NEUROSCIENCE

All-optical electrophysiology in behaving animals

Three different optical strategies enable the manipulation and imaging of neural activity in awake mice.

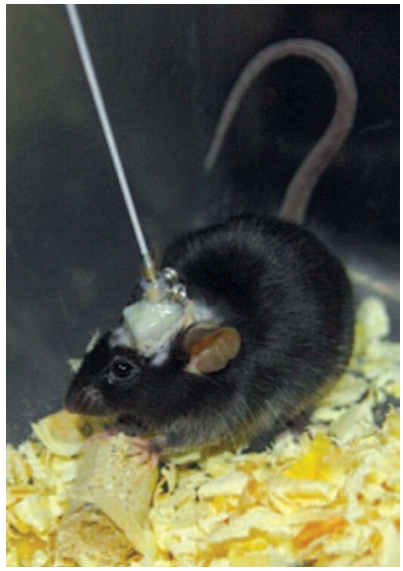
A major goal in neuroscience is the analysis of neural circuits involved in behavior. The optogenetic manipulation of defined neural populations and subsequent imaging of neural activity using calcium sensors can help address this goal. However, several challenges to performing all-optical electrophysiology *in vivo* must be overcome. Valentina Emiliani at the Université Paris Descartes thinks that these challenges are related to the spectral overlap of optogenetic actuators and calcium indicators as well as the requirement for simultaneous photostimulation of multiple targets with cellular resolution. Moreover, genetic tools to express optogenetic actuators are often not specific enough to target defined populations of neurons.

Optical methods to refine the pattern of optogenetic activation may overcome the latter problems, whereas new optogenetic tools, calcium indicators or photostimulation protocols may address the former issue. The groups of Emiliani, David Tank at Princeton University and Michael Häusser at University College London have recently independently developed strategies to simultaneously activate defined neurons with high spatial and temporal resolution in behaving mice.

For targeting individual neurons, the illumination of optogenetic actuators has to be targeted to defined areas with cellular resolution. Häusser and his colleagues used a spatial light modulator to split a laser beam into several individual beams and scan the neurons of interest in a spiral fashion (Packer *et al.*, 2015). With this approach, the researchers could activate multiple neurons at the same time while monitoring the activity of the targeted and other neurons with wide-field scanning at a different wavelength. This was made possible by using an optogenetic activator and a calcium sensor that can be spectrally separated under two-photon conditions. Tank and his colleagues used a different strategy to activate neuronal populations (Rickgauer *et al.*, 2014). They illuminated multiple neurons near-simultaneously by focusing a two-photon laser to a spot with the size of a neural soma and moving between neural targets with the help of fast scanning mirrors. Both groups applied their methods to awake, head-fixed mice running on a treadmill.

"Advantages of these methods compared to our method are the possibility of working [at] a greater depth and a better axial resolution allowed by the use of two-photon excitation, …[but they] are not compatible with the use on freely behaving animals, which was the main goal of our work," says Emiliani. The method established by her and her coworkers takes advantage of computer-generated holography techniques and spatial light modulators to establish defined photostimulation patterns (Szabo *et al.*, 2014). These patterns are then delivered to the brain area of interest with a fiberscope, which is affixed to the mouse skull and is also used to image calcium signals. Because they used one-photon illumination as well as actuators and sensors with overlapping spectral sensitivities, they optimized the imaging conditions to minimize optogenetic activation during imaging. However, it may be possible to use red-shifted optogenetic actuators, which could eliminate spectral overlap and also be helpful in improving imaging speed and the signal-to-noise ratio.

The fiberscope allows a variety of behaviors to be analyzed with all-optical electrophysiology because the animals do not need to be restrained. Emiliani thinks that "performing experiments in freely behaving animals would not only facilitate the experimental procedure … but would also provide a more 'physiological', less stressful environment for the animal (compared to a head-fixed situation)."



Peeking into a mouse brain with a fiberscope. Figure from Szabo *et al.*, Elsevier.

Depending on their experimental needs, researchers interested in all-optical manipulation and imaging of neural activity *in vivo* now have multiple options to choose from. The two photon–based approaches by the Tank and Häusser groups permit the analysis of neural circuits in head-fixed mice, with good axial resolution and at some depth: advantages that are inherent to two-photon microscopy. Emiliani's approach, on the other hand, is more suitable to the analysis of the neural circuitry underlying behaviors in unrestrained animals.

Nina Vogt

RESEARCH PAPERS

Packer, A.M. *et al.* Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution *in vivo*. *Nat. Methods* **12**, 140–146 (2015).

Rickgauer, J.P. *et al.* Simultaneous cellular-resolution optical perturbation and imaging of place cell firing fields. *Nat. Neurosci.* **17**, 1816–1824 (2014).

Szabo, V. *et al.* Spatially selective holographic photoactivation and functional fluorescence imaging in freely behaving mice with a fiberscope. *Neuron* **84**, 1157–1169 (2014).