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Approaches to Optical Neuromodulation from Rodents to Non-Human Primates by Integrated Optoelectronic Devices

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***Abstract* - Methods on rendering neurons in the central nervous system to be light responsive has led to a boom in using optical neuromodulation as a new approach for controlling brain states and understanding neural circuits. In addition to the developing versatility to "optogenetically" labeling of neural cells and their subtypes by microbiological methods, parallel efforts are under way to design and implement optoelectronic devices to achieve simultaneous optical neuromodulation and electrophysiological recording with high spatial and temporal resolution. Such new device-based technologies need to be developed for full exploitation of the promise of optogenetics. In this paper we present single- and multi-element optoelectronic devices developed in our laboratories. The single-unit element, namely the coaxial optrode, was utilized to characterize the neural responses in optogenetically modified rodent and primate models. Furthermore, the multi-element device, integrating the optrode with a 6x6 microelectrode array, was used to characterize the spatiotemporal spread of neural activity in response to single-site optical stimulation in freely moving rats. We suggest that the particular approaches we employed can lead to the emergence of methods where spatio-temporal optical modulation is integrated with real-time read out from neural populations.**

I. Introduction

Targeted spatiotemporal access at single neuron level is desirable to the study of neural circuits and cellular conditions, for basic understanding of their function as well as neural disorders. Modulating neural circuits by electrical microstimulation is a well-established tool in electrophysiology and clinical neurosurgery, even if uncertainties are inherent given the non-selective activation of cells by the complex flow of current pathways [1, 2]. In a broader context of extracting information via external neuromodulation methods, systematic models of how activity in neural circuits occurs, whether normal or aberrant, is made complicated by the nonspecific nature associated with electrical, magnetic and pharmacological methods in general. A new opportunity to neuron-specific stimulation began with the discovery of the light-sensitive ion channel, Channelrhodopsin-2(ChR2) [3], followed by the discovery of the optically activated chloride pump, halorhodopsin (NpHR) [4]. By combining genetic and optical methods these discoveries were rapidly advanced to fundamentally new methods for targeting neurons, now known as 'optogenetics'[5, 6]. Importantly, the light induced neuromodulation offered a targeted means for both cellular excitation and inhibition for well-defined control of neuronal events with millisecond time resolution.

One of the practical advantages of optical methods for neuromodulation is their minimal instrumental interference with simultaneous electrophysiological recordings. Further, the method is technically scalable for accessing potentially multiple target areas as spatiotemporally controlled stimulation across the cortex and deeper brain structures. To advance such prospects, novel "dual-function" implantable devices for both light delivery and electrophysiological recording are essential components. For light delivery, optical fibers have already seen wide use in optogenetics to date, given their abundant commercial availability as flexible (if fragile) low-loss optical waveguides. An optical fiber allows also in principle *in vivo* fluorescence detection in the intact brain for minimally invasive assessment of opsin expression [7]. Recording of the optically evoked neural activity *in vivo* for rodent and non-human primate models has been limited to the use of individual extracellular electrodes (microwires) which are simply adhesively attached in parallel to the optical fiber ([7] for instance). Yet there are a variety of techniques that have been developed in electrophysiology for neural population recording, ranging from intracortical MEAs to ECoGs, thus raising the question of integrating such established devices with fiber optics, as chronic implants in awake or freely behaving animal settings.

Here we describe the design and fabrication the "dual-function" single coaxial optrode devices developed in our laboratories. The single optrode was chiefly motivated the need to create a tool for study of optogenetics in non-human primates, focusing on current research where testing a variety of optogenetic molecular constructs and their efficacy in evoking electrophysiological and possible behavioral impact in primates. We present preliminary

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results on the application of these devices in modulating and recording neural activity in freely moving rodents and anesthetized non-human primates.

II. Device Design and Fabrication

A. Single Coaxial "Optrode"

First we introduce a single-element device, termed the single coaxial optrode, a structure which consists of a gold-coated tapered optical fiber fixed in a stainless steel reinforcing tube (Fig. 1 upper trace). It allows light delivery into the brain through the fiber and simultaneous electrophysiological recording through exposed gold layer at the tip. In addition to performance such as control and overlap of the optical stimulation and recording volumes, we also emphasized in mechanical robustness of the coaxial optrode whereupon e.g. multiple penetrations of the dura mater could be accomplished with the same device.

