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A Microelectrode Array Incorporating An Optical Waveguide Device for Stimulation and Spatiotemporal Electrical Recording of Neural Activity

Jiayi Zhang, Farah Laiwalla*, Jennifer A. Kim, Hayato Urabe, Rick Van Wagenen, Yoon-Kyu Song, Barry W. Connors and Arto V. Nurmikko

Abstract— **Targeted neural excitation coupled with simultaneous multineuron recording is desirable both for studying the real-time dynamics of neural circuits and for prospective clinical treatment of neurological diseases. Optical stimulation of genetically targeted neurons expressing the light sensitive channel protein *Channelrhodopsin* (ChR2) has recently been reported as a means for millisecond temporal control of neuronal spiking activity with cell-type selectivity. This offers the prospect of enabling local (cellular level) stimulation and the concomitant monitoring of neural activity by extracellular electrophysiological methods, both in the vicinity of and distant to the stimulation site. We report here a novel dual-modality hybrid device which consists of a tapered coaxial optical waveguide ("optrode") directly integrated into a 100 element intra-cortical multi-electrode recording array. The dual-modality array device was used in ChR2 transfected mouse brain slices. Specifically, epileptiform events were reliably optically triggered by the optrode and their spatiotemporal patterns were simultaneously recorded by the multi-electrode array.**

I. Introduction

NEURAL stimulation by means of injecting electrical current through brain tissue has been a powerful tool in electrophysiology [1] and clinical neuroscience [2], in spite of the uncertainties associated with complex current paths and non-selective depolarization of axons, dendrites and neuronal cell bodies by volume electrical stimulation [3]. Direct optical stimulation of neural cells in brain tissue genetically modified to express channelrhodopsin-2 (ChR2) has been recently achieved [4] and applied in both *in vitro* and *in vivo* studies [5]. Various light delivery schemes have been reported to match the excitation spectrum of ChR2 at blue-light wavelengths, including band-filtered white light [4], light emitting diodes (LED) [6] or laser-coupled optics [5] for *in vitro* applications, as well as direct epicranial LED implantation for *in vivo* applications. The spatial distribution of the stimulating light at the target brain tissue using the above-mentioned delivery schemes has been limited by the divergence of the light source itself or the numerical apertures of the optical instruments, and is further "blurred" by scattering intrinsic to the tissue. Moreover, recording of the evoked neural activity has been limited to single patch or EEG/EMG recordings. A mechanically simple construct of an optical fiber glued to an extracellular electrode has been recently reported [7] to monitor neuronal activity evoked by optical stimulation in ChR2 transfected mice. Elsewhere, a variety of techniques have been developed to record from a large population of neurons with high spatiotemporal resolution, ranging from multi-electrode arrays to optical imaging methods. Such techniques face significant technical challenges in enabling simultaneous specific modulation and detailed tracking of activity in intra-cortical microcircuits.

A paradigm case of neural network activity where space- and time-dependent excitation/recording is important is the study of epileptogenesis. One-dimensional recordings of epileptic waves in neocortical slices have been demonstrated previously by linear microwire arrays [8], revealing the patterns of wave initiation and propagation across the columnar and laminar architectures in neocortex. It is thus interesting to use the two-dimensional spatiotemporal propagation patterns of epileptic waves, including those under pharmacologically induced seizure conditions, as a model for the application of the newly developed set of tools described in this paper.

We describe a tapered coaxial optical waveguide construct, dubbed as the "optrode", which is embedded within a 100-element "Utah" intra-cortical multi-electrode recording array (MEA). This dual-modality, hybrid, optrode-MEA device is capable of locally delivering light stimuli to neural tissue while simultaneously making multi-site extracellular recordings from an area of approximately 4.0 mm by 4.0 mm centered at the stimulation focus (these are present dimensions, which are extendable to any number of combinations and geometries with advanced MEA and optrode fabrication techniques). The optrode itself is a dual-element device providing simultaneous light delivery and electrical recording capabilities. This optrode-MEA

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*Farah Laiwalla is with the Division of Engineering, Brown University Providence, RI 02912 USA (phone: 401-863-1453; fax: 401-863-9120; email: farah_laiwalla@brown.edu).

Jiayi Zhang is with the Department of Physics, Brown University, Providence RI 02912 USA (email: jiayi_zhang@brown.edu).

Arto Nurmikko is with the Division of Engineering, Brown University, Providence, RI 02912 USA (e-mail: arto_nurmikko@brown.edu).

hybrid device was used to study the 2-D spatiotemporal propagation of optically- induced epileptic waves in disinhibited ChR2 mouse cortical slices. Epileptic waves with robust propagation patterns were demonstrated.

