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A coaxial optrode as multifunction write-read probe for optogenetic studies in non-human primates

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HIGHLIGHTS

- Developed powerful new device tool for optogenetic studies in non-human primates.
- Multifunction "coaxial optrode" was deployed in non-human primates, rats, and mice.
- Device achieved targeted optical stimulation safely with minimal cortical damage.
- Device design and performance correlated well with computational models.
- Device was able to quantify and map local opsin expression in the brain.

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ABSTRACT

Background: Advances in optogenetics have led to first reports of expression of light-gated ion-channels in non-human primates (NHPs). However, a major obstacle preventing effective application of optogenetics in NHPs and translation to optogenetic therapeutics is the absence of compatible multifunction optoelectronic probes for (1) precision light delivery, (2) low-interference electrophysiology, (3) protein fluorescence detection, and (4) repeated insertion with minimal brain trauma.
New method: Here we describe a novel brain probe device, a "coaxial optrode", designed to minimize brain tissue damage while microfabricated to perform simultaneous electrophysiology, light delivery and fluorescence measurements in the NHP brain. The device consists of a tapered, gold-coated optical fiber inserted in a polyamide tube. A portion of the gold coating is exposed at the fiber tip to allow electrophysiological recordings in addition to light delivery/collection at the tip.
Results: Coaxial optrode performance was demonstrated by experiments in rodents and NHPs, and characterized by computational models. The device mapped opsin expression in the brain and achieved precisely targeted optical stimulation and electrophysiology with minimal cortical damage.
Comparison with existing methods: Overall, combined electrical, optical and mechanical features of the coaxial optrode allowed a performance for NHP studies which was not possible with previously existing devices.

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Conclusions: Coaxial optrode is currently being used in two NHP laboratories as a major tool to study brain function by inducing light modulated neural activity and behavior. By virtue of its design, the coaxial optrode can be extended for use as a chronic implant and multisite neural stimulation/recording.

1. Introduction

While optogenetics has rapidly established itself as a powerful tool to study brain function in invertebrates and rodents (Deisseroth, 2011; Yizhar et al., 2011), transition to non-human primates (NHPs) is still at an early stage. Even though robust optical modulation of neural activity has been shown with a variety of optogenetic constructs (Diester et al., 2011; Gerits et al., 2012; Han et al., 2009; Jazayeri et al., 2012), optically-induced behavioral perturbations has been reported only in few studies (Cavanaugh et al., 2012; Gerits et al., 2012; Jazayeri et al., 2012) (also O'Shea et al., 2011; O'Shea et al., 2012; Goo et al., 2012; P.K. et al., 2012 *Soc. Neurosci. Abstr.* 229.03). While these results highlight the potential for optogenetics in NHPs to explore the neural substrate of high-level cognitive functions and sensorimotor processing, further development of entirely novel optoelectronic tools is critically required, with capabilities of simultaneous electrophysiological recording, light delivery and fluorescence collection (Gerits et al., 2012) (also O'Shea et al., 2011). Advanced NHP studies, especially with chamber-mounted access ports to the brain, typically involve multiple penetrations into the same brain region over weeks and months, thereby demanding probes which are also mechanically robust while minimizing brain damage.

So far, most commonly used probe for optogenetics studies in NHPs has been the "dual-pronged optrode", based on simply attaching a glass optical fiber to a metal microelectrode (Anikeeva et al., 2012; Diester et al., 2011; Gradinaru et al., 2007). Typically this axially asymmetric construct induces cortical damage after repeated penetrations and presents the additional risk of optical fiber breakage, confounding the interpretation of the observed results. A more recent device, now with axial symmetry, is a glass-coated tungsten electrode (Tamura et al., 2012), designed for usage in NHPs. This probe is formed by four optical fibers embracing a central metal electrode which were subsequently pulled to a sharp tip. Although the device has the interesting feature of delivering or collecting light from four fibers independently, the overall shank diameter (~500 μm diameter) is much larger than a typical microelectrode. It is unclear how durable such a glass-dominated material structure is against multiple penetrations.

