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Strategies for optical control and simultaneous electrical readout of extended cortical circuits

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HIGHLIGHTS

- We combined optogenetics with μECoG in mouse, rat, and macaque monkey.
- Optical stimulation was targeted to inhibitory as well as to excitatory neurons.
- Photovoltaic artifacts were clearly distinguishable from evoked neural activity.
- Artifacts could be minimized using transparent indium tin oxide electrodes.
- We discuss a broad palette of applications for μECoG combined with optogenetics.

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ABSTRACT

Background: To dissect the intricate workings of neural circuits, it is essential to gain precise control over subsets of neurons while retaining the ability to monitor larger-scale circuit dynamics. This requires the ability to both evoke and record neural activity simultaneously with high spatial and temporal resolution.
New Method: In this paper we present approaches that address this need by combining micro-electrocorticography (μECoG) with optogenetics in ways that avoid photovoltaic artifacts.
Results: We demonstrate that variations of this approach are broadly applicable across three commonly studied mammalian species – mouse, rat, and macaque monkey – and that the recorded μECoG signal shows complex spectral and spatio-temporal patterns in response to optical stimulation.
Comparison with existing methods: While optogenetics provides the ability to excite or inhibit neural sub-populations in a targeted fashion, large-scale recording of resulting neural activity remains challenging. Recent advances in optical physiology, such as genetically encoded Ca^{2+} indicators, are promising but currently do not allow simultaneous recordings from extended cortical areas due to limitations in optical imaging hardware.
Conclusions: We demonstrate techniques for the large-scale simultaneous interrogation of cortical circuits in three commonly used mammalian species.

1. Introduction

Electrophysiological recordings have led to tremendous advances in our understanding of the neural basis of perception, cognition, and action. However, recording constitutes only part of a larger toolkit required to understand the normal or pathological brain in terms of the underlying neural circuit. Precise causal

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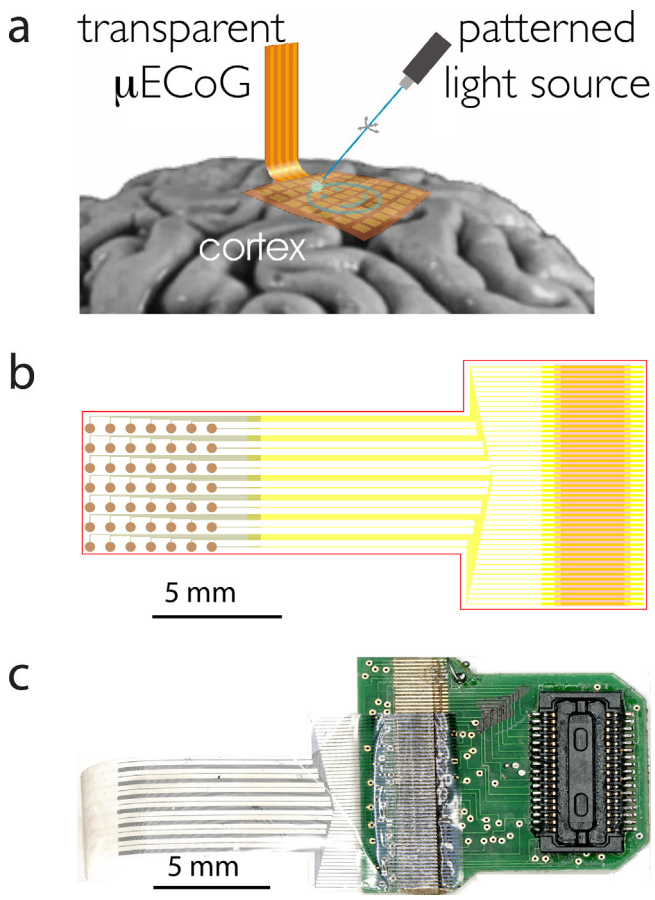

Fig. 1. Integration of μECoG with optogenetics. (a) Patterned light stimulation excites genetically modified neural tissue underneath a transparent μECoG array. Electrical activity recorded by the array could reveal the effects of stimulation, the characteristics of the underlying neural circuits, or be feedback-coupled with the light source to achieve precise control of the activation states on the cortex. © [2011] IEEE. Reprinted, with permission, from conference proceedings (Ledochowitsch et al., 2011b). (b) Computer-aided layout of the μECoG array: metal-traces are shown in yellow, ITO-traces and electrodes are shown in green, red indicates plasma-etched vias for the purpose of conductor exposure and device outline definition. (c) Photograph of transparent μECoG ACF-bonded to PCB equipped with Zif-Clip® compatible Hirose connectors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

intervention is also indispensable, yet we lack tools for modulating specific neural populations while simultaneously monitoring effects across large areas of cortex. Here we describe a broadly applicable strategy for performing such interventions, using a combination of optogenetics and μECoG (Fig. 1a).