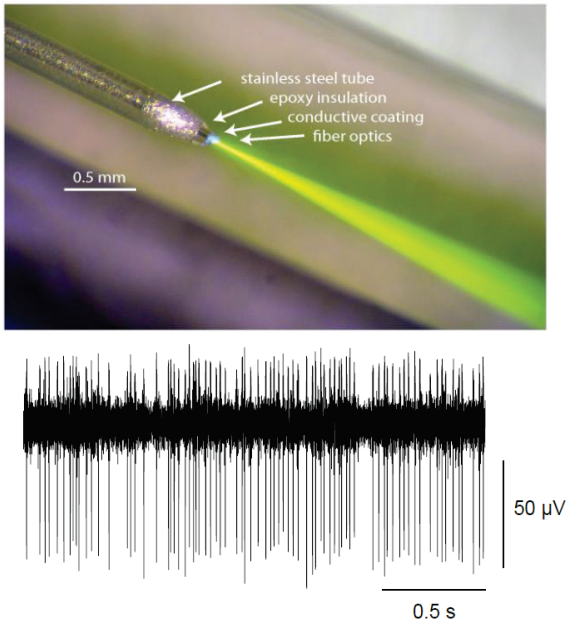

Figure 1 : (Upper trace) Photograph of the illumination pattern from a coaxial single optrode device, imaged from fluorescence induced in an organic dye stain (solution).. (Lower trace) Spontaneous single unit activity (spikes) recorded from the LIP region in an awake non-human primate (penetration of approximately 7mm into the cortex).

The upper panel of Fig. 1 identifies the primary materials used in the fabrication of the single optrode. A multimode optical fiber (125 µm in outer diameter) was tapered with mechanical polishing to a sharp conical tip and coated with thin layers of chromium/gold (25/100 nm). This structure was inserted first into a polyamide tube and then into a chamfered stainless steel tube leading to an overall device diameter of approximately 300 µm. The polyamide tube was used as an insulation layer between the gold layer and stainless steel tube. The gold-coated, tapered tip of the optical fiber was exposed from the chamfered end of the stainless steel tube and fixed by a UV-cured epoxy (Fig. 1). The very tip of the tapered end of the optical fiber was polished once more mechanically for optical transmission from its apex. A typical radiation pattern is shown below in Fig. 1. The exposed gold microelectrode in this design is equivalent in its shape to a conical ring, whose dimensions and area was tailored to provide an impedance in the 500 kΩ range, by employing impedance control in the fabrication process flow.

B. "Optrode-Microelectrode Array"

Previously, we have introduced an initial design of a device where an optical fiber is integrated into an intracortical microelectrode array (MEA) to trigger and map 2D epileptic wave propagation in optogenetically modified mouse cortical slices [8]. Here we report a version of this device which has been chronically implanted in rats, to enable the use in freely moving animals over periods of up to 6 months. In so doing, the device has enabled us to elicit neuromodulation while simultaneously mapping electro-physiological response from population of neurons in ChR2-expressing animal models *in vivo* (Section III). Here, an optrode-MEA consists of one optrode embedded within a silicon-based intra-cortical MEA (Blackrock Microsystems).

