In Vivo Calcium Imaging to Illuminate Neurocircuit Activity Dynamics Underlying Naturalistic Behavior

The brain perceives the outside world, coordinates behavior, regulates emotional states, and forms memories about these experiences. To orchestrate such a diverse array of functions, billions of neurons interact to form circuits with the capacity to compute sensory infor-

mation and rapidly generate appropriate behavior. Understanding these processes and how the dysregulation of circuits contributes to psychiatric illness is a major goal of neuroscience research and has led to the development of novel tools for circuit-level analysis of the

Figure 1. Optical technologies for *in vivo* calcium imaging in freely behaving mice. (a) Mini-epifluorescence microscope attached to a head-mounted base plate that is centered around a microendoscope implanted above a brain region of interest. (b) A fiber probe is implanted above the target brain region of interest to collect bulk changes in calcium-mediated fluorescence using fiber photometry. (c) Animals engaging in naturalistic behavior such as social interaction while fluorescent transients are measured as a read-out of neural activity with the use of microendoscopic imaging (left) or fiber photometry (right).

brain in behaving animals (Jennings and Stuber, 2014). One approach for monitoring circuit-specific activity dynamics is *in vivo* calcium imaging. During periods of increased neural activity, dynamic fluctuations in calcium levels correlate with events such as action potential generation, exocytosis of neurotransmitters, changes in synaptic plasticity, and gene transcription. Thus, monitoring calcium dynamics with genetically encoded calcium indicators, such as GCaMP6, can serve as a proxy for neural activity within defined neurocircuits (Chen *et al.*, 2013). Imaging of neural activity using calcium indicators has largely been accomplished with *in vivo* two-photon microscopy, which provides unprecedented cellular and subcellular spatial resolution; however, two-photon imaging must be performed in head-fixed animals, greatly limiting the assessment of naturalistic behavior (Svoboda and Yasuda, 2006). To circumvent these limitations, new methods for visualizing and quantifying calcium-mediated fluorescent signals have been developed for use in freely moving animals.

Visualizing calcium transients in freely moving mice requires imaging devices that are small enough to fit on an animal's head and light enough to be carried by the animal. In the last few years, two such approaches have been developed: mini-epifluorescence microscopes used in conjunction with gradient index (GRIN) lens microendoscopes (Ziv *et al.*, 2013) and fiber photometry (Cui *et al.*, 2013; Gunaydin *et al.*, 2014) (Figure 1). Mini-epifluorescence microscopes are ideal for measuring somatic calcium activity dynamics. Importantly, with this novel technology, the activity of hundreds of genetically and spatially defined neurons within a single animal can be repeatedly imaged, permitting the analysis of cellular network dynamics that orchestrate behavior as well as the transition to pathological disease states (Ziv *et al.*, 2013). In addition, both microendoscopic imaging and fiber photometry are capable of recording neural activity many millimeters deep within the brain. Fiber photometry utilizes optical fibers to detect bulk changes in

calcium-mediated fluorescence in the soma (Cui *et al.*, 2013) or terminal fields (Gunaydin *et al.*, 2014) of genetically defined neurons. While fiber photometry lacks cellular resolution, it can provide important insight into the synchronous activity dynamics within a defined neurocircuit during both adaptive and aberrant behavioral states. Importantly, the light weight and small size of both these devices allows them to be coupled with a wide array of behavioral paradigms, such as sensory tasks, elevated plus maze for measures of anxiety, tail suspension for research related to depression, as well as complex learning paradigms utilizing operant chambers. Although advances are continuously being made in all aspects of these devices, current models of the mini-epifluorescence microscopes are not yet compatible with behavioral paradigms where mice are submerged in water, such as the forced swim test. Nonetheless, these innovative approaches can elucidate the temporal and spatial activity patterns of molecularly defined neurocircuits during a multitude of adaptive and maladaptive behaviors.

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DREAMM: A Biobehavioral Imaging Methodology for Dynamic *In Vivo* Whole-Brain Mapping of Cell Type-Specific Functional Networks

The human brain contains ~100 billion anatomically and neurochemically heterogeneous neurons that form 100 trillion brain-wide connections giving rise to a complex network whose interconnected activity regulates behavior. A major challenge in neuroscience has been to decipher the discrete functional connectivity underlying normal or pathological behavior.

Until recently, the primary methods for determining whole-brain functional activity as a consequence of discrete manipulation of specific cell populations was to study indirect markers such as c-Fos immunoreactivity in postmortem sections cut throughout the brain, or via *in vivo* blood oxygenation level-dependent measures using functional magnetic resonance imaging (fMRI), which requires experimental subjects to be fully immobilized. In an effort to overcome such limitations, we recently described the development and application of a new *biobehavioral imaging* methodology termed DREADD-assisted metabolic