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Intact skull chronic windows for mesoscopic wide-field imaging in awake mice

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HIGHLIGHTS

- Created bilateral chronic windows for imaging through the intact mouse skull.
- Includes a head-fixing screw for immobilizing the head during awake imaging.
- Preparation is stable for awake, widefield imaging of neuronal activity with GCaMP6.
- Functional connectivity maps were generated from awake spontaneous activity.

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ABSTRACT

Background: Craniotomy-based window implants are commonly used for microscopic imaging, in head-fixed rodents, however their field of view is typically small and incompatible with mesoscopic functional mapping of cortex.
New method: We describe a reproducible and simple procedure for chronic through-bone wide-field imaging in awake head-fixed mice providing stable optical access for chronic imaging over large areas of the cortex for months.
Results: The preparation is produced by applying clear-drying dental cement to the intact mouse skull, followed by a glass coverslip to create a partially transparent imaging surface. Surgery time takes about 30 min. A single set-screw provides a stable means of attachment (in relation to the measured lateral and axial resolution) for mesoscale assessment without obscuring the cortical field of view.
Comparison with existing methods: We demonstrate the utility of this method by showing seed-pixel functional connectivity maps generated from spontaneous cortical activity of GCAMP6 signals in both awake and anesthetized mice in longitudinal studies of up to 2 months in duration.
Conclusions: We propose that the intact skull preparation described here may be used for most longitudinal studies that do not require micron scale resolution and where cortical neural or vascular signals are recorded with intrinsic sensors or in transgenic mice expressing genetically encoded sensors of activity.

1. Introduction

The current generation of optogenetic tools provides unprecedented possibilities for selectively mapping and manipulating network elements within the rodent brain (Emiliani et al., 2015). As a result, these methods are growing in popularity for studying the relationship between neuronal activity and behavior in awake mice. Two approaches for delivering light to the brain are to either implant a light fiber, or to create a cranial window that provides optical access to the underlying cortex. The fiber-based approach can target subcortical structures that are not accessible via cranial windows (Aravanis et al., 2007; Gaffield et al., 2015), and can sample a field of view as large as 2 mm with the use of head-mounted microendoscopes (Jennings et al., 2015). However, mesoscopic mapping of cortical function – a spatial scale where the relationship among several functional regions can be examined (Oh et al., 2014; Silasi and Murphy, 2014) – requires optical access to

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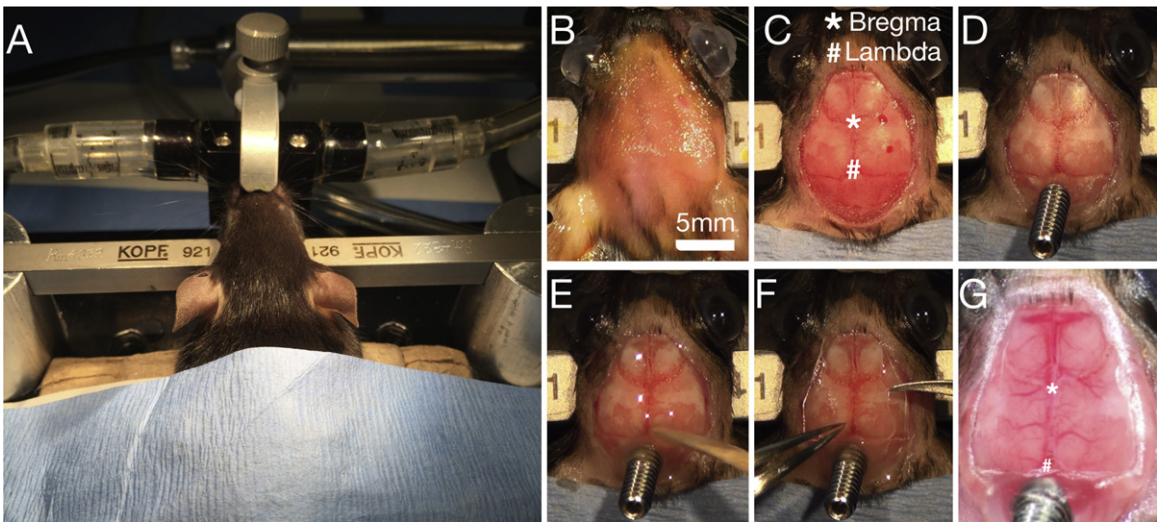

Fig. 1. Surgical procedure for chronic window implant. Anesthetized mice are stabilized in a stereotaxic frame (A), the skin between the ears and eyes is shaved (B) and the skin covering the occipital, parietal and frontal bones is cut away (C). The underlying skull is cleared of fascia and a setscrew is attached with dental cement over the occipital bone (D). A thick layer of dental cement is applied over the exposed (intact) skull (E) and a piece of coverglass is carefully lowered over the cement (F). In contrast to the opaque nature of the dry skull (C, D), once the chronic preparation dries, surface vessels are clearly visible through the intact skull (G).

an even larger area, with preferably non-invasive preparations to resolve map-like activity.