Techniques have been developed for both optical and electrical stimulation as well as optical and electrical recording of neural activity. Electrical stimulation allows for higher temporal resolution than optical methods, as the kinetics of genetically encoded proteins that act as signal transducers may low-pass-filter the light stimulus. However, electrical techniques are limited in spatial and cell-type selectivity. In addition, stimulation artifacts render simultaneous electrophysiological recording and electrical stimulation at the same site very difficult. In parallel, in recent years, optical interrogation of neural activity has made tremendous progress, although the off-kinetics of even the best available Ca^{2+} sensitive fluorescent proteins such as GCaMP6 (Chen et al., 2013) is too slow to resolve single action potentials in vivo. State-of-the-art tools for combined optogenetic stimulation and electrophysiological recordings, such as 'optrodes' (Gradinaru et al., 2007), are destructive to neural tissue and cover only a tiny fraction of the cortex with relatively few stimulation and recording sites. While some studies have attempted to reduce the tissue damage caused by optrode penetrations, reported success was limited to recordings from single sites (Ruiz et al., 2013). In addition, photovoltaic artifacts caused by optical stimulation can also interfere with simultaneous electrophysiology recordings.

Optogenetic stimulation combined with μECoG recording constitutes a versatile and adaptable approach with potential to provide insight into the physiological mechanism of cortical function, and can serve as a platform to develop new stimulation-based therapies for neurological disorders. In 2011, we introduced transparent arrays that consisted of indium tin oxide (ITO) traces embedded in a transparent polymer (Parylene C) as a means of enabling optogenetic manipulation and recording from the surface of the brain (Ledochowitsch et al., 2011b). Building on our work, Kwon et al. (2013) demonstrated 16-channel arrays (ITO traces in Parylene C) with embedded LEDs as a light delivery mechanism in rats. Later, optical windows in mice have been used in combination with 16-channel μECoG arrays comprising platinum traces embedded in Parylene C (Richner et al., 2014). Most recently, Park et al. (2014) used graphene-based transparent arrays for optogenetics applications in rodents. Previous work on the combination of μECoG with optogenetics has mainly focused on engineering of the wafer-level devices and on the use of novel advanced materials. Most published studies either contain little biological data, typically limited to basic device testing in a rodent model (e.g. Park et al., 2014), or use conventional, small channel count μECoG (Richner et al., 2014). Moreover, many practical considerations such as the recognition and avoidance of photovoltaic artifacts, or modifications that meet the needs of different animal models, have not been sufficiently addressed. In this work, we emphasize the versatility of advanced, high-channel count μECoG in combination with optogenetics. Moreover, we show how to tailor experimental specifics to three different species, including non-human primate (NHP), a model in which the combination of μECoG with optogenetics is entirely novel.

We begin by detailing general strategies to avoid photovoltaic artifacts. The small size of rodent brains makes it difficult to confine light to transparent regions of the brain not covered by electrodes. We show that this issue can be solved by optical stimulation through fully transparent electrodes that are impervious to photovoltaic effects. The total area recordable with fully transparent electrodes is limited to approximately 1 cm^2 due to the brittle nature of the transparent conductor used (ITO) (Ledochowitsch et al., 2011b). This can be an issue, for example, for recordings from an entire hemisphere of a rat, and is a serious constraint for use in animal models with larger brains such as NHPs. However, we show that in animals with large cortical surfaces (NHP), the challenge of photovoltaic artifacts can be addressed by array design, without resorting to novel materials, simply by avoiding optical excitation of the electrode material.

Finally, we use transgenic mice to show that with our approach it is possible to capture complex and interesting effects of cell-type-specific optical stimulation in the form of spatially heterogeneous changes to the spectral composition of cortical potentials.

2. Methods

2.1. Characterization of transparent, flexible microelectrode arrays

The computer-aided design (CAD) of the electrode and wiring layout is shown in Fig. 1b. Fig. 1c shows the fully assembled and bonded transparent μECoG array with 49 recording contacts.

The fabrication process for Parylene C-based μECoG with metal electrodes is similar to the fabrication of flexible cables for neural