Both the dual-pronged-optrode and glass-coated tungsten electrode utilize large diameter optical fibers which limits the optical spatial resolution e.g. in fluorescence imaging. In addition, their optical apertures reside at least few hundred micrometers away from the sites of electrophysiological recording, leading to a non-optimal registry between the site of recording and the spatial profile of both light delivery and fluorescence collection. Thus, optogenetics studies in NHPs would benefit from new types of probes with improved capabilities.

With the motivation for developing a new type of optoelectronic tool for optogenetics in NHPs, here we describe here a "triple-function" coaxial optrode with combined capabilities of electrophysiology, light delivery and fluorescence intensity measurement. The device has a mechanically robust, axially symmetric structure with a diameter comparable to conventional metal microelectrodes enabling multiple penetrations into a targeted brain region with minimal tissue damage. Its design, informed by computer models, does not impose any constraints on length making it attractive also to studies of deep brain structures. Numerical simulation of electrophysiological recording capabilities gave a priori comparison with conventional metal microelectrodes, while numerical models based on Monte Carlo approaches for photon diffusion (Chow et al., 2010; Wang et al., 1995, 2012) enabled determination of optical energy distribution in the brain tissue—including the safety concerns of heat generation. Quantifying tissue heating and limits on input optical power delivered into the brain have been largely ignored in in vivo optogenetics studies. (Optically induced photoelectric artifact effects have been subject to discussion (Long and Fee, 2008; Moser et al., 1993; Yizhar et al., 2011), however minimized in our device during fabrication (Section 2.1)). While being published elsewhere, ongoing experiments in our laboratories have taken advantage of the capabilities of the new coaxial optrode to generate optically-induced behavioral modulation in NHPs and rodents.

2. Materials and methods

2.1. Microfabrication of the coaxial optrode

A step-index multimode optical fiber (10/125 μm core/cladding diameters, HPSC10, Thorlabs) (Supplementary Fig. S1(i)) was cut into a desired length (for our applications ~10–15 cm) and tapered at one end by mechanical polishing using a pipette beveler (BV-10, Sutter Instruments) (Supplementary Fig. S1(ii)) where an 18 G stainless steel tube was mounted on the pipette holder (set at ~15–30°) to guide the fiber toward the polishing plate. The fiber was rotated continuously in the tube during polishing to obtain a conical tip shape. Following the tapering, the fiber was ultrasonically cleaned in acetone, methanol and water, and an LC miniature connector (MM-FER2007C-1270, OptoEquip) was mounted at the non-tapered end using a heat-curable epoxy (F-123, Thorlabs).

The connecterized and tapered fiber was left in acetone overnight to soften the polymer jacket for easy removal, and sequentially cleaned with acetone and methanol. Next, the fiber was flatly mounted in the electron beam evaporator for deposition of a chromium layer (~10 nm) followed by a gold layer (~100–300 nm) (Supplementary Fig. S1(iii)).

After metallization, the fiber was first inserted into a polyamide tube (~3 cm shorter than the fiber, 36AWG, Small Parts) and then into a reinforcing stainless steel tube (29 G) (Supplementary Fig. S1(iv)) which had its end facing the fiber tip chamfered with a drill. At this junction, we followed two different pathways in the fabrication process depending on the purported usage of the optrode. If dura penetration was aimed as in NHPs, the chamfered end was fixed at the start of the tapered segment of the fiber (Supplementary Fig. 1(iv), left). Otherwise, the chamfered end was fixed at a desired distance from the tip (Supplementary Fig. S1(iv), right) to obtain a thinner shank diameter (~165 μm).

Next, UV-curable epoxy (NOA81, Thorlabs Inc.) was manually applied to the tip in 2–3 steps to expose a small portion (semi-conical frustum (~60° opening angle) with top and bottom plane diameters of about 30 μm and 80 μm) of the gold coating to the air (Supplementary Fig. S1(v–vi)) as the recording surface and make a smooth transition from the tapered fiber to polyamide and stainless steel tubes (Supplementary Fig. S1(v)). Impedance "tuning" (~100 kΩ precision) was accomplished by controlling the amount of exposed metal during dispensing of the epoxy. For electrical contact, a wire (29 G) was attached to the metal coating exposed