Optoelectronic Devices for Optogenetics: From Rodents to Non-Human Primates

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***Abstract*—By contrast to the constantly expanding libraries of opsins and promoters for optogenetics, there is a need for versatile optoelectronic devices which in particular can (a) be chronically implanted, including non-human primates (NHP), and (b) deliver arbitrary spatio-temporally patterned distributions of light while performing simultaneous multisite neural recording. Here we discuss specific recent approaches where monolithic probes have been microfabricated in our laboratory as cortical actuator/sensor implants in rodents and non-human primates (NHP). These range from multifunctional single channel implants to newly developed optically transparent, electrically conducting intracortical microarrays.**

Keywords—optogenetics, neuromodulation, neural recording,

I. OVERVIEW: SINGLE-CHANNEL OPTRODE-TYPE PROBES FOR IN VIVO EXPERIMENTS.

Since their inception, simple dual-pronged structures represent the most common optoelectronic probe, with light delivered through optical fibers (~ 200 micron diameter) and accompanying metal microelectrodes for extracellular recording (<100 micron diameter) were simply glued to the glass fiber [1-3]. While quite effective, these probes have limitation. For example, optogenetic studies in NHPs with chamber-mounted access ports to the brain involve multiple penetrations over extended periods of time, thereby demanding probes which are both mechanically robust yet cause minimal tissue damage. Second, the light emitting apertures in such dual-pronged, axially asymmetric constructs probes are usually constrained to be separated several hundred micrometers away from the target sites of electro-physiological recording, resulting in a mismatch of spatial registry between the physiological volumes of neural recording and optical stimulus.

Many laboratories have improved this basic construct. In our laboratory, we first implemented a single channel "triple-function" coaxial monolithic optrode specifically for NHPs [4] with a joint capability for electrophysiology, light delivery, and fluorescence measurement within a targeted brain volume of interest with approximately 100μm precision. The compact microfabricated devices (Fig. 1) were designed to be mechanically robust with an overall diameter comparable to conventional metal microelectrodes. This allowed multiple penetrations into a targeted brain region, particularly in NHPs with much reduced tissue damage. As one application we investigated whether optogenetic stimulation of the somatosensory cortex could be detected as a proxy for tactile sensation by NHPs [5].

Fig. 2 shows summary of experiments deploying the single channel coaxial optrode where rhesus monkeys were trained on a simple tactile sensory detection task to report mechanical vibrations which were generated on a touchpad onto which the animals placed their hand upon cue. After learning the vibration task, the mechanical vibrations were replaced by a direct optical stimulus of 500 ms duration (≈2 mW at 561 nm) applied to transduced target within Area 1 of the somatosensory cortex (viral construct AAV5-CamKIIα-C1V1-ts-EYFP from Deisseroth Lab at Stanford). Our results showed that the monkeys could identify the optogenetic stimulation of the somatosensory as summarized in Figs. 2(b)-(c). Importantly, we found that the animals learned to report a touch-like percept induced by the proxy light stimulus at performance rates and reaction times comparable to that induced by mechanical means. Such outcomes, enabled by the multifunctional single-channel microprobe then beg the question expanding such capability across multiple locations of e.g. the cortex. We show in the next two sections how work in our laboratory has addressed this next level challenge in two separate phase of device development.

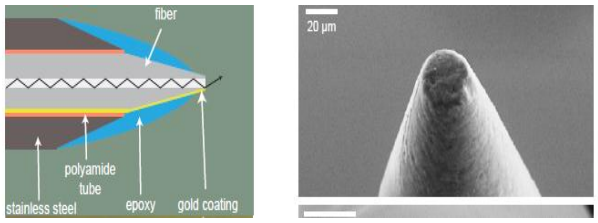

Fig 1. Cross sectional schematic and scanning electron microscope images of the coaxial optrode tip, showing the polished tip and the gold outer electrode layer [4].

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II. MULTICHANNEL RECORDING OF OPTICALLY MODULATED NEURAL POPULATION DYNAMICS

