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Windows and periscopes into primate behavior

Ryan A. Canfield,[1] Amy L. Orsborn,[1,2,3] and Gregory D. Horwitz[1,2,4,*]
[1]Department of Bioengineering, University of Washington, Seattle, WA, USA
[2]Washington National Primate Research Center, Seattle, WA, USA
[3]Department of Electrical and Computer Engineering, University of Washington, Seattle, WA, USA
[4]Department of Physiology and Biophysics, University of Washington, Seattle, WA, USA
*Correspondence: ghorwitz@uw.edu
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Calcium imaging of neurons in monkeys making reaches is complicated by brain movements and limited by shallow imaging depth. In a pair of recent studies, Trautmann et al., 2021 and Bollimunta et al. (2021) present complementary solutions to these problems.

Animal behavior is a result of the coordinated activity of neurons. Revealing relationships between neural activity and behavior requires longitudinal, stable recordings from multiple neurons simultaneously (or nearly so), ideally of known transcriptional type and location. Neuronal calcium imaging satisfies these demands. Applied to rodent models, calcium imaging has revealed much about the neural basis of sensory representations, motor representations, and neural plasticity (Chen et al., 2013). New efforts are underway to optimize calcium imaging for the macaque monkey (Tang et al., 2018; Benvenuti et al., 2018; Choi et al., 2018). The macaque monkey has a diverse, human-like behavioral repertoire, which makes it a scientifically and clinically important model, but its larger brain, greater cortical thickness, and susceptibility to intracranial infection present new challenges. Recently, two complementary studies met and overcame these challenges with distinct technical approaches.

Trautmann et al., 2021 imaged GCaMP6f-expressing neurons in primary motor cortex with a table-mounted 2-photon microscope (Figure 1A). Obtaining stable images in this preparation required significant technical innovations. A major challenge was to minimize movements of the brain relative to the microscope objective; the brain movements were particularly severe during the reaching behavior studied. The monkey's head was held using a customized three-point fixation system, and a tissue stabilizer applied gentle pressure to the imaged cortex. These technical advances were critical for stable and high-resolution imaging of individual neuronal somata and processes. Although imaging was feasible only at the cortical surface (< 600 μm), the high spatial resolution opened a window onto the activity of deep-layer neurons via calcium signals in their apical dendrites. This innovative approach broadens the utility of calcium imaging for studying deep-layer neurons and sets the stage to study dendritic computations in behaving monkeys.

Bollimunta et al. (2021) imaged neurons in the dorsal premotor cortex (PMd) with a surgically implanted, miniature endoscope (Figure 1B). One end of the endoscope interfaced with a camera that could be attached and removed in a matter of minutes. The other end terminated in a 1-mm-diameter prism lens that reflected light, periscope-like, from the top to the side and vice versa. One-photon excitation of GCaMP6f through this endoscope was used to image calcium signals from approximately 100 neurons simultaneously. Although the monkeys studied by Bollimunta et al. were free to move their heads, the imaged neurons moved remarkably little relative to the lens. The basis of this impressive stability is not entirely clear, but one possibility is that the lens served as an anchor, tethering the brain to the overlying dura mater and skull.

The strengths of both techniques are augmented by their compatibility with histological analysis. This analysis provides a detailed snapshot of the transcriptomic type, laminar position, and morphology of the recorded neurons and is a valuable complement to the dynamic activity captured by calcium imaging. Trautmann et al. used CLARITY volumetric imaging to reveal the 3-dimensional structure of recorded neurons and to trace the dendrites imaged in the superficial layers back to their deep-layer somata. Bollimunta et al. examined thin, fixed tissue sections to confirm that their recordings were from lower layer 3 and upper layer 4, which is deeper than can be imaged from the surface.

