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A transparent epidural electrode array for use in conjunction with optical imaging

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HIGHLIGHTS

- We present a method to produce an exceedingly transparent electrode array.
- The electrode possesses epidural recording/stimulation capabilities.
- Optical signals from the cortex can be detected using the electrode array.
- Sensory-evoked LFP recordings were simultaneously performed with optical imaging.
- Neural activity was visualized following anodal/cathodal direct cortical stimulation.

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ABSTRACT

Background: Combining optical imaging and direct cortical stimulation can be a powerful technique for high-resolution functional mapping of the cortex. However, stimulating electrodes often obstruct the field of view, resulting in a lack of functional images behind the electrodes.

New method: To overcome this problem, we developed a transparent electrode array with 16-contact grids for epidural cortical stimulation. Using a commercially available indium tin oxide (ITO)-coated polyethylene terephthalate (PET) sheet, the electrode array was fabricated using a photolithography process. Because a complete circuit pattern, including the electrode contact itself, was formed in the transparent metal ITO layer, our electrode array was completely transparent and therefore could be used with optical imaging.

Results: Cortical stimulation was performed using the transparent electrode array. Evoked neural activity was successfully monitored through the array using a voltage-sensitive dye and optical imaging. The newly developed electrode array made it possible to detect optical signals from directly below the stimulating electrode. The electrode array could also be used for epidural recording of somatosensory evoked potentials.

Comparison with existing methods: A variety of surface electrodes for cortical recording and stimulation exist. However, this study aimed to make electrodes as transparent as possible. We provided a simple and low-cost fabrication process for producing the transparent electrode arrays.

Conclusions: Our transparent epidural electrode can be used for both stimulation and recordings without interfering with the detection of optical signals from the underlying cortex.

1. Introduction

A combination of intrinsic signal (IS) optical imaging and multielectrode array (MEA) recording represents a powerful method with which to investigate the functional architecture of the cortex *in vivo*. The good spatial resolution of IS imaging, and good temporal resolution associated with MEA recording, are in a mutually complementary relationship. The epidural and subdural MEAs used for this method should be transparent to prevent obstruction of the underlying cortex; a variety of MEAs endowed with transparency have been developed to date (Theriot et al., 2006; Ledochowitsch et al., 2011; Richner et al., 2014). In one embodiment, Theriot et al. (2006) fabricated a semi-transparent MEA by patterning a platinum electrode circuit onto a glass substrate, which reduced the

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proportion of non-transparent platinum traces to <5% in the final MEA. Because the effect of obstructions during optical imaging is negligible, these authors' transparent MEA successfully produced optical and electrical recordings *in vivo* (Theriot et al., 2012). However, this and other similar MEAs employing opaque conductive metals as electrode contacts have reportedly not been used as stimulation electrodes during optical imaging. One issue that should be considered is that low-impedance stimulation electrodes will require a larger contact-pad area, resulting in obstruction of the wider cortical imaging area. Therefore, to date few studies have used stimulating MEAs in conjunction with *in vivo* optical imaging.

There are two primary optical imaging techniques: IS- and voltage-sensitive dye (VSD)-based methods. The majority of previous *in vivo* studies have utilized IS optical imaging together with MEA recording (Stieglitz et al., 2010; Theriot et al., 2012). In contrast, *in vitro* electrophysiological studies have frequently combined VSD optical imaging with MEA dishes (Kim et al., 2014). For example, VSD imaging has been used to compensate for the reduced spatial resolution associated with MEA recording (Mapelli et al., 2010; Ferrea et al., 2012), and to characterize the overall relationship between membrane currents and voltage within functioning neural networks (Tominaga et al., 2001; Mann et al., 2005). Similarly, *in vivo* VSD imaging provides information that cannot be obtained using IS imaging, such as inhibitory neural activity in the cortex (Takashima et al., 2001). Therefore, combining VSD imaging with stimulating MEAs would likely be useful, but the benefits of such an approach remain to be demonstrated. Here we focused on a transparent MEA fully compatible with *in vivo* VSD imaging that can be used to stimulate the surface of the cortex.

Direct cortical stimulation is an important brain-mapping technique used during neurosurgical operations. The purpose of intraoperative mapping is to prevent impairment in language, motor, and sensory functions, while simultaneously allowing for the removal of the greatest possible amount of tumor. For historical reasons, the classical 50–60 Hz bipolar Penfield technique is commonly employed to map speech-related cortical areas, whereas both monopolar and bipolar cortical stimulation are used for motor mapping (Talacchi et al., 2013). Bipolar stimulation is generally considered more focal; however, because the actual dispersion of stimulus current has not been consistently studied, the degree to which stimulation selectivity depends on stimulus parameters remains poorly understood. In an important study by Haglund et al. (1993), the monkey visual cortex was stimulated with bipolar electrodes and the degree of cortical activation was monitored using IS optical imaging. The imaging results obtained successfully demonstrated the extent of the spread of stimulation, but unfortunately the electrode placed on the brain surface obstructed the cortex below it, thereby preventing quantification of the degree of activation directly under the stimulation site (Haglund et al., 1993).

