論文の内容の要旨

Optogenetic study of neural activity and connectivity in deep brain structures of non-human primates

（非ヒト霊長類の脳深部構造における
神経活動および神経結合の光遺伝学的研究）

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SUMMARY

The optogenetic approach to studying the primate brain has unparalleled potential for uncovering circuit-based mechanisms of cognitive functions. Here, I describe a series of works on the application of optogenetics to deep brain structures in primates. I developed a minimally invasive combined optical and electrical probe, (optrode), a minimally invasive combined injection needle and electrode, (injectrode), and an ultrasmall syringe-pump system, and demonstrated that these pieces of equipment were effective *in vivo*. Fiber-optic fluorescence measurements made with the optrode clearly detected the expression of channelrhodopsin-2-enhanced-yellow-fluorescence-protein (ChR2-EYFP) *in vivo*. Functional magnetic resonance imaging (fMRI) performed during photostimulation of the rat thalamus revealed focal activation of the cortical projection target. Light-responsive single units in the monkey thalamus were isolated and categorized according to the spike discharge probability in response to the high-frequency light-pulse stimulation. These methodologies will advance future studies of deep brain neural circuits in macaque monkeys.

The optogenetic approach to studying the primate brain is a breakthrough research model that has huge potential to advance our understanding of the neural mechanisms of cognitive functions. However, the precision and invasiveness of the virus injection, photostimulation and electrophysiological recordings involved in optogenetics limit the use of this approach to study macaque deep brain structures due to the large size of the macaque brain and the small number of animals trained to perform cognitively demanding tasks. For virus injection, photostimulation and electrophysiological recording, the target region-of-interest must be precisely localized in each animal without miss-localization or injection fault. In addition, procedures should be minimally invasive to allow the collection of reproducible results over long experimental periods from individual animals. Therefore, the devices used in these procedures need to be custom designed for the deep brain structure of monkeys. The effects of optogenetic photostimulation can be analyzed at the single-cell level by electrophysiological recordings and at the whole brain level by fMRI.

Optimization of the optogenetic approach and basic *ex vivo* tests

Glass-coated tungsten optrode: Optrodes are used for photostimulation and electrophysiological recording in regions of the brain that are far from the surface. I developed a new optrode by integrally coating a bare tungsten needle and multiple sharpened optical fibers with insulation glass (Fig. 1). The glass-coated optrode had the following characteristics: (1) the tip was sharp and smooth to reduce friction that can cause tissue damage and optical deterioration with repeated penetrations (Fig. 1A); (2) the shank was sufficiently long, straight, and stiff for correct insertion into deep targets; and (3) the area illuminated by photostimulation of multiple optical fibers was wider than that illuminated by photostimulation of a single optical fiber and had no shadow as revealed by *ex vivo* test (Fig. 1B).

Metal-backboned injectrode and stick-shaped syringe-pump: A virus vector is usually injected into the brain through an injection needle by pressure provided by a microsyringe. I developed a new injectrode that had a long, rigid metal backbone to enable the injectrode to be inserted deeply into the brain, and a thin and smooth tip to minimize tissue damage and reduce the backflow of the solution. I connected a stick-shaped ultrasmall syringe-pump directly to the injectrode without any tubing that reduces control accuracy. This stick-shaped syringe-pump was mounted onto the conventional hydraulic manipulator that is used to electrophysiologically map the target regions, allowing the depth of the injectrode to be finely adjusted based on online monitoring of unit activity and preventing injection faults in deep regions of the macaque brain.