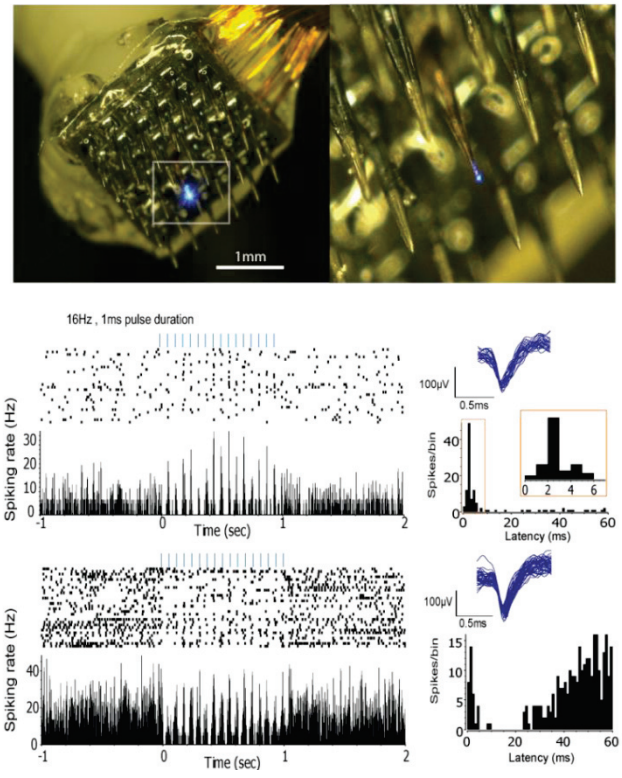

Figure 2 : (Upper trace) Photographs of the 6x6 multi-electrode array device with one element being replaced by an optrode (frame). The spacing between neighboring electrodes is 400 µm and shank length is 1mm. (Lower trace): Raster plots and PSTHs from two different neurons activated by the blue laser pulse trains (blue ticks, 473 nm), with excitatory and inhibitory response to the same stimulation. The spike count histograms (right panels) show the time-locked evoked spikes (randomly selected spike waveforms indicated).

This device allows local optical stimulation at one or more sites while mapping the neural activity (spikes and LFPs) across the array.

Photograph of a 6x6 element version of the device is shown in the upper trace of Fig. 2, designed for in vivo work for a rat model. An important consideration for the design of the form factor of the single optrode was that their shape and dimensions closely matched that of the individual intra-cortical MEA electrodes for minimizing any tissue damage. Some of the fabrication steps are described in [8]; here, the commercially available MEAs were further processed by ablative laser drilling to remove one silicon electrode at a chosen site (usually near the center) while leaving a clear round hole in the structure of 200 μm diameter. A wire-bonded Au-bundle from the microelectrodes was laid out on the planar back of the array so as to detour the drilled hole site. A Teflon cannula was centered with and bonded to the open hole on the backside of the MEA. The function of the cannula was to provide a spatially stationary alignment guide for the viral injections and subsequent employment of the optrode as a local light delivery tool, respectively.

III. Examples of Applications in Non-Human Primate and Rat Rodent Models

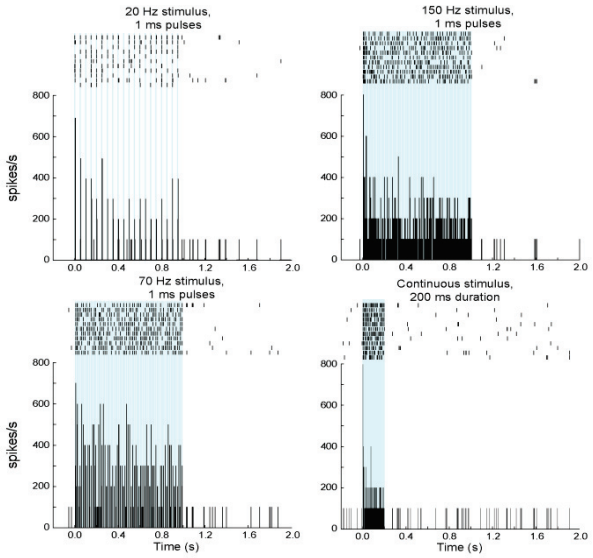

Figure 3: Blue light modulation as rasters and peri-stimulus time histograms of neuronal firing in response to blue and yellow (control) light pulse train stimulation in S1 of a ChR2-expressing primate under anesthesia. The figures show photoinduced spiking at different repetition rates of 473 nm light pulse trains.

We employed the single coaxial optrode in anesthetized adult rats and young anesthetized non-human rhesus macaque primates (2.8 kg) to modulate neural activity optically. Several AAV-5-virally packaged optogenetic constructs were employed (ChR2 and NpHR), and injected to multiple sites of the cortex of the animals., We employed specially designed precision hollowed-out Ti-screws in the skull of the primate which acted as cannula for the viral injection by a hypodermic needle and subsequent repositioning of the optrode in subsequent (repeated) experiments over weeks. The injection protocol employed hydraulic minipumps in a setup where the injected viral volumes per site (along cortical columns) were 1 μL with typical viral titers were ~10^{10} IU/mL. The single coaxial optrode device was deployed in number of cortical areas, while simultaneously reporting optical neuromodulation, to locate and characterize sites with maximal photoactivated response. As an example, Fig. 3 shows rasters and peri-stimulus time histograms of a representative firing activity of one neuron in primate LIP (expressing ChR2) in response to blue laser (473nm) pulse train stimulation at various frequencies. A number of experiments are currently under way aiming to advance the understanding of the microcircuit-level mechanisms which are involved in optical neuromodulation in primates – and in so doing bridge neurophysiological experiments to behavioral and brain models. Experiments are being extended to include the deployment of the optrode-MEA devices, of which we next show an example as chronic implants in freely moving rats.

