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.

ACKNOWLEDGEMENTS

We thank Joshua Jennings for assistance in preparing the figure. We thank the Brain and Behavior Research Foundation, The Foundation of Hope, The Klarman Family Foundation, the National Institute on Drug Abuse (DA032750, DA038168) (GDS), and the Carolina Institute for Developmental Disabilities (T32HD040127) (SLR) for support.

Shanna L Resendez^{1,2} and Garret D Stuber^{1,2}

¹Departments of Psychiatry & Cell and Molecular Physiology, UNC Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA;

²Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
E-mail: gstuber@med.unc.edu

FUNDING AND DISCLOSURE

G.D. Stuber has received prototype versions of a mini-epifluorescence microscope system from Inscopix Inc. for testing purposes. The authors declare no conflict of interest.

Chen T-W, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A *et al.* (2013). Ultrasensitive fluorescent

proteins for imaging neuronal activity. *Nature* **499**: 295–300.

Cui G, Jun SB, Jin X, Pham MD, Vogel SS, Lovinger DM *et al.* (2013). Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature* **494**: 238–242.

Gunaydin LA, Grosenick L, Finkelstein JC, Kauvar IV, Fenno LE, Adhikari A *et al.* (2014). Natural neural projection dynamics underlying social behavior. *Cell* **157**: 1535–1551.

Jennings JH, Stuber GD (2014). Tools for resolving functional activity and connectivity within intact neural circuits. *Curr Biol* **24**: R41–R50.

Svoboda K, Yasuda R (2006). Principles of two-photon excitation microscopy and its applications to neuroscience. *Neuron* **50**: 823–839.

Ziv Y, Burns LD, Cocker ED, Hamel EO, Ghosh KK, Kitch LJ *et al.* (2013). Long-term dynamics of CA1 hippocampal place codes. *Nat Neurosci* **16**: 264–266.

Neuropsychopharmacology Reviews (2015) **40**, 238–239; doi:10.1038/npp.2014.206

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

Figure 1. Sagittal (a) and horizontal (b) views of a three-dimensional image reconstruction of DREAMM showing functional activation in the extended amygdala (ExA) and weaker activation in cingulate cortex (CG) and olfactory tubercle (OT) regions after remote chemogenetic activation (hM4Di) of prodynorphin-expressing periamygdaloid cortex neurons in behaving rats.

mapping (DREAMM) (Anderson *et al*, 2013; Michaelides *et al*, 2013). DREAMM leverages the ability of designer receptor exclusively activated by designer drug (DREADD) chemogenetic technology, which enables remote, *in vivo* activation or inhibition of distinct sets of neurons that result in discrete perturbations in brain activity and behavior (Armbruster *et al*, 2007), along with the unique capacity of positron emission tomography (PET) and [^{18}F]fluoro-2-deoxyglucose (FDG) for non-invasive, whole-brain, dynamic functional imaging in behaving humans and laboratory animals (Michaelides *et al*, 2013; Schulz *et al*, 2011; Villien *et al*, 2014). DREAMM is thus the first technique that provides simultaneously quantitative, dynamic, whole-brain and regionally unbiased, cell type-specific functional circuit mapping captured during the awake, freely moving state.

DREAMM affords the unique flexibility of combining multiple strategies—molecular, viral, transgenic, and pharmacological (inert ligands that specifically target designer receptors)—to probe, in a mechanistic

and unbiased manner, whole-brain functional networks and concomitant behaviors recruited upon stimulation or inhibition of a region- and neurochemically-specific cell type. In a typical DREAMM experiment, subjects are first exposed to a genetic engineering manipulation (eg, viral vector-based or transgenic model), resulting in regional or global expression of stimulatory or inhibitory DREADDs in targeted cell types. Subjects then undergo chemogenetic DREADD modulation by administering an inert ligand such as clozapine-*N*-oxide that specifically activates the DREADD and is combined with an FDG-PET behavioral imaging strategy. Either a ‘snapshot’ of whole-brain metabolic activity can be obtained during the DREADD-activated freely moving condition prior to imaging in a conventional PET scanner (Michaelides *et al*, 2013) or using head-mounted mobile PET scanner technology whole-brain metabolic activity is simultaneously measured during behavioral monitoring (Schulz *et al*, 2011).

