A flexible head fixation system for optical imaging and electrophysiology in awake mice

Martin Thunemann[1], Phillip Mächler[1], Natalie Fomin-Thunemann[1], Yichen Lu[2], Xin Liu[2], Duygu Kuzum[2], Anna Devor[1,3,4,5]

[1]Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093
[3]Department of Electrical and Computer Engineering, University of California, San Diego, La Jolla, CA 92093
[3]Department of Biomedical Engineering, Boston University, Boston, MA 02215
[4]Department of Radiology, University of California, San Diego, La Jolla, CA 92093
[5]Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA 02129
Author e-mail address: martin@thunemann.de

Abstract: We describe a head fixation system allowing chronic microscopic imaging through cranial windows and optically transparent implanted microelectrode arrays in awake mice.

1. Introduction

An ongoing effort in many experimental neuroscience laboratories is to perform chronic/longitudinal optical imaging and neurorecording in awake behaving animals. In many cases, the animal needs to be head-fixed during data acquisition. The fixation system typically requires a headpost permanently attached to the animal's skull providing mechanical stability. Recent advances in nanofabrication technology led to the development of microelectrode arrays, which are largely or entirely transparent (e.g., [1,2]). These arrays, in combination with neurophotonics methods, allow for simultaneous acquisition of multimodal datasets. Here, we present a modular headpost system for optical imaging and electrophysiology (OIE) that allows chronic installation of microelectrode arrays. Our design needed to fulfil the following criteria: (1) Chronic implantation of microarrays with a lifetime of up to 6 months. (2) Access with microscope objectives of different size. (3) Headpost and headpost holder provide unhindered access to whisker pad for sensory stimulation. (4) The design can be adapted for different brain regions and larger exposures.

2. Methods

All parts were designed using CAD (Autodesk Fusion 360) and machined from titanium (OIE-headpost), stainless steel and aluminum (OIE-headpost holder). Parts that secure microelectrode arrays were produced using stereolithography. Animal surgery was performed as previously described [3]. The following procedure led to best results: After removal of the scalp and cleaning of the bone, skin is glued to the bone using VetBond (3M). Then, the skull is treated with etchant gel (35% phosphoric acid, Kerr); after etchant removal, the bone is covered with a thin layer of UV-curable primer (Optibond, Kerr). The bone where the craniotomy will be performed is spared from etchant and primer treatment. The headpost is installed using UV-curable dental cement (Kerr or Tectonics). After craniotomy, the glass window is fixed using UV-curable dental cement.

3. Results

Figure 1a shows the headpost with a central opening of 9 mm targeting the barrel field. We manufactured the OIE-headpost from titanium at a thickness of 1 mm. The OIE-headpost has an additional notch for installation of a reference screw over the cerebellum and three 2-64-UNF-threaded holes (red arrows in Fig. 1a). During implantation surgery, an installation aid attached to a stereotaxic arm allows reproducible placement of the headpost relative to the skull. The headpost holder consists of bottom and top part; the bottom part is installed on four Ø1/2-inch optical posts (Fig 1b). The top part of the OIE-holder is fastened with two 3-56 UNF machine screws to the bottom part (Fig 1b). We tested different approaches to install and protect microelectrode arrays (Fig 1c) on the OIE headpost. First, the microarray is glued to the glass window using optical-grade glue (Fig 1c) and secured together with the window to the skull using dental cement. Then, the connector is placed inside a round cap fastened to the headpost (Fig 1e). For recordings, the cap is removed, and the array is connected directly to the recording equipment. Alternatively, the connector can be joined to a miniature interface board placed inside a protective housing that is secured to the headpost. We observed that OIE-headposts with microarray connectors remain intact for at least 3 months. The additional weight carried by the animal (3-6 g) does not impair well-being (weight loss or other signs of distress) under standard housing conditions.

4. Discussion

Combined optical imaging and electrophysiological recordings bridges the new world of genetically encoded probes of neuronal activity to 'gold standard' measures of spikes and field potentials. We generated a platform that combines these measurement modalities for chronic experiments in awake mice to be applied in the future for studying brain function in health and disease.

5. Acknowledgements

This work was supported by NIH grants (EY030727, MH111359, and NS057198). Philipp Mächler was supported by the Swiss National Science Foundation.

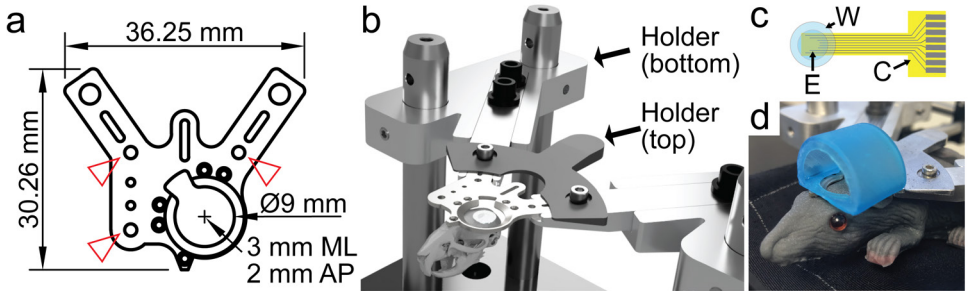

Figure 1. a) Headpost layout with notch for reference screw. Red arrows point towards 2-64-UNF-threaded holes. Center coordinates are given relative to bregma (ML, medial-lateral AP, anterior-posterior) **b)** Headpost with holder. The bottom part of the holder is mounted on optical posts, the top part secures the headpost to the holder. **c)** Surface microarray structure; the active part of the array with electrode pads (E) is glued onto the cranial window (W). For recording, the array is connected through the connector interface (C). **d)** A plastic cap (blue) securing the array (not shown) is fastened to the headpost; the cap is removed for recordings.

6. References

[1] Thunemann M, Lu Y, et al. (2018) Deep 2-photon imaging and artifact-free optogenetics through transparent graphene microelectrode arrays. Nat Commun 9:2035.

[2] Ganji M, et al. (2019) Selective Formation of Porous Pt Nanorods for Highly Electrochemically Efficient Neural Electrode Interfaces. Nano Lett 19:6244–6254.

[3] Desjardins M, Kılıç K, et al. (2019) Awake Mouse Imaging: From Two-Photon Microscopy to Blood Oxygen Level–Dependent Functional Magnetic Resonance Imaging. Biol Psychiatry Cogn Neurosci Neuroimaging 4:533–542.