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Chronically Implanted Hyperdrive for Cortical Recording and Optogenetic Control in Behaving Mice

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Abstract— **Neural stimulation technology has undergone a revolutionary advance with the introduction of light sensitive ion channels and pumps into genetically identified subsets of cells. To exploit this technology, it is necessary to incorporate optical elements into traditional electrophysiology devices. Here we describe the design, construction and use of a "hyperdrive" capable of simultaneous electrical recordings and optical stimulation. The device consists of multiple microdrives for moving electrodes independently and a stationary fiber for delivering light to the tissue surrounding the electrodes. We present data demonstrating the effectiveness of inhibitory recruitment via optical stimulation and its interaction with physiological and behavioral states, determined by electrophysiological recording and videographic monitoring.**

I. INTRODUCTION

Neural engineering inherits from basic neuroscience the division of brain processes into sensory and motor components. However, in many contexts the division is artificial. For example, during active sensing, organisms adapt their behavior while extracting information from the environment, such as when we scan our eyes over a scene, or our fingers over an object. In this case, the output motion is part of a closed loop with the sensed input, and neither can be fully understood without reference to the other [1]. Much of neuroscience consists of "correlative" studies, that determine changes in neural activity subsequent to a stimulus in the case of sensation, and changes preceding a movement in the case of motor control. In "causal" studies, one instead alters neural activity through direct neural stimulation, and determines the impact on later behaviors. Given the closed-loop nature of many sensorimotor behaviors, the implication is that appropriate stimulation of sensory areas could inject information into this circular stream. It is likely that future neural prosthetics will need to combine sensory and motor components to achieve high functionality, for example, adjusting timing of stimulation to be consistent with the organism's motion into a virtual object [2].

Here we describe methods we have developed to combine stimulation of cortical areas with extracellular recording and behavioral monitoring, as a step towards real-time feedback control in behaving animals. We use the mouse whisker tactile sensory system to exploit an expanded range of genetic methods in an excellent model for active sensing. Our approach consists of designing an appropriate hyperdrive implant, expressing channelrhodopsin-2 (ChR2) in somatosensory cortex (SI), implanting the hyperdrive, and, after recovery, running repeated behavioral sessions.

II. METHODS

A. Hyperdrive design

Our design was adapted from hyperdrives previously employed to measure hippocampal activity in freely moving rats [3], [4], so we concentrate only on the changes from the standard approach. Briefly, a plastic base piece printed with stereolithography (American Precision Prototyping, Inc) was manually fitted with multiple microdrives (also called "top pieces") consisting of cannulae joined to a miniature screw with dental acrylic. Each microdrive can move independently. We loaded the drives with standard 4-wire twisted electrodes, or tetrodes, although using single wire electrodes or stereotrodes would require only minimal modifications. The tetrodes were connected via a solderless pin system to a custom printed circuit board (Sunstone Circuits, Sunnyvale CA), also called an electrode interface board (EIB), housing a surface mounted connector (Mill-Max, Oyster Bay, NY) for the electrophysiology acquisition system (Neuralynx, Inc, Bozeman MT).

The hyperdrive was modified from the original rat design to reduce the size and weight to acceptable scales for a mouse. We used smaller screws (#0000-160) and limited the number of microdrives to seven (six tetrodes and one reference), as well as eliminated as much material as possible from the drive body. The principal modification for optogenetic use was the incorporation of a optical fiber with a miniature ferrule connector (Doric Lenses, Inc, Quebec,

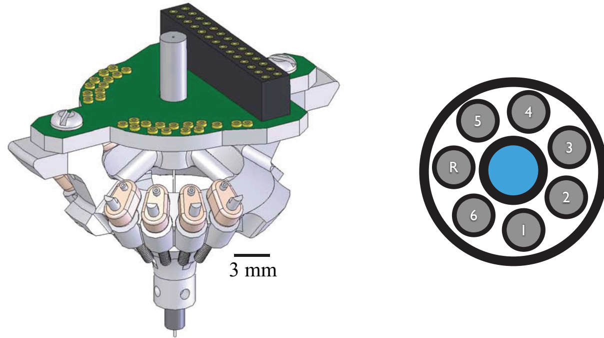

Fig. 1. *Left*: schematic rendering of the hyperdrive, showing the optical and electrical connectors, custom printed circuit board, STL base piece, and top pieces and screws for individually moveable microdrives. *Right*: schematic view of drive bottom, showing central optical fiber ringed by six guide tubes for tetrodes and one reference electrode.