As the first step towards multichannel optogenetics, we addressed the question of increasing the number of electrophysiological recording sites based on widely deployed intracortical microelectrode arrays. Constructs such as mechanically assembling multiple optical fibers or waveguides with standard electrophysiological microprobes [6] have technical limitations as they typically involve physical integration of two or more distinctly separate material blocks (optical, electrical) leading to outcomes of awkward geometries and large physical sizes. Monolithic, purely optical multisite stimulating devices have also been reported but without recording capabilities, such glass arrays [7] or microprobes with multiple optical waveguides [8] (see Section III). Our intermediate step towards full multichannel optoelectronic capability was to first device means of "writing-in" cortical stimulus by single site optical neuromodulation while reading out consequent neural population microcircuit dynamics by intracortical microelectrode arrays in chronically implanted NHPs and rodents. We developed a polymer-fiber/microelectrode array (POF-MEA) which, following validation in rats [9], was transitioned to NHP as chronic implants. [10]. The POF-MEA is a 10x10 microelectrode silicon (optically opaque) array integrated with a tapered polymer optical fiber at the center (Fig. 3a). A highly flexible polymer fiber (260 μm diameter) was inserted into a laser drilled hole and bonded. Both the electrical (wirebundle) and optical wiring emerged from the device parallel to its plane and threaded through the cranium to separate skull mounted connector pedestals. As detailed in Ref 10, we injected the viral construct AAV5-CamKIIα-C1V1-ts-EYFP in either motor cortex or ventral premotor cortex of two monkeys with subsequent implantation of the POF-MEA. The monkeys were trained on a

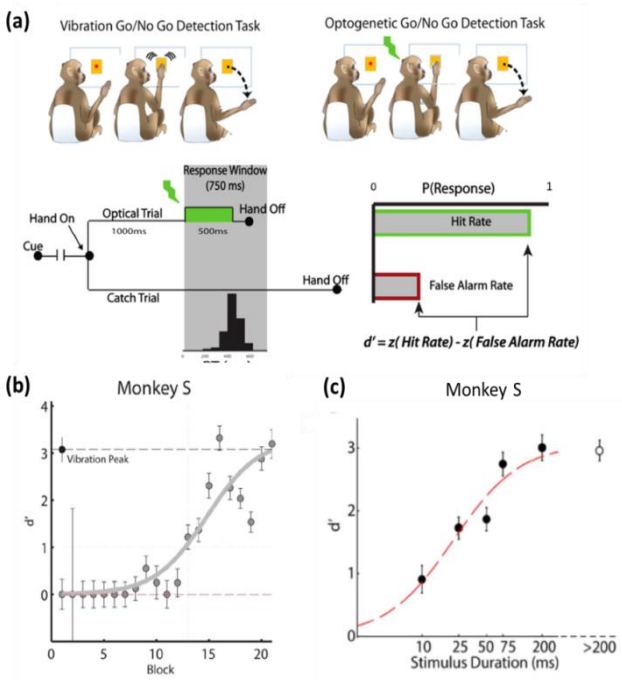

Fig 2. *Coaxial Optrode for somatosensory stimulation in NHPs:* ***(a)*** *Two monkeys were trained on a go/no go mechanical vibration detection task on a touchpad. The task was to detect the vibrations and respond by quickly removing a hand (<750 ms). On approximately 50% of trials (Catch Trials), no vibration occurred and the monkey was rewarded for retaining its hand on the touchpad longer, for at least 1.5 seconds. The monkeys were then introduced to optogenetic stimulation delivered directly into the somatosensory cortex as a proxy for mechanical stimulus (500 ms pulses of light).* ***(b)*** *Performance in one monkey in reporting optically induced sensation. Every block included n=100 trials. Peak performance in detecting mechanical vibration is shown as a reference to indicate that the monkey learned to detect the optical stimulation approaching the performance level of the real physical vibration.* ***(c)*** *Varying stimulus duration between 10 and 200 ms shows that the optogenetic stimulation is detected with increasing accuracy as stimulus duration increases. Stimulus durations as short as 10 ms can be detected (number of trials n= 5133 [5].*

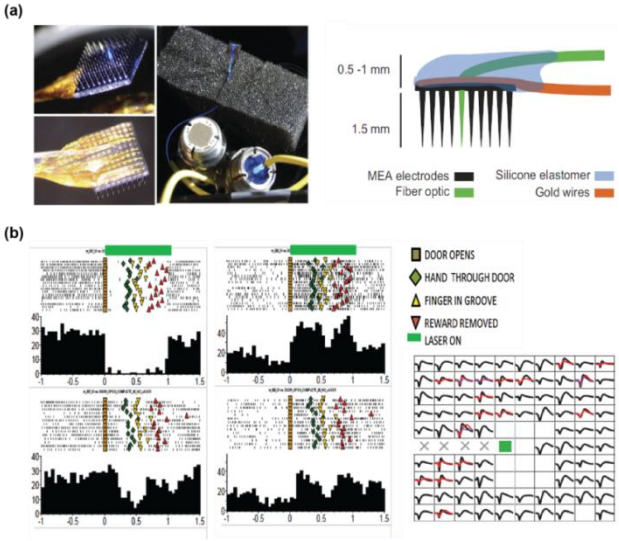

Fig 3**. (a)** POF-MEA attached to two skull-mounted pedestals for optical and electrical connections, respectively. An optical fiber (green) was integrated to the center of the 10x10 silicon MEA. **(b)** Spike waveforms from chronically implanted monkey of isolated units are shown in the right bottom panel. About 30% of these units responded to optical stimulation delivered at the center of the array (green square).The activity of two selected units are shown as raster plots and peristimulus time histograms (left two colums). The bottom rows represent the spiking activity during the task without optical stimulation. The top rows show the activity during task in the presence of optical stimulus (green bars) [10].