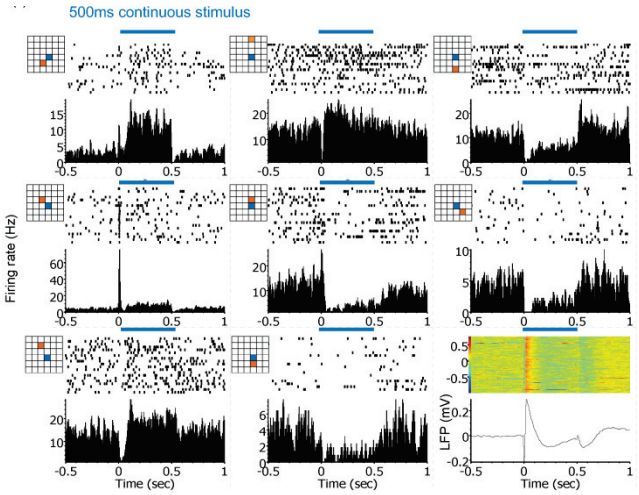

Figure 4: Examples of activation patterns of single units at selected sites across the optrode-MEA hybrid array (insets indicate recording locations as blue squares while the fixed location of photoexcitation is labeled as a red square), in response to 500ms duration of continuous blue light stimulation. The last plot in lower right hand corner is an example of an evoked LFP response.

In the rodent experiments, the optrode-MEA was chronically implanted e.g. in somatosensory areas of adult rats. The animals were free to move in a cage which also included facilities for behavioral tasks (not detailed here). The neural activity was modulated during free behavior while monitoring its time course within the cortex. In broad terms, we observed that single site optical stimulation could generate a variety of activity patterns in the surrounding cells, consisting of periods of inhibition, excitation or both (e.g. lower trace Fig. 2). In this instance, given the relatively large area of cortical real estate covered by the MEA, this type of complex network response is not entirely surprising, which is further illustrated in Fig. 4 through actual spike-histogram maps at a number of locations (individual

microelectrodes of the MEA). In all ChR2 expressing animals, we could optically evoke neural response with strongly modulated spike firing and LFP activities (an example is shown in Fig. 5). While leaving the interpretation of the underlying neuroscience elsewhere, the data is shown chiefly to demonstrate the utility of the device a chronic implant, scalable to primate use.

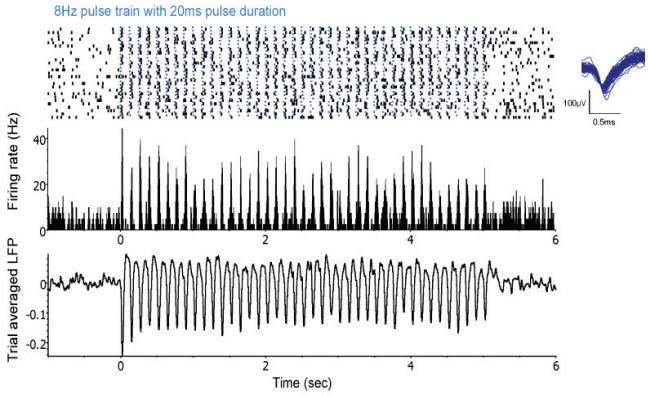

Figure 5: Examples of synchrony in light activation of single units and local field potential (LFP) from a light-responsive single unit. Vertical blue lines in the upper trace across the raster plots indicate the timing of the pulse train stimulation. Randomly selected spike waveforms are shown on the right. Lowest trace shows how trial averaged LFPs have negative deflections with a positive rebound in response to each pulse of blue laser stimulation, in synchrony with the laser excitation and spiking activity in posterior parietal cortex of an awake rat.

IV. Conclusion

Through selected examples, we have demonstrated that, with a new suite of hybrid optoelectronics devices, it is possible to effectively modulate neural activity in the intact brain of primates and rodents in ways that augments the existing methods in optogenetics research. In addition to being tools for neuroscience research, a grand motivating goal of these devices is to provide one starting point for developing treatments for a variety of neurological diseases and brain injuries.

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