In addition to mapping, cortical stimulation can be utilized to monitor motor-evoked potentials (MEPs) to assess the intraoperative integrity of the corticospinal pathway. Changes in MEP latency and amplitude serve as warning criteria during surgery and are also of prognostic value. To achieve reliable MEP recording, Gorman (1966) demonstrated that anodic stimulation requires a lower level of stimulation intensity to trigger MEPs compared to cathodic and bipolar stimulation. Taniguchi et al. (1993) further reported that high-frequency (300–500 Hz) anodic stimulation was more effective in lowering stimulation intensity, and only five pulses were required to trigger an MEP under general anesthesia. Although neurophysiological parameters used during MEP recording are currently determined based on these reports, the most suitable and safe stimulus parameters remain to be elucidated.

In recent years, cortical stimulation has also been used during clinical treatment. Specifically, clinical trials have demonstrated that epidural motor cortex stimulation is an effective treatment for neuropathic pain (Holsheimer et al., 2007) and for motor deficits after stroke (Kim et al., 2008). However, since the underlying treatment mechanisms are unclear, there are many uncertainties concerning the most effective electrode configuration and stimulus parameter selection. Therefore, in clinical practice anodal/cathodal monopolar or bipolar stimulation are occasionally chosen (Lefaucheur et al., 2009); optimal stimulus conditions may need to be empirically determined on a case-by-case basis (Lefaucheur, 2013). For neurosurgeons and clinicians, one of the most problematic technical issues associated with direct cortical stimulation is the fact that the spatial extent, and intensity of evoked neural activity after direct cortical stimulation, cannot be seen.

In this paper, we describe a method for producing an exceedingly transparent MEA using a commercially available indium tin oxide (ITO)-coated polyethylene terephthalate (PET) sheet. We demonstrate that our electrode has epidural recording/stimulation capabilities without field-of-view obstruction when combined with optical imaging. Our electrode array appears particularly promising when used for stimulation purposes because it enables visualization of all neural activity in the cortex, that it itself elicits through direct cortical stimulation. Here, we show the spatiotemporal spread of neural activity that originates just below the anodal or cathodal stimulating electrode. This is the first VSD imaging report to unveil neural activity under a stimulating electrode placed on the cortical surface, revealing distinctive features of cortical activation depending on the stimulation polarity. Following the intraoperative MEP monitoring procedure, we applied high-frequency anodic stimulation to the brain surface and demonstrated that it effectively induces neural activation in the sensorimotor cortex. Immediate visualization of activation after applying different stimulation parameters will be useful for the optimization of these parameters; therefore, imaging results obtained using animal models might inform the design of efficacious and safe protocols for electrical cortical stimulation in humans.

2. Materials and methods

2.1. Electrode fabrication

We used ITO, a transparent conductive polymer, as an electrode material to construct a circuit pattern comprising electrode contacts, terminals, and interconnecting wires between them. We used a conventional photolithography method to fabricate custom-made MEAs (Fig. 1). An ITO-coated PET film (749745-1EA; Sigma-Aldrich, St Louis, MO, USA) was used as the electrode array substrate. The PET film thickness was 0.127 mm. The ITO coating had a thickness of 0.1 μm and sheet resistance of 200 Ω/sq. The ITO-coated PET film was highly transparent for optical imaging (transmittance: >80% at 480–680 nm) and flexible enough to follow the curvature of the cortex.

The fabrication procedures used were as follows. First, the 1-μm-thick positive photoresist (FPPR-200; Fuji Chemicals Industrial, Tokyo, Japan) coating was added onto the substrate by a spin-coating method: a small amount of photoresist material was applied to the center of the substrate, which was then attached to a magnet and rotated at high speed (1000 rpm) using a laboratory magnetic stirrer. After prebaking at 90 °C for 2 min on a hot plate, the electrode pattern was exposed to ultraviolet (UV) light (emission peak at 380 nm and light intensity of 3.2 lumen; BOX-S1100; Sunhayato Corp., Tokyo, Japan) for 5 min through mask #1 (Fig. 1A(2) and B). This mask included the complete electrode circuit pattern. The mask was designed using the Corel Draw software package (Corel Corp., Ottawa, Ontario, Canada) and then printed onto a clear transparent label (thickness = 0.14 mm) for use with