NEUROSCIENCE

A bright future for voltage imaging

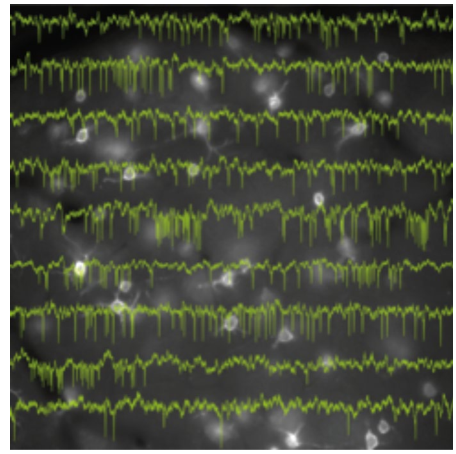
The hybrid voltage-indicator Voltron combines the voltage sensitivity of microbial rhodopsins with the brightness and photostability of chemical dyes.

While genetically encoded calcium indicators are widely used to monitor neuronal activity in the brain, they do not provide insights into subthreshold events and are often not capable of reporting individual action potentials. Voltage indicators can overcome these challenges. Yet, "a real limitation of voltage imaging was the fluorophores, [they are] limited in terms of brightness and photostability," says Eric Schreiter, from Janelia Research Campus, Virginia. He further explains that "you are really photon-limited when trying to image voltage, so getting more photons out would be impactful."

Schreiter and his collaborators, in particular postdoctoral researcher Ahmed Abdelfattah, built on existing FRET-based voltage sensors and decided to replace the fluorescent protein-based FRET donor in this class of voltage indicators with a self-labeling protein tag and a fluorescent dye. Coincidentally, Schreiter's office is next door to Luke Lavis, who developed the Janelia Fluor dyes, which are much brighter and more photostable than fluorescent proteins. The researchers tried a variety of different enzyme and dye combinations, but settled on the HaloTag and Janelia Fluor dyes with a HaloTag ligand handle. While other enzymes and dyes could be incorporated into sensors that worked in culture, Abdelfattah found that labeling with those molecules is much harder in vivo.

Abdelfattah remembers the moment when he thought the strategy would work for in vivo voltage imaging. "The one day that I would remember […] is that when I tried to label fish, I just put the dye in the water and the neurons that had sensors were […] brightly labeled," says Abdelfattah.

The sensor with the best signal-to-noise ratio, which the researchers called Voltron, consists of the Ace2 rhodopsin fused to a HaloTag. This sensor had at least a tenfold increase in photon yield compared to existing genetically encoded voltage indicators. The researchers demonstrated the capabilities in a variety of different experiments. They imaged spontaneous voltage dynamics in the mouse hippocampus as well as visually evoked activity in the mouse visual cortex. Furthermore, they imaged neuronal activity in the midbrain of fictively swimming zebrafish larvae, as well as in the *Drosophila* mushroom body.



Voltron enables imaging of voltage signals from dozens of neurons in mouse cortex. Credit: Ahmed Abdelfattah and Eric Schreiter.

Schreiter acknowledges "the wonderful collaborations we have with the biology labs here at Janelia" as testing Voltron in this breadth of situations was a team effort. But the environment is conducive to this type of work. "I think there is a good general understanding between biology labs and tool development labs here that we are in this together and we are willing to put in time and effort to help each other out," says Schreiter.

In the future, Schreiter and his team plan to further explore the idea of hybrid fluorescent indicators using the bright, photostable dyes as components of the indicators. Given that Voltron like other opsin-based voltage indicators is not compatible with two-photon imaging, "one possible future development that we are actively working on right now is also two-photon compatibility of those sensors," says Abdelfattah.

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Research paper

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