Both methods enable investigations of cortical population dynamics during rich, dynamic behaviors, including arm movements. Bollimunta et al. imaged PMd activity bilaterally in a monkey reaching to food rewards. They found that neurons within the imaging window had heterogeneous relationships to movements: neurons tuned for rightward reaches were immediately adjacent to neurons tuned to leftward reaches, and there were mixed preferences for the contralateral or ipsilateral arm. Imaging from the surface, Trautmann et al. found similarly heterogeneous tuning among neuronal somata and processes. Together, this pair of studies provides complementary insights into the organization of movement representation across the cortical sheet (Trautmann et al., 2021) and across layers (Bollimunta et al., 2021). These data contribute to continued debate about the spatial organization of motor cortex (Omrani et al., 2017), which calcium imaging can resolve more decisively than electrophysiology.

Both studies also showed that calcium signals can be used to accurately decode the animal's reach goals, and this is valuable for brain-computer interfaces. Additionally, Trautmann et al. developed a real-time pipeline to classify reach

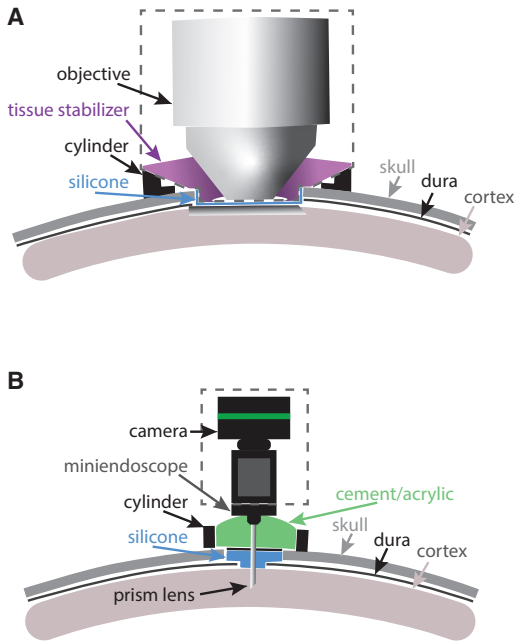

Figure 1. Schematic cranial implants for calcium imaging

A) The recording chamber of Trautmann et al., 2021 consists of a titanium cylinder (black), silicone artificial dura (blue), and tissue stabilizer (magenta).

B) The miniscope system of Bollimunta et al. (2021) consists of a miniature endoscope (black and gray), silicone elastomer (blue), aluminum cylinder (black), and cement/acrylic (green).

In both panels, dashed outlines enclose components that are not permanently attached to the skull.

direction as the monkey performed the task. This pipeline highlights the potential to incorporate calcium imaging methods into closed-loop approaches for interrogating neural function (Orsborn and Pesaran, 2017). However, additional advances will be needed to optimize decoding algorithms to calcium signals, the temporal statistics of which differ greatly from those of conventional electrophysiological signals.

Although both studies achieve similar goals, they use notably distinct methodological approaches with significant repercussions for flexibility and ease of use. The microscope objective used by Trautmann et al. was mounted to an external support and required daily calibration, whereas the endoscope used by Bollimunta et al. was attached to the animals' skulls and was essentially "plug-and-play." Endoscope data could be collected by relatively untrained personnel, and the self-contained implant design minimized the risk of infection. This ease-of-use comes at the cost of flexibility; once implanted, the endoscope cannot be easily moved. In contrast, the system developed by Trautmann et al. requires trained personnel who must open the chamber periodically, and this increases the risk of infection. A major benefit of this system is experimental flexibility; it can be used to image different neuronal populations each day over a relatively large area, to record electrical signals, and to modulate neural activity pharmacologically, electrically, or optically.

By providing distinct means to overcome the challenges of calcium imaging in behaving macaques, Bollimunta et al. and Trautmann et al. have laid a valuable foundation for future studies harnessing the strengths of optical methods. Fluorescent sensors of neurotransmitters and neuromodulators can be incorporated into either workflow to examine the dynamics of chemical signal transmission in behaving monkeys (Bi et al., 2021). The fact that many optical indicators can be encoded genetically opens the door to the targeted interrogation of specific cell types (Liu et al., 2020). Further work is needed to optimize decoding algorithms for calcium imaging to fully leverage its spatial resolution and stability. Nevertheless, these studies mark an exciting foray into the use of calcium imaging in behaving macaques, and they chart a course to new discoveries into the neural mechanisms of movement and cognition.

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