Cranial windows, such as those developed for chronic 2-photon microscopic imaging, were initially applied in optogenetic studies (Dombeck et al., 2007; Holtmaat et al., 2009), however, the relatively small field of view limits their application in mesoscopic brain mapping, and the invasiveness of the preparation may produce inadvertent brain damage and inflammation (Yang et al., 2010). Several research groups began to take advantage of the semi-transparent nature of the mouse skull by generating large, bihemispheric windows through either the intact (Guo et al., 2014), or partially thinned skull (Silasi et al., 2013). Although retraction of the skin alone can have some unwanted effects, such as significant brain cooling in anesthetized mice (Kalmbach and Waters, 2012), the reduced invasiveness offered by transcranial windows has made this the preparation of choice in a number of imaging applications (Cang et al., 2005; Yang et al., 2010; Yoder and Kleinfeld, 2002) including wide-field imaging of hemodynamic signals in anesthetized preparations (Kalchenko et al., 2014; White et al., 2011), and targeted photostimulation in awake (Hira et al., 2009) or behaving mice (Hira et al., 2015). So far, however there have been no detailed methodological descriptions for these preparations, nor has this technique been applied for chronic, wide-field functional imaging in awake mice.

Here we describe a chronic transcranial window preparation that we have previously used for anesthetized imaging (Vanni and Murphy, 2014), and demonstrate its feasibility and stability for bi-hemsipheric wide-field imaging of spontaneous activity in awake mice. Seed pixel functional connectivity (correlation) maps generated from spontaneous activity in awake mice with chronic windows show similar patterns of connectivity to the anesthetized state, indicating that our preparation can facilitate functional cortical mesoscopic mapping in different brain states.

2. Method

2.1. Animals

All procedures were approved by the University of British Columbia Animal Care Committee and conformed to the Canadian Council on Animal Care and Use guidelines. Transgenic GCaMP6s mice ($n = 11$) were produced by crossing Emx1-cre (B6.129S2-Emx1$^{tm1(cre)Krj}$/J, Jax #005628), CaMK2-tTA (B6.Cg-Tg(Camk2a-tTA)1Mmay/DboJ, Jax #007004) and either TITL-GCaMP6s (Ai94; B6.Cg-Igs7$^{tm94.1(tetO-GCaMP6s)Hze}$/J, Jax #024104) or TITL-GCaMP6f (Ai93; B6.Cg-Igs7$^{tm93.1(tetO-GCaMP6f)Hze}$/J, Jax #024103) strains (Madisen et al., 2012). The presence of GCaMP expression was determined by genotyping each animal with PCR amplification. This crossing is expected to produce stable expression of GCaMP6 specifically within all excitatory neurons across all layers of the cortex (Vanni and Murphy, 2014). Control experiments, assessing the effects of movement-induced changed in cerebral blood volume and consequent filtering of fluorescence, were performed in Thy1-GFP-M mice ($n = 2$; Jax #007788).

2.2. Surgical procedures

2.2.1. Sterile surgery and animal preparation

A sterile field was created by placing a surgical drape over the previously cleaned surgical table, and surgical instruments were sterilized with a hot bead sterilizer for 20s (Fine Science Tools; Model 18000-45). Mice were anesthetized with isoflurane (2% induction, 1.5% maintenance in air) and then mounted in a stereotactic frame in with the skull level between lambda and bregma (Fig. 1A). The eyes were treated with eye lubricant (Lacrilube; www.well.ca) to keep the cornea moist, and body temperature was maintained at 37 °C using a feedback-regulated heating pad monitored by a rectal probe. Lidocaine (0.1 ml, 0.2%) was injected under the scalp, and mice also received a 0.5 ml subcutaneous injection of a saline solution containing burprenorphine (2 mg/ml), atropine (3 μg/ml), and glucose (20 mM). The fur on the head of the mouse (from cerebellar plate to near the eyes) was removed using a fine battery powered beard trimmer, and the skin was prepared with a triple scrub of 0.1% Betadine in water followed by 70% ethanol (Fig. 1B). Respiration rate and response to toe pinch was checked every 10–15 min to maintain surgical anesthetic plane.

2.2.2. Chronic through-bone window implant

Prior to starting the surgery, a No. 1 circular cover-glass (Marienfeld, Lauda-Konigshofen, Germany; Cat#:0111520) was cut with a diamond pen (ThorLabs, Newton, NJ, USA; Cat#:S90W) to the size of the final cranial window (~9 mm diameter, Fig. 1). A skin flap