two-way grasping task to pick up food pellets from a groove either parallel or perpendicular to their hand movement direction. Optogenetic stimulation (single pulse of 1s duration, 6mW, 561 nm) was applied during the task performance. Our results showed that single site stimulation of the motor cortex could induce strong neuromodulation in the motor cortex, which encompass firing rate changes in about 30% of the recorded neurons (Fig. 3b) and induction of traveling gamma waves (40-80 Hz) in the local field potentials [10]. Importantly, the amplitude of optogenetically induced gamma oscillations were reduced during task-engaged motor movement. These observations indicated that task-engaged neural activity in motor cortex could override the effects of single-site optogenetic stimulation. By contrast, while single-site optogenetic stimulation generated well-modulated neural population activity in the motor cortex, the optical perturbation was, however, insufficient to override the task-engaged cortical/brain overall neural activity within optical power levels which were considered safe.

III FULLY TRANSPARENT INTRACORTICAL OPTOELECTRONIC ARRAYS

With the learning gained from the above research (Sections I and II), we have recently advanced to a device which enables multisite optical stimulation and simultaneous multichannel neural recoding, implemented as a single crystal monolithic functional device. We exploit physical properties of the so-called wide bandgap semiconductors: a relatively rare combination of high electrical conductivity and optical transparency across the visible spectrum. Employing n-type bulk single crystal ZnO as starting material we designed and fabricated micro-optoelectronic array (MOA) devices (Fig. 4a) [11]. For a first prototype, we chose a geometry and form factor similar to the opaque Utah/Blackrock silicon intracortical MEAs, i.e. array of tapered microelectrodes with 1.0-1.5 mm long shanks at 400 μm pitch. Due to the non-silicon like material properties of ZnO, we developed *de novo* microfabrication process for the MOAs featuring microscale optical/electrical interconnections to the arrays. The multistep fabrication involved precision mechanical dicing followed by multistep specific wet chemical processes was invented. We also developed uniform exposed optrode tip deinsulation process by utilizing PDMS as an etch mask layer to ensure biocompatible parylene-C layer insulating coverage of all surfaces except the tips of the ZnO optoelectrodes. Transparent conductive protection layer of indium tin oxide (ITO) was sputtered on the ZnO tips. Finally, multichannel electrical connection was achieved as a semitransparent flexible electrical cable embedding Au traces within a 17 μm-thick polyimide cable with mechanical flexibility and optical transparency. The exiting Au-based wiring traces were designed not to obstruct the input/output optical pathways, as shown. Focused laser pulses (70-80 μm diameter) targeted each individual optical aperture (>100 μm) in turn by a computer-controlled MEMS mirror-based custom scanning system. Fig. 4b shows incident light coupled into and guided through the tapered ZnO shanks to the very end of the tip for delivery from the micrometer size exit aperture. In initial proof-of-concept experiments, we performed multichannel optical stimulation and electrophysiological recording in Thy1-ChR2 transgenic mice by optical stimulation at 473 nm. Focused laser pulses (70-80 μm diameter) were sequentially delivered onto each individual (≈100 μm) optical aperture. The MOA device recorded neural activity at all channels while optical stimulus was spatially and temporally delivered to selected group of optoelectrodes, such as shown in Fig. 4c. Operating in the 'low' power regime (1-10 μW), we reproduced natural activity patterns generated by whisker stimulation (Fig. 4d). In the 'medium' power regime (1-10 μW), the connectivity within a local neural circuitry could be revealed. In the 'high' power regime (0.6-1.2 mW) optical stimulation of motor cortex led to specific muscle movements to reconstruct the functional maps of the motor cortex (Fig. 4e). These demonstrations, so far in a rodent model, show how the transparent MOA is capable of spatiotemporally selective light delivery in the brain during multisite electrophysiological recordings. In addition to applying the new devices to more comprehensive rodent studies, next steps need to include device advanced for transitioning the MOAs to chronic use in NHPs. Such research can hopefully exploit optogenetics further and to bring new insights about light modulated neural microcircuit dynamics.