I. Device Fabrication

The single optrode is a tapered glass optical fiber with a sub-micron sized aperture at its tip formed by gold metallization cladding (Figure 1(a)). The sharp taper was fabricated from a multimode optical fiber (Fiber Instrument Sales Inc, 62.5 µm core, 125 µm cladding) by wet etching in aqueous hydrofluoric acid (HF, 49%) at room temperature (22~24°C). The etched optical fiber was rinsed in de-ionized water and a 500 nm thick gold film was deposited on the tapered surface using a high vacuum thermal evaporator (Angstrom Engineering). A magnet wire (Alpha Wire Company) was attached to the metalized part of the optrode by means of silver epoxy (H20E; Epoxy Technology) and served as the electrical conduit to external recording electronics. With the exception of the final 50 µm of the tapered tip, the metalized part of the fiber was insulated using a UV-curable epoxy (Norland Optical Adhesive 74). Figure 1(b) shows scanning electron microscope (SEM) images of an optrode tip. Thermally evaporated grains of gold metal are obvious in the close-up image of the tip.

Figure 1(c) shows a schematic of the fully integrated optrode-MEA device. The starting 100-element MEA was fabricated by Blackrock Microsystems, based on the Utah array. This multi-electrode array is a three dimensional silicon based structure comprising a 10 × 10 grid of 1 mm long tapered microelectrodes based on a 4.2 mm × 4.2 mm × 0.2 mm thick substrate. The inter-electrode distance is 400 µm. The array was then further processed as follows: A hole with a diameter of approximately 203 µm was created by removing one silicon electrode shank using ablative laser machining and drilling (Gateway Laser Services). The fiber optic optrode element was mechanically fixed through the hole with UV curable epoxy (Norland Optical Adhesive 74); its tip was precisely aligned to be in plane with the tips of the Pt-coated silicon microelectrodes, and it was attached with thermally conductive epoxy (H70-E; Epoxy Technology) onto an aluminum rod. In this first implementation the contacts to the optical fiber are separate from those to the MEA electrodes, the latter extending as a bundle from the side of the array (Figure 1(d)).

II. Optical and Electrical Characteristics of Single Optrode

The design of the single optrode element had two objectives: the local delivery of light stimulation and the simultaneous electrical recording of proximal, associated neuronal activity suitable for both *in vitro* and *in vivo* applications. The light scattering properties of the brain tissue can vary vastly depending on incident wavelengths, physiological status of the tissue, and even the age of the subject. In order to quantify light scattering in brain tissue, the optical output of the optrode was placed in a brain slice derived from a postnatal day 28 (P28) mouse (to maintain consistency with other brain slice experiments) and a blue laser diode (440 nm; Nichia Corp., within a homemade package) was coupled to the fiber end of the optrode. Figure 2(a) shows a Differential Interference Contrast (DIC) image of the scattered light from an optrode placed at a penetration depth of around 50 µm in the slice. The radial intensity distribution is shown in the inset of Figure 2(a); similar quantification in the axial dimension is prevented by limited optical access due to the 'shadow' of the optrode.

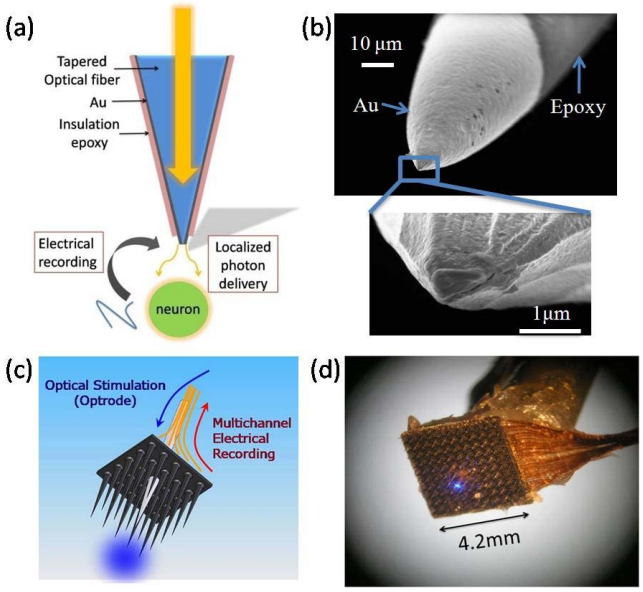

Figure 1 Single optrode and optrode-MEA device. (a) Concept schematic of single optrode. (b) SEM images of the optrode tip. The exposed metallic part of the tip is approximately 50 µm, appearing brighter in the upper image. (c) Schematic of the hybrid device. The optrode is coupled to the MEA through a laser drilled hole. (d) An optical microscope image of the device, showing blue laser light emanating from the tip of the optrode.