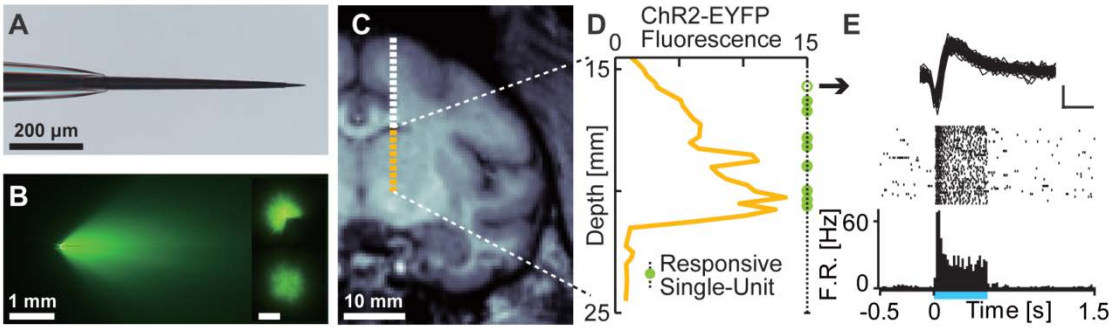

Figure 1. The glass-coated tungsten optrode for primate deep brain structures. (**A**) The tip of the glass-coated tungsten optrode. Sharpened optical fibers and a tungsten wire are integrally coated by the insulation glass except for the tip of 50 µm. Note that transparent parts on the left end are the thinned optical fibers but not the glass-coating. (**B**) A lateral view of a light cone made by the glass-coated tungsten optrode. Insets, an axial view of the light cone observed in a single-fiber illumination condition (top) and that in the four-fiber condition (bottom). (**C**) An MR-image showing the putative position of the optrode recording track in the macaque thalamus injected with lentiviral vector carrying ChR2-EYFP. Yellow region indicates the recording depth range. (**D**) Depth distributions of the ChR2-EYFP fluorescence measured by the optrode (yellow trace) and the light-responsive single-units (green circles) along the optrode track shown in (C). Light-responsive cells were found around the depth with higher ChR2-EYFP expression. (**E**) An example light-responsive single-unit isolated at the depth indicated by the open circle in (D). Top, superimposed spike waveforms. Scales: vertical, 25 µV; horizontal, 1000 µs. Middle, raster plot; bottom, PSTH; blue horizontal bar, photostimulation period.

Optogenetic study of *in vivo* models

Fiber-optic detection of gene expression in vivo: when using optogenetics in long-term experiments of chronically-prepared animals, the expression of the transgenes introduced by the virus injection should be checked before performing photostimulation in behavioral tests. I constructed a fiber-optic fluorescence measurement system with high-sensitivity photodetectors. Histological analysis of the rat thalamus showed that this system accurately measured the fluorescence distribution *in vivo*. In the monkey thalamus, a substantial peak of enhanced yellow fluorescent protein (EYFP) fluorescence was visualized around the site where the lentiviral vector carrying ChR2-EYFP transgene had been injected (Figs. 1C and 1D).

fMRI of the photoactivated thalamocortical system: In *in vivo* optogenetic activation studies, the impact of photostimulation of a specific area of the brain on other areas of the brain is of major interest to understand the causal contribution of a specific brain region in a global network. To examine the impact of photostimulation with the glass-coated optrode on the activation of whole-brain circuits, I performed fMRI of the rat thalamocortical system. When the thalamic posterior medial nucleus (POm) was photoactivated by the glass-coated optrode, there was a focal increase of blood-oxygenation-level-dependent (BOLD) fMRI signal in the putative secondary somatosensory area (SII). Subsequent histological analysis revealed a cluster of POm-derived axon

terminals mainly in the granular layer of area SII, in a pattern that was consistent with the BOLD signal response.

Recording of photoactivated single units: To be suitable for use as a microelectrode, the glass-coated optrode must be able to isolate photoactivated single units. In the monkey thalamus that had been injected with a lentiviral vector carrying ChR2-EYFP transgene, light-responsive single units were successfully isolated around the peak of the ChR2-EYFP fluorescence (Figs. 1C to 1E). Spike waveforms of the isolated units recorded during light-off and light-on periods were similar to each other, and distinct from the waveforms of other units. Light-responsive single units were categorized into two groups according to the spike discharge probability: putative directly photoactivated cells reliably generated spikes even at light-pulse frequencies up to 40 Hz, whereas putative synaptically-activated cells generated spikes only at lower frequencies of light pulses.

In summary, I have developed fundamental optogenetic infrastructure that is optimized for chronic experiments in primate deep brain structures. A part of this study has been published [1] and has had a substantial impact on other researchers in and out of the primate neuroscience community [2, 3]. These methodologies will enhance the investigation of higher order brain functions by enabling optogenetics to be used to study complex neural circuits in the macaque brain.

REFERENCES

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