Canada) along the center axis of the drive. The fiber was cut flush with the base of the drive, such that it could illuminate the cortical surface without penetrating into the tissue. Finally, a foil-and-plastic cone was fixed to the outside of the hyperdrive, in order to protect the tetrodes and provide electrical shielding. A rendering of the drive without protective cone is shown in Fig 1. Also shown is a schematic of the guide tube layout, looking into the bottom of the drive, with six tetrodes and a reference electrodes surrounding the optical fiber.

B. Optogenetic expression

In the experiments described in this paper, we expressed the light sensitive ion channel channelrhodopsin-2 (ChR2) in pavalbumin-positive (PV+) fast spiking inhibitory interneurons. We used a double-floxed virus (University of North Carolina Vector Core) [5] injected into the whisker-responsive "barrels" region of primary somatosensory cortex (SI) of PV-Cre transgenic mice (Jackson Labs, Bar Harbor ME) [6], [7]. Mice were injected one to six months prior to hyperdrive implantation, to allow ChR2 expression to reach maximum levels.

C. Implantation

Hyperdrives were implanted over SI following standard procedures [8]. Following resection of the scalp, a ground screw and up to 4 support screws were inserted into the skull. A craniotomy and durotomy were performed over left SI, adjacent to the injection site. The hyperdrive was aligned with the craniotomy, a drop of water-soluble gel (Surgilube) was placed around the base, and the implant site was sealed with dental acrylic. Following a 2-3 day recovery period, the electrodes were lowered into the brain. The reference electrode was targeted to white matter, while tetrodes were slowly advanced (e.g. around 80μm per day) from superficial to deep cortical layers during 1-4 months of recording.

D. Behavioral sessions

At the start of each behavioral session, a fiber optic tether with a matching ferrule was mated to the hyperdrive via a zirconia sleeve (Doric Lenses, Quebec, Canada), and a 24-channel headstage (Neuralynx, Bozeman, MT) was plugged into the other connector on the EIB. Once connected, all channels were briefly inspected to check appropriate gain and filtering. On some sessions, we also manually estimated tuning of neural activity to specific whiskers. After these initial checks, the mouse was placed in a polycarbonate arena, to engage spontaneous exploration of this "novel" environment; although the featureless arena is the same from day to day, we found animals robustly explored the environment for up to 30 minutes daily for up to several weeks.

We used a mini-DV camcorder (Sony, Inc) to monitor general behaviors throughout a session, and high speed videography (pco.1200hs, Cooke Corp.) to monitor exploratory whisker behaviors at higher resolution in short epochs [9]. Synchronization was accomplished by storing frame triggers and/or marker times on the physiology acquisition system, including laser pulse triggers. We stimulated ChR2 with a 473nm laser (Shanghai Dream Lasers or OptoEngine, Inc) directed into the fiber optic tether with a lensed coupler (Thorlabs, inc). Laser control was provided by Matlab (Mathworks, Natick, MA) routines and a PCI interface board (National Instruments).

E. Data analysis

Most analyses used custom routines written in Matlab. Tetrode recordings were separated into spike files (bandpass 600-6000 Hz, with 1 ms waveforms from all four wires saved to disk whenever any wire passed a voltage threshold) and local field potentials (LFP, bandpass 1-9000 Hz continuously sampled at 32 kHz from an individual wire). Note that although LFPs are typically lowpass filtered around 400 Hz and sampled at a reduced rate, we did not lowpass filter LFP channels during acquisition so that we could assess filter effects offline. Single unit isolation on the spike files was performed with MClust [10]. Video was manually scored for behaviors using custom GUIs in Matlab, although semi-automated whisker tracking could also be used [11], [12], [13].

III. RESULTS

A. Recruitment of inhibitory network

As an initial characterization of stimulation effects, we allowed mice to freely explore the behavioral arena, while providing optical stimulation at times unconnected to the behavior of the animal. In this way we sampled a range of behavioral "states" (e.g. actively exploring, quiescent, grooming) and responses to stimulation without explicit training or reward. After pilot sessions to determine effective ranges of stimulation parameters, we fixed the form of stimulation as brief (1 ms), high power (80 mW/mm^2) light pulses, delivered as single pulses or in trains up to 200 Hz.