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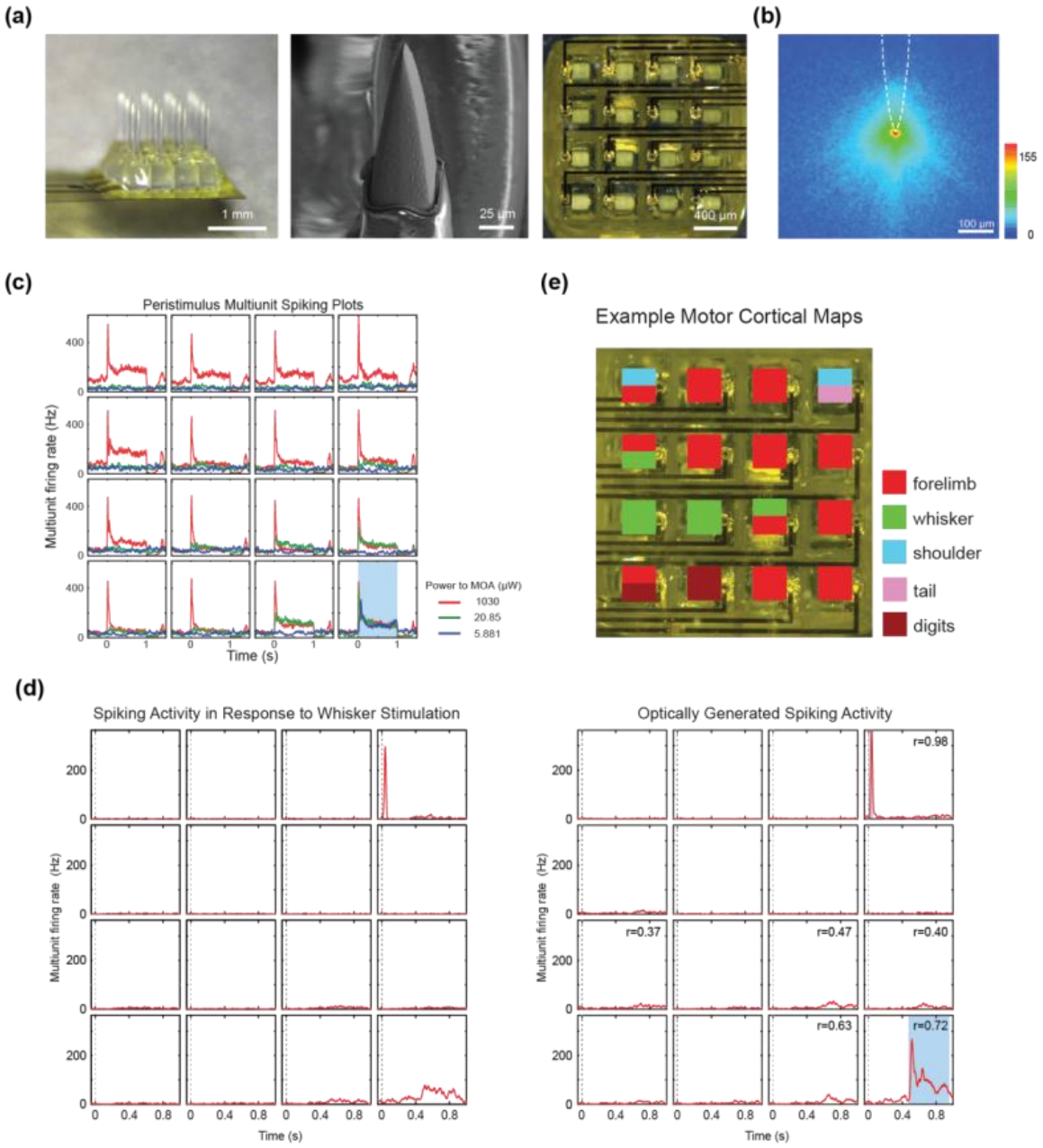

Fig 4**. (a)** Photo of a fully transparent 4×4 ZnO MOA for rodent experiments (left). Magnified scanning electron microscope image (middle panel) shows the smooth surface of the sharp tip. Right panel shows the image of back surface of the MOA through the assembled ribbon cable with metal traces separated from optical aperture. **(b)** Spatial distribution of light emission from optoelectrode tip immersed in fluorescent dye solution. **(c)** Peristimulus time histograms of multiunit spiking activity recorded in anesthetized transgenic mouse under range of optical stimulation power levels. **(d)** Left: peristimulus spiking histograms in the barrel cortex in response to whisker stimulation. Nearly the same neural dynamics could be reproduced by sequential optogenetic stimulation through optoelectrodes 4 and 16 (Right panel) **(e)** Functional map of mouse motor cortex from optical stimulation through each optoelectrode [from 11].

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