DREAMM is currently the only imaging technology that relies on a

direct and well-understood process of cellular function (ie, glucose utilization) as its functional output. Moreover, DREAMM is not limited to FDG, and can be implemented along with a variety of other PET probes (even in the same subject) that target alternative/complementary cellular processes such as neurotransmitter dynamics, receptor signaling, and enzymatic processes, thereby allowing investigation not only of the global long-range functional networks involved in cell type-specific behaviors, but also of the underlying neurochemistry. Another unique advantage of DREAMM is its potential to be performed *completely* non-invasively via the use of transgenic animal models with cell type-specific DREADD expression. Finally, the non-invasive nature of DREAMM when capturing whole-brain *in vivo* functional activity allows for longitudinal assessments in the same subject and for it to be combined with a variety of model- and disease-specific behavioral paradigms (Figure 1).

To date, DREAMM has been used to map novel whole-brain functional activity patterns to changes in motor behavior induced by remote modulation of neighboring, yet heterogeneous basal ganglia pathways (eg, ventral striatonigral and striatopallidal medium spiny neurons). This approach using viral vectors with engineered DREADD receptors under the control of promoters specifically expressed in different striatal pathways illustrated the distinct recruitment of discrete whole-brain functional anatomical signatures and motor behavior upon remote activation of each striatal network (Michaelides *et al*, 2013). DREAMM has also been used to reverse translate the functional relevance of deficits in neuronal populations that have been virtually unstudied, such as periamygdaloid cortex prodynorphin neurons, in which disturbances were phenotypic of human heroin abusers and major depressive subjects. Novel insights obtained by DREAMM, in translational rodent models, revealed that remote inhibition of periamygdaloid prodynorphin neuronal activity

resulted not only in negative affective behavioral regulation, but also in a marked, selective functional activation of the extended amygdala circuit, a brain network well linked to negative affect (Anderson *et al*, 2013) (Figure 1).

In addition to experimental rodents, DREAMM is now being used in non-human primates and has the potential to be implemented in the future in humans (pending advances in gene-therapy safety/efficacy currently under development). The clinical relevance and translational therapeutics potential of DREAMM holds promise as an application for repeated, longitudinal, non-invasive assessment and monitoring of disease/therapy progression in patients undergoing DREADD-based neuromodulatory therapy. Furthermore, DREAMM would also have the capacity to be utilized alongside advanced cellular genetic engineering approaches such as combined DREADD and cell replacement therapeutics (Dell'Anno *et al*, 2014) to assess the efficient functional integration of such transplanted cells within neuronal networks.

In sum, DREAMM holds significant promise as a powerful, reverse-engineering strategy to visualize *in vivo*-specific neuronal ensemble networks associated with normal and pathologic behavior and to thus enhance knowledge about distinct neuronal circuits relevant to neuropsychiatric disorders.

Michael Michaelides^{1,2} and Yasmin L Hurd^{1,2}

¹Fishberg Department of Neuroscience, Friedman Brain Institute; ²Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA
E-mail: yasmin.hurd@mssm.edu

FUNDING AND DISCLOSURE

The authors declare that the work was funded by grants from NIDA (DA015446, DA023214, DA030359). MM was supported by the NIDA Postdoctoral Training Program at Icahn School of Medicine at Mount Sinai (DA007135). MM has received compensation from Metis Laboratories and owns stock in the company. YLH declares no potential conflict of interest.

Anderson SA, Michaelides M, Zarnegar P, Ren Y, Fagergren P, Thanos PK *et al*. (2013). Impaired periamygdaloid-cortex prodynorphin is character-

istic of opiate addiction and depression. *J Clin Invest* **123**: 5334–5341.

Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL (2007). Evolving the lock to fit the key to create a family of G protein-coupled receptors potentially activated by an inert ligand. *Proc Natl Acad Sci USA* **104**: 5163–5168.

Dell'Anno MT, Caiazzo M, Leo D, Dvoretzskova E, Medrihan L, Colasante G *et al*. (2014). Remote control of induced dopaminergic neurons in parkinsonian rats. *J Clin Invest* **124**: 3215–3229.

Michaelides M, Anderson SA, Ananth M, Smirnov D, Thanos PK, Neumaier JF *et al*. (2013). Whole-brain circuit dissection in free-moving animals reveals cell-specific mesocorticolimbic networks. *J Clin Invest* **123**: 5342–5350.

Schulz D, Southekal S, Junnarkar SS, Pratte JF, Purschke ML, Stoll SP *et al*. (2011). Simultaneous assessment of rodent behavior and neurochemistry using a miniature positron emission tomograph. *Nat Methods* **8**: 347–352.

Villien M, Wey HY, Mandeville JB, Catana C, Polimeni JR, Sander CY *et al*. (2014). Dynamic functional imaging of brain glucose utilization using fPET-FDG. *Neuroimage* **100C**: 192–199.

Neuropsychopharmacology Reviews (2015) **40**, 239–241; doi:10.1038/npp.2014.233

More than a Gut Feeling: the Microbiota Regulates Neurodevelopment and Behavior

The realization of the importance of the gut microbiota in health and disease has been one of the most exciting areas in biomedical research over the past 5 years. We are teeming with microorganisms in and on our body, having > 150 times more microbial genes (the microbiome) than mammalian genes. Studies are revealing how variations in the composition of the gut microbiota influence all aspects of physiology, including brain function and even behavior. In terms of neuroscience and psychiatric disease the field is still very much in its infancy, but evidence is accumulating that it has a key role. Factors that shape the bacterial landscape include being born by Caesarian delivery, not being breastfed, environment, gestational age, host genetics, exposure to infections (both maternal and infant), and antibiotic usage. Moreover, stress, especially that in early life and prenatally, can have marked effects

on microbiota composition (Borre *et al*, 2014).

A variety of strategies have been used to investigate the role of this so-called microbiota–gut–brain axis in health and disease. Germ-free mice (mice that never have been exposed to any bacteria) have been used to show that microbiota is crucial for hypothalamic–pituitary–adrenal axis function. Moreover, these mice have widespread neurodevelopmental changes in the brain, including alterations in monoaminergic neurotransmission and behavioral changes in anxiety (Foster and McVey Neufeld, 2013). More recently, we have shown that these mice also exhibit autism-like traits, such as deficits in sociability, social cognition, and increased repetitive behaviors (Desbonnet *et al*, 2014). Interestingly, as in autism and other neurodevelopmental disorders, these effects are much more pronounced in males than females. Moreover, studies in germ-free mice can be expanded to enable research on the ‘humanization’ of the gut microbiota, that is, transplanting faecal microbiota from specific human conditions or from animal models. Indeed, it has been shown that behavioral traits can be transplanted between strains of mice (Collins *et al*, 2013).

Administration of various potential probiotic strains in rodents or humans has also been shown to have beneficial effects in rodents. Major strain and species differences occur in terms of their ‘psychobiotic’ effects. Studies have shown that some *Bifidobacteria* and *Lactobacilli* species as well as *Bacteroides fragilis* can have positive effects on anxiety, depression, cognition, and autism-related behaviors (Hsiao *et al*, 2013). Prebiotics, non-digestible food ingredients that promotes the growth of beneficial gut microorganisms, are also being shown to affect brain BDNF levels. Human studies of prebiotics and probiotics have lagged behind to date, although there is interesting neuroimaging studies emerging clearly showing that such bacterial-based dietary interventions can affect brain function.