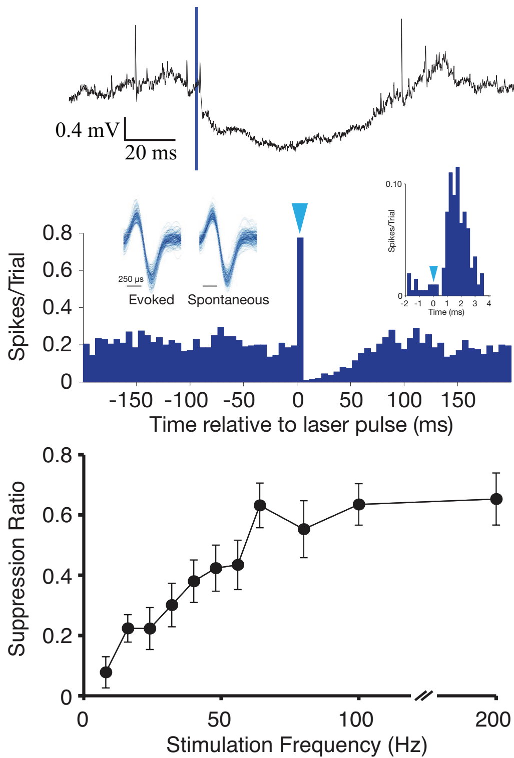

Fig. 2. *Top*: An example of a fast onset, long lasting LFP deflection following a single laser pulse (indicated by the vertical blue bar). The reduction in multi-unit activity appears in the smoothness of the trace post-stimulus; two spikes from a large single unit are also visible, as is a short-latency evoked spike. There is a mild rebound in activity following the inhibitory deflection. *Middle*: Laser pulse aligned peri-stimulus time histogram, showing short-latency, low-jitter evoked spikes (high peak at time zero, and expanded in *right inset*), followed by longer lasting reduction in multiunit spiking activity. The *left inset* shows Evoked waveforms, and waveforms found by template matching during Spontaneous (unstimulated) intervals. *Bottom*: Spike suppression ratio across a range of pulse train frequencies (n=34 regular-spiking units, 3 animals), showing further reductions in spike rate with increasing stimulation. Suppression ratio was calculated by dividing the average number of spikes during the 1 sec pulse train by the number of spikes in the 1 sec pre-stimulus interval.

Single pulses reliably recruited inhibitory activity, as determined by (i) fast onset, LFP deflections lasting 50 ms or more, (ii) selective short-latency recruitment of fast-spiking units, and (iii) a reduction in single and multi-unit spike rate following the pulse. These effects are shown in Fig. 2, and were not seen in sham-injected controls. We note also that selective recruitment of only inhibitory neurons (and hence inhibition onto principal cells) is difficult or impossible to achieve with electrical microstimulation [2]. The timescale of inhibitory effect is consistent with recruitment of inhibition for 50 to 100 ms following whisker stimulation in anesthetized animals [14], although we likely recruited the PV inhibitory network with greater synchrony than seen following natural inputs. There also appeared to be a slight rebound of activity at the end of the inhibitory period. Because of this, activation of the inhibitory network may be effective in aligning the spike times of post-synaptic targets rather than simply suppressing activity.

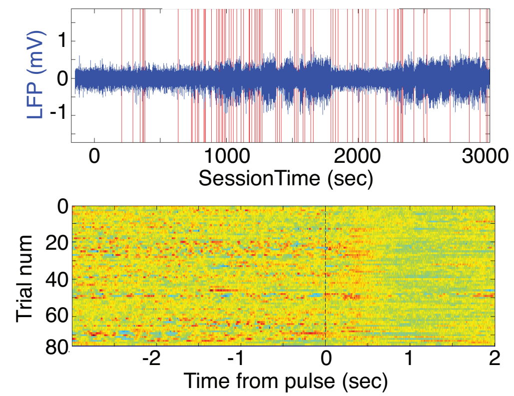

Fig. 3. *Top*: Example LFP over one full session, and motion onsets determined from video shown as vertical red bars. At this time scale, periods of large amplitude oscillations (predominately corresponding to quiescent behavior) appear as increased variance. *Bottom* LFPs shown as a colormap with red positive, aligned to all motion onset events in the session. The rapid cessation of low frequency oscillations at motion onsets is evident.