The capability of the optrode as a probe for recording extracellular electrophysiological neural activity is determined by its bandwidth and root-mean-square noise, which in turn are dictated by its electrical impedance. Figure 2(b) shows the average impedance spectrum of eight separately fabricated optrodes. The plot of impedance versus frequency indicates that impedance has a reactive element and is thus frequency dependent, varying from 2MΩ to 100kΩ over the plotted range of frequencies. Of particular significance to electrophysiological neural recordings are impedances at f≒1 kHz, which lie between 112 kΩ and 671 kΩ in the case of the optrode. Moreover, the electrical impedance is relatively invariant around 300-

2kHz for spike recordings and 10-300Hz for local field potential (LFP) recordings, making the optrode a viable device for both types of neural data recording. It has also been shown that the optrode impedances remain the same when light is coupled into it. Figure 2(c) shows 24 spontaneous spike waveforms recorded by a single optrode in a test experiment using mouse retina (experiment details not shown), overlaid in a 2 ms window. The consistency in shapes and amplitudes of the waveforms indicate that these are single-unit spikes from one ganglion cell. The peak-to-peak amplitudes of the spikes are between 100 and 500 µV, while RMS noise is 14.3 ± 44.6 µV. Thus, the *SNR* for the optrode-recorded spikes lies between 1.12 and 5.60, which is comparable with that of standard extracellular electrodes in the average scenario.

