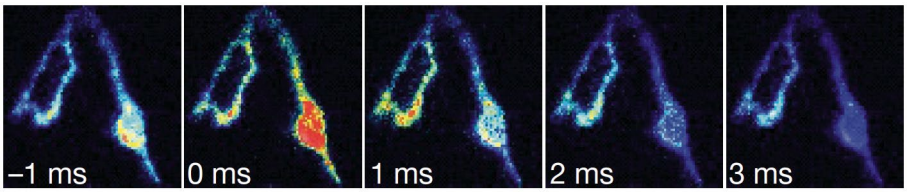
NEUROSCIENCE

Voltage imaging in vivo

Developments in genetically encoded voltage indicators and imaging strategies enable the recording of multiple neurons with good signal-to-noise ratios in behaving mice.



Voltage imaging in two neurons in the CA1 region of the hippocampus. Adapted with permission from Adam et al. (2019), Springer Nature.

Whereas genetically encoded calcium indicators are well-established workhorses in neuroscience, their voltage-sensitive counterparts are more finicky and have not seen much use in vivo. Reasons for their limited uptake include the need for high-speed imaging and their low signal-to-noise ratio in in vivo applications.

Adam Cohen from Harvard University has been working on getting voltage sensors ready for prime time for several years, even though he says that "I originally came into voltage imaging almost by happenstance." He was initially interested in the photophysics of microbial opsins and then made the jump to voltage imaging in neuroscience.

Cohen has been developing derivatives of Archaerhodopsin as voltage sensors. These worked well in cultured cells, "but then the big challenge was to get it working in tissue," says Cohen. Difficulties to address included poor trafficking of the voltage indicators to the plasma membrane and background fluorescence. In Cohen's lab, "much of the effort over the last five years has been chipping away at all of these different sources of systematic artifacts and signal-to-noise problems to overcome the ten- to thirtyfold improvement in signal-to-noise ratio needed in order to get this to work in live animals," says Cohen.

Adam Cohen and his team, including first author Yoav Adam, have now overcome these diverse challenges. Starting from the published QuasAr2, they worked to improve the expression levels and trafficking behavior of the sensor. In addition, they included a point mutation that had been shown to lead to photoswitching in another opsin. These modifications resulted in photoactivatable QuasAr3, or paQuasAr3. This sensor can be converted by blue light from a dark state D_1 to another dark state D_2, which can then exhibit fluorescence in a voltage-dependent manner. While this photoswitching property may appear unique at first glance, "many opsins have unusual photocycles and unusual photoswitching behaviors," says Cohen.

The researchers utilized this photoswitching behavior to devise an imaging strategy in which samples are illuminated simultaneously by both blue and red light, which results in improved signal-to-noise ratio. Specifically, "by intersecting the blue beam and the red beam, we can achieve a degree of optical sectioning akin to what's found in two-photon microscopy," explains Cohen, thereby reducing out-of-focus background. Furthermore, they patterned the red illumination such that only cell bodies were targeted, which further increased the signal-to-background ratio.

The researchers typically imaged in bouts of one to two minutes, but they also managed to record neurons for up to ten minutes, indicating that photobleaching during imaging is not prohibitive. The researchers could also image the same neurons on multiple days, suggesting that phototoxicity was minimal. However, Cohen points out that a bigger problem with long recording sessions is the amount of data being accumulated, with file sizes approaching 30–50 gigabytes.

In addition to the file sizes, analysis of the data is not trivial. "One of the trickier aspects of doing the voltage imaging is making sure that the extracted subthreshold voltages are actually reflective of the voltage in the cell being imaged rather than reflective of some population average activity in the background," says Cohen. Commonly used algorithms cannot be used for this purpose, as they typically assume that signals are not correlated—an assumption that is violated in these analyses, as subthreshold activity is often correlated between different neurons. Hence, the researchers had to adapt off-the-shelf algorithms to their purposes.

Using paQuasAr3 and their imaging strategy, Cohen and his colleagues monitored membrane potentials in multiple neurons of the mouse hippocampus while the animals were walking on a treadmill. They could detect both action potentials and subthreshold activity, and observed differences in the activity patterns during resting and active periods. To determine the contributions of excitatory and inhibitory inputs, the researchers combined their voltage imaging approach with optogenetic stimulation, and found stronger inhibition during walking.

While the approach worked well, "there is still a lot of room for improvement," acknowledges Cohen. He points out that "we are still limited both in the number of cells that we can record simultaneously and in the depth to which we can image." These hurdles are currently being addressed in his lab.

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Published online: 27 June 2019
https://doi.org/10.1038/s41592-019-0478-8

Research papers

Adam, Y. et al. Voltage imaging and optogenetics reveal behavior-dependent changes in hippocampal dynamics. *Nature* **569**, 413–417 (2019).