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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 goals of our recent studies are 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. Fine-scale spatio-temporal dynamics of the neuronal activity in mouse motor areas during a voluntary movement

Functional clustering of neurons is frequently observed in the motor cortex. However, it is unknown if, when, and how fine-scale (<100 μm) functional clusters form relative to voluntary forelimb movements. In addition, the implications of clustering remain unclear. To address these issues, we conducted two-photon calcium imaging of mouse layer 2/3 motor cortex, the rostral forelimb area (RFA) and the caudal forelimb area (CFA), during a self-initiated lever-pull task. In the imaging session, after 8-9 days of training, head-restrained mice had to pull a lever for ~600ms to receive a water drop, and then had to wait for > 3s to pull it again. 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 (primary cluster in Figure 1). 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 higher fidelity of activity was related to higher trial-to-trial correlations of activities of pairs within the cluster (Figure 2). We propose that strong recurrent network clusters may represent the execution of voluntary movements.

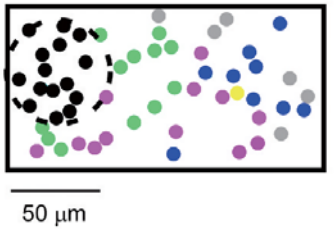

Figure 1. An example of imaged fields in the mouse motor areas. Black, green, blue, magenta, yellow, and grey circles indicate primary-clustered pull cells, other clustered pull cells, non-clustered pull cells, post-pull cells, other task-related cells, and non-task-related cells, respectively. Dotted circles surround the primary clusters.

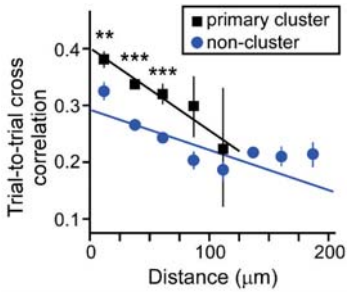

Figure 2. Trial-to-trial cross correlations relative to cellular distances for 1075 pairs of primary-clustered cells (black squares) and 1680 pairs of non-clustered cells (blue circles) across 30 fields that included the primary cluster and ≥3 non-clustered cells (16 mice). Regression lines are shown in the same colors as the cluster types. ** $p < 0.001$ and *** $p < 0.0001$.

II. *In vivo* optogenetic tracing of functional corticocortical connections between motor forelimb areas

Interactions between distinct motor cortical areas are essential for coordinated motor behaviors. In rodents, the motor cortical forelimb areas are divided into at least two distinct areas, the RFA and the CFA. The RFA is thought to be an equivalent to the premotor cortex in primates, whereas the CFA is believed to be an equivalent to the primary motor cortex. Although reciprocal connections between the RFA and the CFA have been anatomically identified in rats, it is unknown whether there are functional connections between these areas that can induce postsynaptic spikes. We used an *in vivo* Channelrhodopsin-2 (ChR2) photostimulation method to trace the functional connections between the mouse RFA and CFA. This was done in either ChR2 transgenic mice, where the L5b neurons express ChR2-EYFP, or in mice where both the upper layers and L5b were transfected with an adeno-associated virus that encoded ChR2-EYFP. Simultaneous electrical recordings were utilized to detect spiking activities induced by synaptic inputs originating from

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2013. The former title is indicated by an asterisk (*).

photostimulated areas (Figure 3). This method demonstrated that neurons in the upper layers, but not L5b, of the CFA induce strong postsynaptic responses in L5 of the RFA, and that RFA neurons in L5b induce strong postsynaptic responses in L5 of the CFA. The onset latency of electrical responses evoked in remote areas upon photostimulation of the other areas was approximately 10 ms, which is consistent with the synaptic connectivity between these areas.

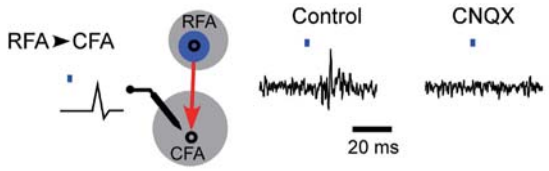

Figure 3. Left, a schematic of the experimental arrangement for photostimulation (blue) of the RFA (gray) with simultaneous electrical recording in the CFA. RFA photostimulation evoked postsynaptic firing activities in the CFA via the synaptic projection (red). Right, representative data showing evoked activities in the CFA when the RFA was photostimulated. Control conditions and application of CNQX to the CFA are shown.

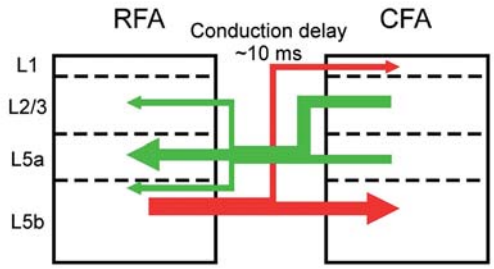

Figure 4. Asymmetric synaptic connectivity between the RFA and the CFA.