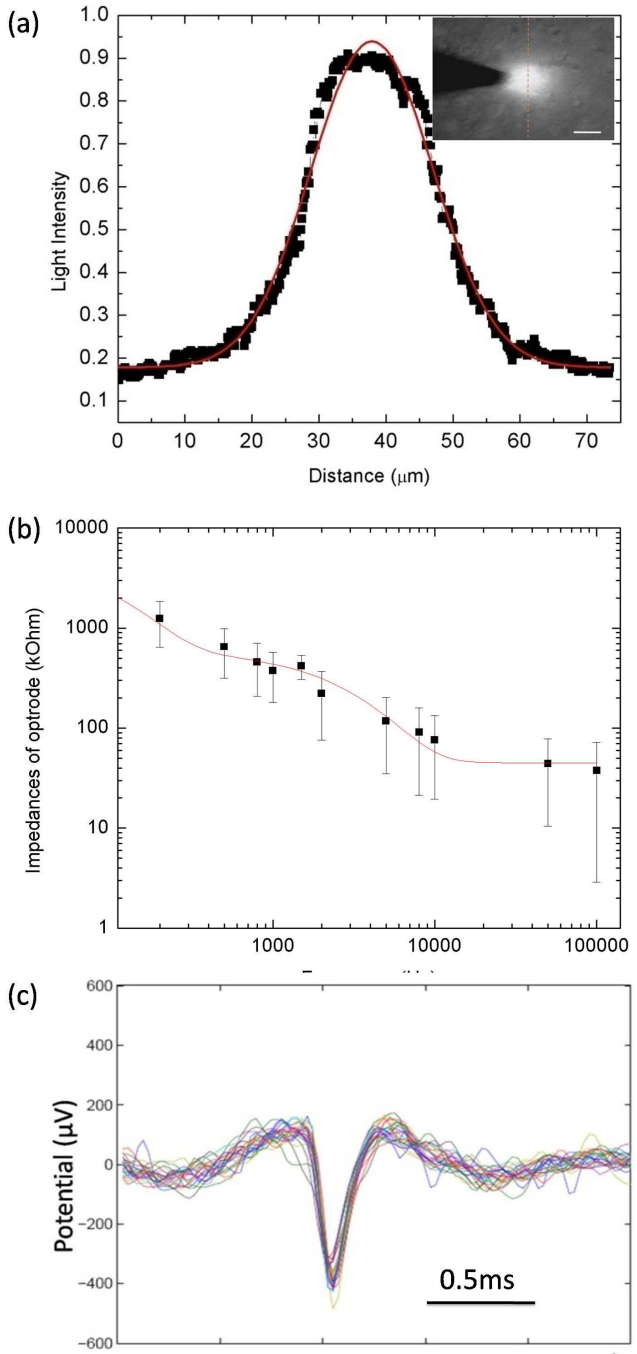

Figure 2 The electrical and optical characteristics of single optrode. (a) A DIC image of the scattered 440 nm light from the optrode in a mouse brain slice. The orange dotted line shows the axis along which the relative scattered intensity distribution is analyzed. Gaussian fit variance is 18 µm. The scale bar in is 20 µm. (b) Average impedance spectrum of optrodes (n=8). The solid line represents a double exponential fit. (c) 24 spike waveforms from mouse retinal ganglion cells (experiment details not shown) overlaid in a 2 ms window. All signals are bandpass-filtered at 250 Hz-4.8 kHz and thresholded at -300 µV.

I. SPATIOTEMPORAL MAPPING OF LIGHT INDUCED EPILEPTIC WAVE IN CHR2 MOUSE BRAIN SLICES

The functionality of the hybrid optrode-MEA device was verified in a disinhibited mouse cortical slice. Synaptic interactions have been shown to be critical for synchronization of neuronal bursting activity [9]. Thus, in a neuronal network where the inhibitory synapses are blocked, the activation of a localized neural population can lead to bursting events propagating throughout the entire network. Pharmacological agents serving to block cortical inhibition are widely used to model epileptogenesis in *in vitro* studies, and are typically coupled with the use of electrical stimulation as a trigger to evoke epileptiform activity in a controlled manner. The latter is achieved by passing square, bipolar current pulses (typically hundreds of µs long and ~10-100 µA in amplitude) through the disinhibited tissue. Use of electrical stimulation, however, is complicated by technical problems such as the presence of large current transients ("stimulation artifact") and the non-specific targeting of neural tissue, which make the development and use of optogenetic technique with light stimulation a promising alternative.

P14 mice (CD-1 Charles River) were transfected by intracranial injections of ChR2-EYFP(Enhanced Yellow Fluorescence Protein) lentivirus solution and the experiment was carried out two weeks after the injection. The ability of a single optrode to excite ChR2 expressing pyramidal neurons was characterized in a mouse cortical slice, where neural response was recorded from a single cell by patch-clamp methods (data not shown).

The functionality of the hybrid optrode-MEA device was verified in a disinhibited mouse cortical slice. One significant advantage of using this device for studying epileptiform wave propagation in an *in vitro* setup is its ability to simultaneously sample a large area of neocortex, while also probing other accessible areas of the brain such as white-matter axon bundles and hippocampus. Since epileptiform waves require synchronous population bursts, it is necessary to "externally bias" the intrinsic neuronal

network by applying pharmacological agents, such as the blocker, Picrotoxin (PTX), which we have employed in our work.

We hypothesized that direct optical stimulation of a small population of light-sensitive cortical neurons would be able to evoke epileptiform waves in the neocortical neural network. This assumption was tested in ChR2 transfected cortical slices, where the optrode-MEA device was used to study the 2-D spatiotemporal propagation of optically-induced epileptiform waves.

