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A glass-coated tungsten microelectrode enclosing optical fibers for optogenetic exploration in primate deep brain structures

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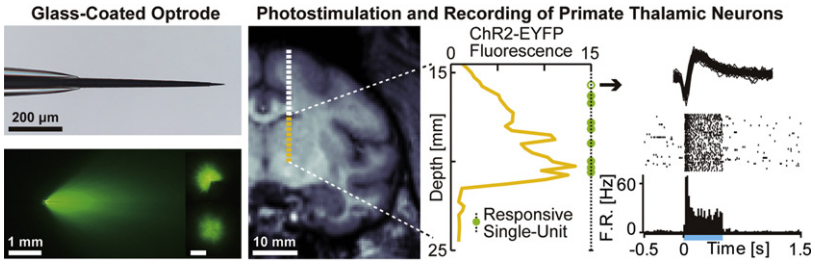
HIGHLIGHTS

- We have developed a glass-coated tungsten optrode enclosing optical fibers.
- The optrode was sharp and smooth to reduce tissue damage during insertion.
- ChR2-EYFP expression was detected *in vivo* by fiber-optic fluorescence measurement.
- Photostimulation-responsive single-units were isolated in the monkey thalamus.
- Our optrode enables the optogenetic dissection of primate deep brain structures.

GRAPHICAL ABSTRACT



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ABSTRACT

The optogenetic approach to primate brain circuitry has unparalleled potential for uncovering genetically and temporally resolved neuronal mechanisms of higher brain functions. In order to optogenetically investigate the large and complex primate brain, an optical-/electrical probe, or "optrode", must be inserted deeply, which requires the optrode to be not only long and stiff, but also sharp and smooth to reduce possible tissue damage. This study presents a tungsten microelectrode-based optrode that encloses optical fibers within its insulation glass. Optical fibers and a tungsten wire were tightly bound to each other and integrally coated with a smooth, thin layer of glass. This design satisfied the structural requirements for use in deep brain structures. The performance of the optrode was then examined in the thalamus of the rat and macaque monkeys which were injected with lentiviral vectors carrying the channelrhodopsin-2-enhanced yellow fluorescent protein (ChR2-EYFP) transgene. With fluorescence measurements *via* the optical fiber, ChR2-EYFP expression was detected clearly *in vivo*, which was confirmed by histological analysis in the rat. With photostimulation and extracellular recording, photoresponsive single-unit activities were isolated in the monkeys. The depth distribution of these units and the peak of the EYFP fluorescence profile overlapped consistently with each other. Thus, by developing a new probe, optogenetic methodology was successfully applied to a primate subcortical structure. This smooth glass-coated optrode is a promising tool for chronic *in vivo* experiments with various research targets including deep brain structures in behaving monkeys.

Abbreviations: ChR2, channelrhodopsin-2; Deg, degree; Em_{535}, emission in 535-nm band; *Ex*, excitation; EYFP, enhanced yellow fluorescent protein; GFAP, glial fibrillary acidic protein; MRI, magnetic resonance imaging; PSTH, peri-stimulus time histogram; Rf_{469}, reflection in 469-nm band; ROI, region-of-interest.

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1. Introduction

To understand the neural mechanisms of various cognitive functions, the optogenetic approach to the primate brain is a breakthrough research model. With optogenetic methodology, the causal relationship between the activity and function of a specific neuronal circuit is elucidated with high temporal and cell-type specificity (Baier and Scott, 2009; Goard and Dan, 2009; Miesenbock, 2009; Johansen et al., 2010; Kaneda et al., 2011; Tsubota et al., 2011; Tsunematsu et al., 2011; Yizhar et al., 2011a). Pioneering works have successfully applied the technique to primates in the dorsal areas of neocortex (Han et al., 2009; Diester et al., 2011).

To optically manipulate and electrophysiologically record neuronal activity *in vivo*, optrodes, combined optical/electrical probes, have been utilized (Gradinaru et al., 2007; Zhang et al., 2009; Kravitz et al., 2010; Royer et al., 2010; Lechasseur et al., 2011). Among previous studies conducted with rodents, the majority of optrodes have been fabricated by directly gluing a bare optical fiber to the outside of an independent electrode. While this is a simple design for coupling the two materials, there are some concerns about its invasiveness in recording: smoothness of the surface and the tip, safety of a chemical glue, and robustness of the coupling. The invasiveness of electrode penetration is a primary concern in chronic experiments in behaving monkeys in which individual animals and their brain tissues should be kept in a good condition during months or years of experiments. Another factor to be considered is the size of the monkey brain: it is larger than the rodent brain, and its cortical gyri, sulci, and subcortical nuclei are often located tens of millimeters from the skull. Given these issues on the invasiveness and the brain size, investigation in the deep areas of primate brain in chronic experiments (*e.g.*, inferior temporal cortex) (Miyashita, 2004; Hirabayashi et al., 2010; Takeuchi et al., 2011) requires an optrode incorporating the following characteristics (Quintana and Fuster, 1986). First, the tip should be sharp and smooth to reduce friction that causes tissue damages. Second, the shank should be sufficiently long, straight, and stiff for correct insertion into deep targets. Third, the illuminated area by the optrode should be wide for manipulating substantial population of neurons (Diester et al., 2011; Kravitz and Kreitzer, 2011).

