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Microelectrode array stimulation combined with intrinsic optical imaging: A novel tool for functional brain mapping

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HIGHLIGHTS

- Visualize cortical circuitry.
- Combined imaging and electrophysiological recording.
- Multiple simultaneous or sequential stimulation sites.

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ABSTRACT

Background: Functional brain mapping *via* cortical microstimulation is a widely used clinical and experimental tool. However, data are traditionally collected point by point, making the technique very time consuming. Moreover, even in skilled hands, consistent penetration depths are difficult to achieve. Finally, the effects of microstimulation are assessed behaviorally, with no attempt to capture the activity of the local cortical circuits being stimulated.

New method: We propose a novel method for functional brain mapping, which combines the use of a microelectrode array with intrinsic optical imaging. The precise spacing of electrodes allows for fast, accurate mapping of the area of interest in a regular grid. At the same time, the optical window allows for visualization of local neural connections when stimulation is combined with intrinsic optical imaging.

Results: We demonstrate the efficacy of our technique using the primate motor cortex as a sample application, using a combination of microstimulation, imaging and electrophysiological recordings during wakefulness and under anesthesia.

Comparison with current method: We find the data collected with our method is consistent with previous data published by others. We believe that our approach enables data to be collected faster and in a more consistent fashion and makes possible a number of studies that would be difficult to carry out with the traditional approach.

Conclusions: Our technique allows for simultaneous modulation and imaging of cortical sensorimotor networks in wakeful subjects over multiple sessions which is highly desirable for both the study of cortical organization and the design of brain machine interfaces.

1. Introduction

The cerebral cortex is functionally diverse, with specific regions being responsible for different sensory, motor, and higher cognitive functions. Its electrical excitability has lent itself well to mapping with electrical stimuli, something that led to the first proof of the so-called localization theory of brain function. Early studies included Hitzig's experiments on the victims of the Franco Prussian war (1870–1871) and, later, his and Fritsch's work in dogs (Koehler, 2010). These experiments were carried out using DC currents and large surface electrodes, which provided only crude maps of cortical function. The next few decades saw a number of refinements in the technique and resulted in the publication of the first detailed homunculus by Penfield and Boldrey (1937). Since then, microelectrode mapping of the cortical surface has remained a staple for localizing functional areas in the clinic as well as being an important experimental tool (Dum and Strick, 2002; Bruce et al., 1985; Bonini et al., 2014).

In addition to mapping, electrical microstimulation has proven useful for controlled modulation of sensory percepts and behaviors (Graziano et al., 2002; Murphey and Maunsell, 2007; Romo et al., 2000; Salzman et al., 1990; Tehovnik and Slocum, 2009) and for

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brain-machine interface applications (Tehovnik et al., 2009; Chase et al., 2012). However, despite the exciting advances in brain stimulation technology, the understanding of circuits underlying these behavioral effects remains indirect and limited.

To associate stimulation induced behavioral effects with underlying neural circuitry, one approach is to develop an *in vivo* functional tract tracing method. Unlike traditional anatomical tract tracing, *in vivo* functional tract tracing opens new avenues for studying cortical connections without sacrifice of the animal or time-consuming anatomical reconstruction, and, furthermore, enables study of circuits activated by the stimulation sites which induced behavioral effects. Such methods have been developed in conjunction with intrinsic signal optical imaging (Lieke et al., 1989; Godde et al., 2002; Brock et al., 2013; Stepniewska et al., 2011), voltage sensitive dye imaging (Sawaguchi, 1994; Kunori et al., 2014; Suzurikawa et al., 2009), and fMRI (Tolias et al., 2005; Moeller et al., 2008; Ohayon et al., 2013) following single site stimulation. These methods have revealed both local intra-areal and distant inter-areal connection patterns (Brock et al., 2013; Kaas et al., 2013).

Here, we further adapt this approach by using the Utah microelectrode array. Multielectrode arrays have introduced the possibility of mapping in a systematic grid with sufficient density to reveal local functional organization. The construction of multielectrode arrays in the early 1980s consisted of bundles of a few tens of wire electrodes. However, hand-building such a device was very time consuming and their utility was limited by the throughput of the computers used at that time to record the data at the necessary high sample rates. A few years later, a number of probes were developed using microlithographic materials processing, including the flat Michigan probe with contacts along its shank used to sample various cortical layers and the rectangular Utah probe, which is better suited for sampling millimeters of cortical area at a particular depth as well as flexible designs for surface stimulation (Hambrecht, 1995; Drake et al., 1988; Jones et al., 1992). Unlike probes designed solely for recording, these arrays are treated with a sputtered iridium oxide film (SIROF), making it possible to alternate between stimulation and recording at the same sites without degrading the electrode tip (Davis et al., 2012; Slavcheva et al., 2004).

