a drop of water from a spout near their mouth. With the reward at the cessation of the lever pull, a magnet-controlled solenoid pole would quickly push the lever back to the wait position. The mice then had to wait 3 s before they could receive another reward for a lever pull. During 8-9 days of the training sessions, the task difficulty was increased by gradually increasing the lever-pull time (100–1,000 ms) and mice were able to either increase or maintain the number of successful trials. Furthermore, the interval time between successful trials decreased, indicating that mice successfully learned how to pull the lever and also understood that they had to wait >3 s to pull the lever again and obtain another reward.

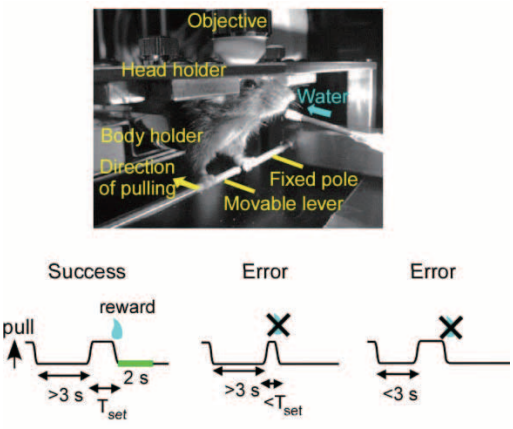

Figure 1. The lever-pull task in head-restrained mice. The head of the mouse was fixed under an objective, and the right forelimb was available to grasp and pull the movable lever. Before pulling the lever, mice had to wait for more than 3 s. After the lever pull was maintained for T_{set}, a drop of water was dispensed.

II. Identification of the mouse motor forelimb areas with photostimulation mapping

We used channelrhodopsin-2 (ChR2) mice to determine the forelimb motor areas over the broad neocortical surface by optogenetic stimulation mapping (OSM) using blue-laser scanning of the cortical surface. Laser illumination induced forelimb movements in two distinct areas: RFA (the rostral area) and CFA (the caudal area) (Figures 2). These motor forelimb areas corresponded to those reported by intracortical microstimulation in the rat and mouse cortex.

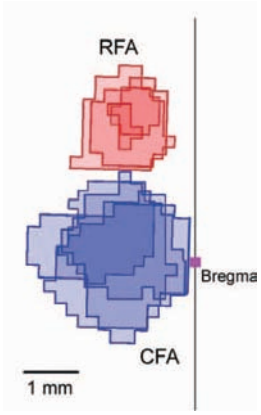

Figure 2. Dorsal view of left cortical hemisphere. Overlaid red and blue contours show the RFA and CFA, respectively, as revealed by OSM.

III. Spatio-temporal representation of motor information in the brain

We performed two-photon calcium imaging in layer 2/3 of the left RFA or CFA while mice executed the task (Figure 3). Motion-corrected calcium transients reflected spiking activity, and particularly the burst firing of individual cells. We found two types of task-related cells in the mice: cells whose peak activities occurred during lever pulls (pull cells) and cells whose peak activities occurred after the end of lever pulls (post-pull cells). The activity of pull cells was strongly associated with lever-pull duration. In approximately 40% of imaged fields, functional clusterings were temporally detected during the lever pulls. Spatially, there were ~70 μm-scale clusters that consisted of more than four pull cells in approximately 50% of the fields. Ensemble and individual activities of pull cells within the cluster more accurately predicted lever movement trajectories than activities of pull cells outside the cluster. This was likely because clustered pull cells were more often active in the individual trials than pull cells outside the cluster. This

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

higher fidelity of activity was related to higher trial-to-trial correlations of activities of pairs within the cluster. We propose that strong recurrent network clusters may represent the execution of voluntary movements.

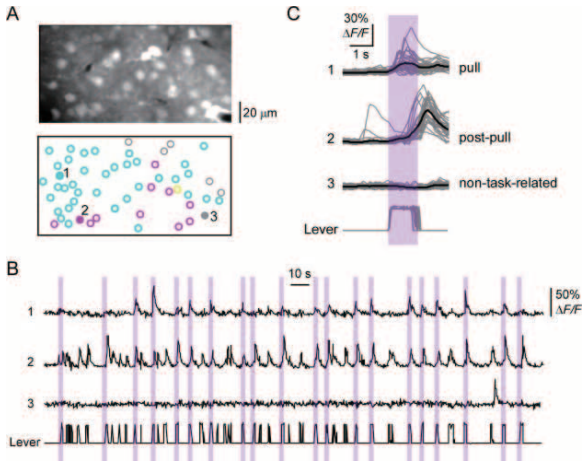

Figure 3. Activities of layer 2/3 motor cortex cells during task performance. A. A representative example of a two-photon imaged field in the RFA. Circles surround reconstructed neurons. Parts of fluorescent traces of numbered, closed circles are shown in (*B*). Cyan, magenta, yellow, and grey circles indicate pull cells, post-pull cells, other task-related cells, and non-task-related cells, respectively. B, 270-s traces of motion-corrected calcium transients of the numbered cells shown in (***A***). The trace indicated as Lever shows the lever trajectory. Shaded boxes indicate successful trials. C, The motion-corrected traces of cells 1, 2, and 3 are aligned with the start of all 30 successful lever pulls during the imaging session. Black thick lines indicate the mean traces. The lever trajectories in individual trials are overlaid in the bottom trace.

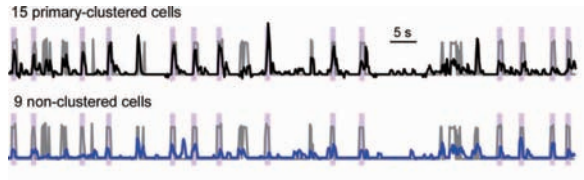

Figure 4. Prediction accuracy of the lever movement trajectory relative to neural ensemble activity of pull cells. The linear model prediction of the lever trajectory (grey) from 15 primary-clustered cells (black) and nine non-clustered cells (blue) in the imaged field shown in Figure 3. One of the test segments of the trajectory is shown. The correlation coefficients between the predicted and real trajectories for this segment were 0.55 and 0.40 in the 15 primary-clustered and nine non-clustered cells, respectively.

Publication List

〔Original paper〕

- Kimura R., Saiki A., Fujiwara-Tsukamoto Y., Ohkubo F., Kitamura K., Matsuzaki M., Sakai Y., and Isomura Y. (2012). Reinforcing operandum: rapid and reliable learning of skilled forelimb movements by head-fixed rodents. J. Neurophysiol. *108*, 1781-1792.