The glass-coated tungsten microelectrode (Levick, 1972; Merrill and Ainsworth, 1972) has been widely used in single-unit recordings in chronically prepared animals. This popularity is partly because the metal tip is more robust and enables stable recording with a high signal-to-noise ratio compared to other electrodes, such as glass micropipette electrodes, and also because the glass has more suitable characteristics as a coating material with regard to its impedance, durability, and surface smoothness compared to resins such as epoxy and parylene (Sugiyama et al., 1994). In a method for fabricating a glass-coated tungsten microelectrode, a glass capillary into which a sharpened tungsten wire is inserted is pulled by a pipette puller to collapse the glass onto the tungsten wire for insulation. This method is potentially general enough to enclose other materials in addition to the tungsten wire.

In the present study, we have adapted this fabrication method to develop an optrode especially suited for the primate brain: a tungsten wire symmetrically surrounded by four sharpened optical fibers is integrally coated with glass in order to reduce friction and illuminate the region around the tip from multiple directions. We demonstrate the test performance on illumination, *in vivo* optical fiber-based fluorescence measurements, and the extracellular recording of optogenetically manipulated single-unit activities in the monkey thalamus.

2. Methods

All animal procedures complied with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Review Committee of the University of Tokyo School of Medicine. All surgical procedures were performed under anesthesia, and all efforts were made to minimize the suffering and number of animals used.

2.1. Fabrication of the glass-coated tungsten optrode

In the first process, optical fibers and tungsten wires were sharpened. To obtain sharpened optical fibers that efficiently conduct light to the tip, the graded index silica fibers (cladding 125 μm, numerical aperture 0.275; GIF625, Thorlabs) were selected and pulled by a micropipette puller. The pulled fiber had a conical tip with a 5 mm taper. The tungsten wires (diameter 50 μm; #715550, A-M Systems) were electrochemically etched. In the second process, four optical fibers were thinly coated with an appropriate amount of silicon lubricant (see below) and assembled together with the tungsten wire in polyimide tubes (i.d. 300 μm/o.d. 350 μm; #120, MicroLumen) and a glass capillary (i.d. 400 μm/o.d. 500 μm, PYREX) so that these fibers symmetrically surrounded the centered tungsten wire (Fig. 1A, right). The assembly was fixed at the base with cyanoacrylate adhesive. In the third process, by pulling the capillary, the optical fibers and the tungsten wire were integrally coated with a thin layer of glass (Fig. 1B). Finally, the glass coating at the tungsten tip was removed so that the optrode had the impedance suited for electrophysiological recording.

In the glass-coating process, the amount of lubricant was critical. Excess lubrication prevented the glass coating from adhering to the tungsten, while insufficient lubrication caused cracking of the optical fibers due to adhesion. The specifications of the optrode were adjusted as follows: exposed tip length, 30–70 μm; impedance, 0.2–1.2 MΩ (1 kHz); inter-tip distance (tungsten wire to optical fibers), 300–700 μm; and total length, 4 cm (for rats) or 10 cm (for monkeys). The basal part of the optrode was reinforced with a heat shrink tube. The optrode could be held and manipulated by a conventional manipulator.

2.2. Inspection of the illumination property

The light emission property was examined by a stereoscopic fluorescence microscope (VB-G05, Keyence) and ImageJ software (NIH). For the lateral view, the optrode was observed in fluorescein solution. For axial views, the optrode was positioned so that its tip lightly touched a fluorescent screen made of two small fragments of cover glass sandwiching a drop of fluorescein solution (Fig. 2C). The light spot on the screen was observed from the opposite side in five different illumination conditions: four single-fiber conditions in which the light is conducted to only one of the fibers 1, 2, 3 or 4; and an all-fibers condition in which the light was conducted to all of four fibers (Fig. 2D). Emission from a single optical fiber was observed in the air.

The spatial illumination pattern was analyzed in the axial images. In every single-fiber condition, a triangular dark quadrant was inevitably generated due to shading by the tungsten tip. In order to analyze the spatial distribution of the intensity, a cross-shaped region-of-interest (cross-ROI) consisting of two long rectangular ROIs (2 mm × 0.2 mm) crossing at right angles was defined. The cross-point of the cross-ROI located on the center of the spot and one of the four arms of the cross-ROI divided the dark quadrant in half. Small ROIs (0.2 mm × 0.2 mm) were defined on every arm of the cross-ROI at the point 0.5 mm from the cross-point (named 0-, 90-, 180-, and 270-deg ROI; the ROI on the dark