Here, we demonstrate the feasibility of combining the use of the Utah multielectrode array with chronic optical imaging *in vivo* both in the anesthetized and the awake behaving monkey. We hope this capability will open new avenues for investigation, including the ability to reveal in parallel: (1) the functional architecture of a local (several millimeter) cortical region, (2) the functional architecture of connection patterns arising from multiple points within this local cortical region, (3) the relationship of these connection patterns to specific sensorimotor behaviors and (4) the modulation of cortical activation patterns in response to electrically stimulated behavioral modulation.

2. Methods

2.1. Surgical procedures

All procedures were performed in accordance with NIH guidelines and with the approval of the Vanderbilt Institutional Animal Care and Use Committee. Two rhesus macaque (*Macaca mulatta*) monkeys were sedated with ketamine (10 mg/kg), intubated and placed in a stereotaxic frame. The animals were ventilated with a 1–3% mixture of isoflurane in oxygen. Vital signs, such as expired CO_2, body temperature, heart rate and blood oxygen saturation were monitored continuously. A craniotomy and durotomy were performed to expose the brain for implantation of the array. A low-density functional map of the hand representation in premotor and primary motor cortical areas was obtained using a few (<10) microstimulation penetrations with a conventional parylene-coated tungsten microelectrode with an impedance of 1 MΩ (World Precision Instruments, cf. (Kaas et al., 2013)). The general location of the implanted array, chamber and major anatomical landmarks are shown in Fig. 1(A). When the appropriate area was located, the array (96 channels, 400 micron spacing, 1 mm shank length) was placed on the surface of the brain and its wire bundle was contoured with rubberized tweezers to conform to the curvature of the brain and minimize the torque on the array itself. A pneumatic injector (Blackrock Microsystems) was then lowered until it barely touched the array and ventilation was briefly stopped to minimize respiration-related brain pulsations during the injection process. The array was then pneumatically injected 1 mm into the brain (Fig. 1(B)). The wire bundle exiting the array was enclosed in rapid-curing biocompatible silicone (World Precision Instruments Kwik-Cast) *in situ* up to its point of termination in an implantable steel connector, which was secured to the head with bone screws. The silicone made it easier for the array to be removed *post mortem* from the surrounding cranioplastic cement. A custom-made rigid nylon chamber (20 mm outer diameter, 1 mm wall thickness, 6 mm wall height) was placed into the craniotomy and secured with bone screws and cranioplastic cement (Fig. 1(C) and (D)). Finally, a custom-made flexible hat-shaped silicone (Shin Etsu Chemical Co. KE-1300T) artificial dura was inserted inside the chamber with the edges tucked under the edges of the durotomy, allowing a clear view of cortex and the microelectrode array (Fig. 1(C) and (D)). The chamber was closed with a threaded cap and sealed with bone wax (Ruiz et al., 2013; Chen et al., 2002).

Post-surgical care included analgesic (buprenorphine) and anti-inflammatory agents (dexamethasone) for 3 days. The chamber was opened and cleaned under aseptic conditions at least once per week and maintained with a prophylactic antibiotic (Amikacin Sulfate).

2.2. Experimental procedure

We performed a series of experiments to demonstrate the utility of our technique. These included: (1) microstimulation with the Utah array under anesthesia to characterize the different movements evoked in each specific region of the brain covered by the array, (2) microstimulation with the Utah array combined with intrinsic signal imaging under anesthesia to visualize the cortical connections between the areas stimulated in nearby regions, and (3) multi-channel recordings of neuronal activity in an awake behaving animal.

2.2.1. Electrical microstimulation

Electrical microstimulation was performed under 0.5–1% isoflurane-oxygen/nitrous oxide mixture. To minimize contamination of imaged data by noise due to body movement, we conducted electrical stimulation evoked mapping and optical imaging acquisition at separate times, although it is possible to combine the two (Stepniewska et al., 2011). Cortical sites were stimulated using a programmable multi-channel microstimulator (Blackrock Microsystems CereStim), connected to the Utah array through an implanted pedestal. Stimulation consisted of biphasic 300 Hz trains of 100 pulses with a 200 μs pulse width and a 53 μs interphase interval (Stepniewska et al., 2011). For intrinsic imaging of cortical motor circuitry, the amplitude was set at 10 μA, high enough to generate a signal but in most cases too low to evoke movements. For characterization of movements, which was done in a separate series of experiments, the stimulation current was stepped until a just noticeable motor movement was produced; the level was identified as the threshold current level (Burish et al., 2008). Thresholds were typically lower in primary motor cortex than premotor