B. Opto-electric artifacts

Pulsed illumination of electrodes in saline can produce waveforms resembling RC charging and discharging [15], [5], [16]. Apparent deflections of LFPs during sustained illumination, or spikes during a short pulse, could be artifacts. In separate tests, we found artifact size depended on the surface area of uninsulated conductor in saline that is directly illuminated, and illumination duration (data not shown). Because our implanted electrodes advance away from a fixed fiber, the conducting tips are "in shadow" and less susceptible to artifacts, except at the most shallow depths. Moreover, with short pulses such artifacts were limited to before the onset of inhibition and were generally small (< 10 μV).

C. Interaction with behavior

Mice would typically begin a session by actively exploring throughout the arena, then exhibit increasingly long periods of grooming and immobility. We observed the well-known phenomenon of large amplitude, low frequency (around 10 Hz) oscillations generally observed during periods of immobility, e.g. spindles [17]. We found the incidence of spindling increased on average over the course of a session, but abruptly terminated at the onset of motion of the animal (Fig. 3).

Can mice "feel" the inhibitory network? Mice can learn to respond to optogenetic stimulation of excitatory SI neurons [18], and stimulation of the local PV population could be detectable due to its influence on network activity. However, given the high variability of behavioral and physiological states evident in Fig. 3, it is unlikely that every stimulation pulse will evoke the same effect (similar to, e.g. microstimulation in restrained rats [19]). Moreover, we did not train the mice to produce any particular response to stimulation. Nevertheless we sometimes observed apparently evoked, short latency changes in behavioral and physiological state similar to sensory-induced startles or freezes. Figure 4 shows three examples taken from a 30 minute session of single pulse

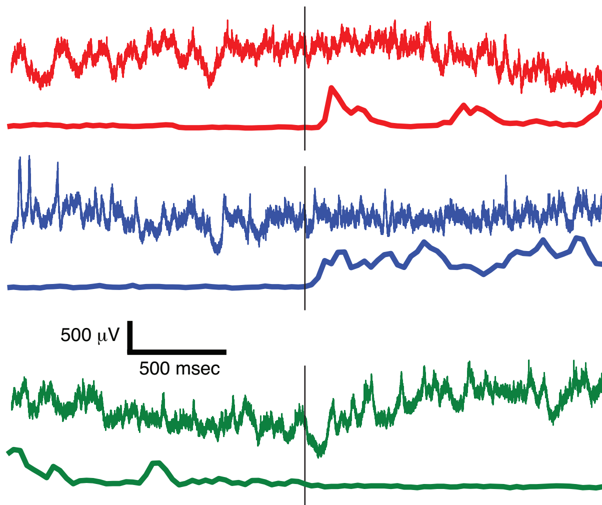

Fig. 4. Each pair of traces is an LFP (top) and motion signal (bottom, arbitrary units) aligned to a single pulse (vertical bar). In the top two examples, low frequency oscillations disappear upon stimulation, and motion begins with short latency. In the bottom example, motions cease following a light pulse, and low frequency oscillations arise.

stimulation at random intervals (mean interstimulus interval 8 sec). Responses were highly variable, including epochs of behavioral inactivity paired with ongoing low frequency oscillations, during which stimulation had little to no effect on either signal. Also, apparent behavioral responses could take many forms (e.g. whisking bouts, head motion, or postural changes), making it difficult to assess stimulation induced behaviors statistically. Future work will test these behavioral effects quantitatively via operant training.

IV. CONCLUSIONS

We adapted hyperdrive recording methods to include optical fibers for optogenetic stimulation of cortex. Using this approach, we activated fast-spiking inhibitory interneurons in freely behaving animals, and observed the effects on network activity and exploratory behaviors. The methods presented here are sufficiently general that they could be used to target other brain regions and, in combination with available optogenetic tools, excite and/or inhibit a wide range of different cell types. Because most of the hyperdrive parts can be manufactured through rapid-prototyping processes, customized designs can be created quickly and inexpensively. With slight modifications to the design, it will be possible to stimulate one region while recording from another, or incorporate multiple fibers and recording sites into the design.

We target the rodent whisker system as a powerful model for the study of active sensing, neural coding, and cortical organization and dynamics [6], [12], [20], [21]. Combined with real-time feedback and control systems, the methods we outline here should foster substantial advances in understanding sensory neuroscience. By leveraging the speed and specificity of optogenetic interventions with the fast readout provided by electrophysiology, we will be able to interact with neural circuits on their functional temporal scales.

V. ACKNOWLEDGMENTS

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