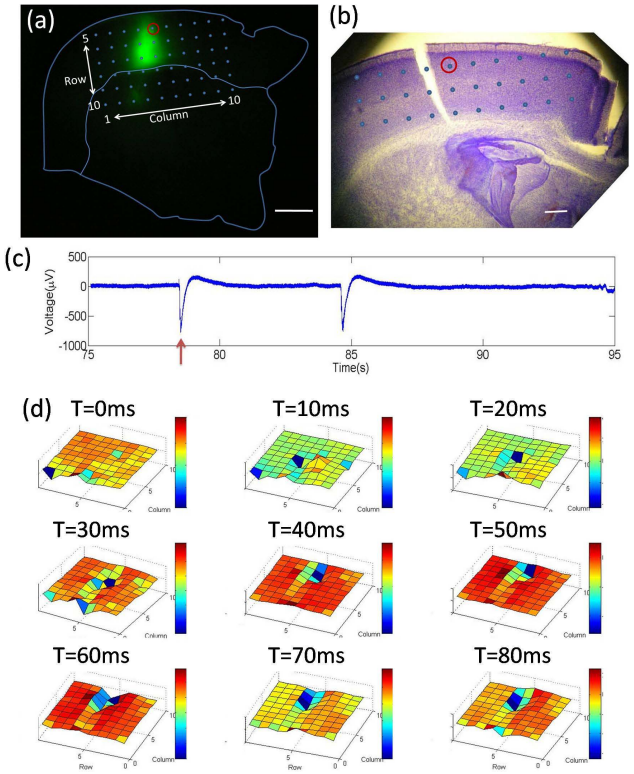

Figure 3 (a) EYFP fluorescence and the positions of the electrodes in the coronal slice. Scale bar is 1mm. (b) Histology of the ChR2 transfected slice with Nissl staining shows the locations of the electrodes in the neocortex. The red circle represents the position of the optrode. Scale bar is 400 μm. (c) A typical electrophysiological recording of epileptic waves (captured on an MEA electrode). The red arrow indicates the onset of the light stimulus. The first event on the plot is in response to the optical trigger, while the second one is spontaneous. (d) The spatiotemporal propagation of an epileptic wave from a time-lapse movie. The light triggered epileptic wave first appears at the T=78.46s frame, 400 μm below the optrode.

A fluorescent region of about 300 μm width was identified as the excitation target in the somatosensory cortex (Figure 3(a)). The array was aligned such that the "optrode" was level with the transfected tissue while several other MEA sites rested in the surrounding neocortical region. Histological analysis was used post-experimentally to determine the placement of electrodes in the MEA (Figure 3(b)).

Optical stimulation pulses (440 nm) with durations of 500 μs and incident peak power of 15 mW were coupled into the fiber and delivered to the center of the fluorescent region. Inter-stimulus intervals were about 60 s. Figure 3(c) shows two sample waveforms; one of the light-evoked seizure waves and one of a spontaneous wave. The 2D spatiotemporal propagation of the light-evoked epileptiform activity are detailed in the time-lapse movie of Figure 3(d). A negative deflection of 300 μV was detected at 400 μm below at T=78.46 s, 5 ms after the onset of the stimulus. The experimentally extracted velocity of the wave propagation from backward direction (to the left) was 3.3±0.3 cm/s while that in the forward direction (to the right) was 4.6±0.1 cm/s.

We have designed and fabricated a hybrid neural sensor and stimulation device integrating a tapered optical fiber (optrode) with a planar multi-electrode array, and demonstrated its use for controlled optical stimulation and spatiotemporal recordings of optogenetically transfected cortical tissue *in vitro*. Our initial case study of modulation of the initiation and propagation of epileptiform activities is one example of an application which is achieved by combining the optrode-MEA device with optogenetic methods.

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REFERENCES

[1] S. J. Cruikshank, T. J. Lewis, and B. W. Connors, "Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex," *Nature Neuroscience,* vol. 10, pp. 462-468, Apr 2007.

[2] H. S. Mayberg, A. M. Lozano, V. Voon, H. E. McNeely, D. Seminowicz, C. Hamani, J. M. Schwalb, and S. H. Kennedy, "Deep brain stimulation for treatment-resistant depression," *Neuron,* vol. 45, pp. 651-660, Mar 2005.

[3] P. Blomstedt and M. I. Hariz, "Hardware-related complications of deep brain stimulation: a ten year experience," *Acta Neurochirurgica,* vol. 147, pp. 1061-1064, Oct 2005.

[4] E. S. Boyden, F. Zhang, E. Bamberg, G. Nagel, and K. Deisseroth, "Millisecond-timescale, genetically targeted optical control of neural activity," *Nature Neuroscience,* vol. 8, pp. 1263-1268, Sep 2005.

[5] L. Petreanu, D. Huber, A. Sobczyk, and K. Svoboda, "Channelrhodops in-2-assisted circuit mapping of long-range callosal projections," *Nature Neuroscience,* vol. 10, pp. 663-668, May 2007.

[6] L. Campagnola, H. Wang, and M. J. Zyka, "Fiber-coupled light-emitting diode for localized photo stimulation of neurons expressing channelrhodops in-2," *Journal of Neuroscience Methods,* vol. 169, pp. 27-33, Mar 2008.

[7] V. Gradinaru, K. R. Thompson, F. Zhang, M. Mogri, K. Kay, M. B. Schneider, and K. Deisseroth, "Targeting and readout strategies for fast optical neural control in vitro and in vivo," *Journal of Neuroscience,* vol. 27, pp. 14231-14238, Dec 2007.

[8] D. J. Pinto, S. L. Patrick, W. C. Huang, and B. W. Connors, "Initiation, propagation, and termination of epileptiform activity in rodent neocortex in vitro involve distinct mechanisms," *Journal of Neuroscience,* vol. 25, pp. 8131-8140, Sep 2005.

[9] R. Miles and R. K. S. Wong, "Single Neurons can Initiate Synchronized Population Discharge in the Hippocampus," *Nature,* vol. 306, pp. 371-373, 1983.