NEUROSCIENCE

Imaging deep in the cortex and beyond

Combining red calcium indicators with an optimized illumination strategy enables neuronal activity to be imaged in deep layers of the mouse cortex and hippocampus.

It is now routine to image neuronal activity in superficial layers of the cortex using two-photon illumination and green, genetically encoded calcium indicators, but reaching deeper layers remains challenging, as scattering and aberrations degrade imaging quality. Illuminating with longer wavelengths as in three-photon microscopy, or wavefront shaping via adaptive optics can counteract these problems. Masanori Matsuzaki and colleagues from the University of Tokyo employ yet another strategy that builds on conventional two-photon microscopy.

The numerical aperture (NA) of an objective defines the range of angles over which light is emitted and accepted. Low-NA objectives emit light at a small angle, which shortens the path to the focal spot and minimizes scattering. However, this comes at the cost of a low collection efficiency due to the small acceptance angle. High-NA objectives can collect more light, but they are also more sensitive to scattering effects. Matsuzaki and colleagues take advantage of the high collection efficiency of high-NA objectives while effectively reducing the NA in the emission path by underfilling the back aperture with a narrow laser beam. This illumination strategy restricts scattering but also reduces axial resolution. The resolution is sufficient to image single neurons, however.

The researchers expressed red calcium indicators in the mouse medial prefrontal cortex or hippocampus and employed their illumination strategy to image neuronal activity up to a depth of 1,200 microns. They could image deep in the cortex for half an hour without inducing obvious signs of tissue damage. The researchers performed calcium imaging with their approach in head-fixed awake mice during a simple conditioning task. Imaging neuronal activity in hippocampus was possible as well; however, this structure could only be accessed in young mice, as the white matter above the hippocampus becomes more strongly myelinated with age, and this increases scattering.

The deep-imaging strategy of the Matsuzaki group is straightforward to implement and should be widely applicable.

Nina Vogt

RESEARCH PAPERS

Kondo, M. *et al.* Two-photon calcium imaging of the medial prefrontal cortex and hippocampus without cortical invasion. *eLife* **6**, e26839 (2017).