We also determined the anatomical connections between the RFA and the CFA. The anatomical results supported the idea that the RFA receives strong functional projections from layer 2/3 and/or layer 5a, but not from layer 5b, of the CFA. Further, the CFA receives strong projections from layer 5b neurons of the RFA. Our results suggest that neuronal activities in the RFA and the CFA during movements are formed through asymmetric reciprocal connections (Figure 4).

III. *In vivo* photoinhibition mapping method using halorhodopsin

We are developing *in vivo* photoinhibition methods as well as the *in vivo* photostimulation method using ChR2. We examined whether the Thy1-eNpHR2.0 (enhanced halorhodopsin) transgenic mice are suitable for *in vivo* photoinhibition. For this purpose, we illuminated the motor cortex of these mice with green light (559 nm wavelength) while measuring neuronal activity and forelimb movement. Spontaneous multiunit activity (MUA) was recorded from a single electrode that was inserted into the motor cortex. Illumination near the tip of the electrode clearly inhibited spontaneous MUA (Figure 5), with a rapid recovery of activity afterwards. In contrast, illumination 1 mm away from the recording site had no effect on MUA. To examine the spatial range of photoinhibition mediated by eNpHR2.0 *in vivo*, we measured upward local field potentials (LFPs) that presumably reflect chloride ion influx into eNpHR 2.0-expressing neurons (Figure 5). Illumination near the tip of the recording electrode (location 1) induced large LFPs, while illumination at more distant sites produced smaller LFPs (locations 2 and 3). By scanning the position of the light spot, while measuring LFPs, we could make a two-dimensional map of the spatial range of photoinhibition (Figure 5). The width of the area exhibiting light-induced LFPs was 0.65 mm in the experiment shown in Figure 5.

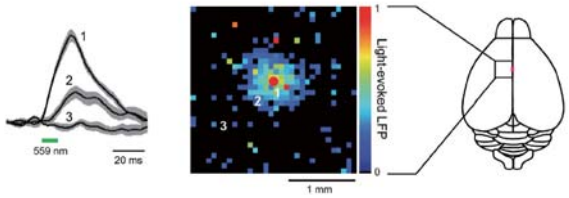

Figure 5. Left, LFPs caused by activation of eNpHR2.0, recorded at the location indicated by the red circle in the middle, in response to light spots positioned at the numbered locations in the middle. Middle, map of amplitudes of LFPs evoked when eNpHR2.0 was activated. Each of the 32 x 32 pixels in the map was illuminated (559 nm) and the amplitude of the LFP evoked at each pixel was encoded into the pseudocolor scale shown at right. Right, schematic dorsal view of the cortical surface; boxed region is the photostimulation mapping area and the magenta square denotes the bregma.

We also examined *in vivo* photoinhibition of limb movements induced by intracortical microstimulation in Thy1-eNpHR2.0 mice. Stimulation of the right motor forelimb area in the motor cortex produced movements of the left forelimb. Whole-field illumination of the right cortical surface with orange light (594 nm) clearly inhibited left forelimb movement and movements were restored rapidly once the light was turned off. These results show that cortical activity and limb movement can be photoinhibited *in vivo* using the Thy1-eNpHR2.0 mouse, indicating that this mouse is an excellent tool for disruption of neural circuit activity *in vivo*.

Publication List

〔Original papers〕

- Asrican, B., Augustine, G.J., Berglund, K., Chen, S., Chow, N., Deisseroth, K., Feng, G., Gloss, B., Hira, R., Hoffmann, C., Kasai, H., Katarya, M., Kim, J., Kudolo, J., Lee, L., Lo, S., Mancuso, J., Matsuzaki, M., Nakajima, R., Qui, L., Tan, G., Tang, Y., Ting, J.T., Tsuda, S., Wen, L., Zhang, X., and Zhao, S. (2013). Next-generation transgenic mice for optogenetic analysis of neural circuits. Front. Neural Circuits *7*, 160.
- Hayama, T., Noguchi, J., Watanabe, S., Takahashi, N., Hayashi-Takagi, A., Ellis-Davies, G.C.R., Matsuzaki, M., and Kasai, H. (2013). GABA promotes the competitive selection of dendritic spines by controlling local Ca^{2+} signaling. Nature Neurosci. *16*, 1409-1416.
- Hira, R., Ohkubo, F., Ozawa, K., Isomura, Y., Kitamura, K., Kano, M., Kasai, H., and Matsuzaki, M. (2013). Spatiotemporal dynamics of functional clusters of neurons in the mouse motor cortex during a voluntary movement. J. Neurosci. *33*, 1377-1390.
- Hira, R., Ohkubo, F., Tanaka, Y.R., Masamizu, Y., Augustine, G.J., Kasai, H., and Matsuzaki, M. (2013). In vivo optogenetic tracing of functional corticocortical connections between motor forelimb areas. Front. Neural Circuits *7*, 55.