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New methods could help researchers watch neurons compute

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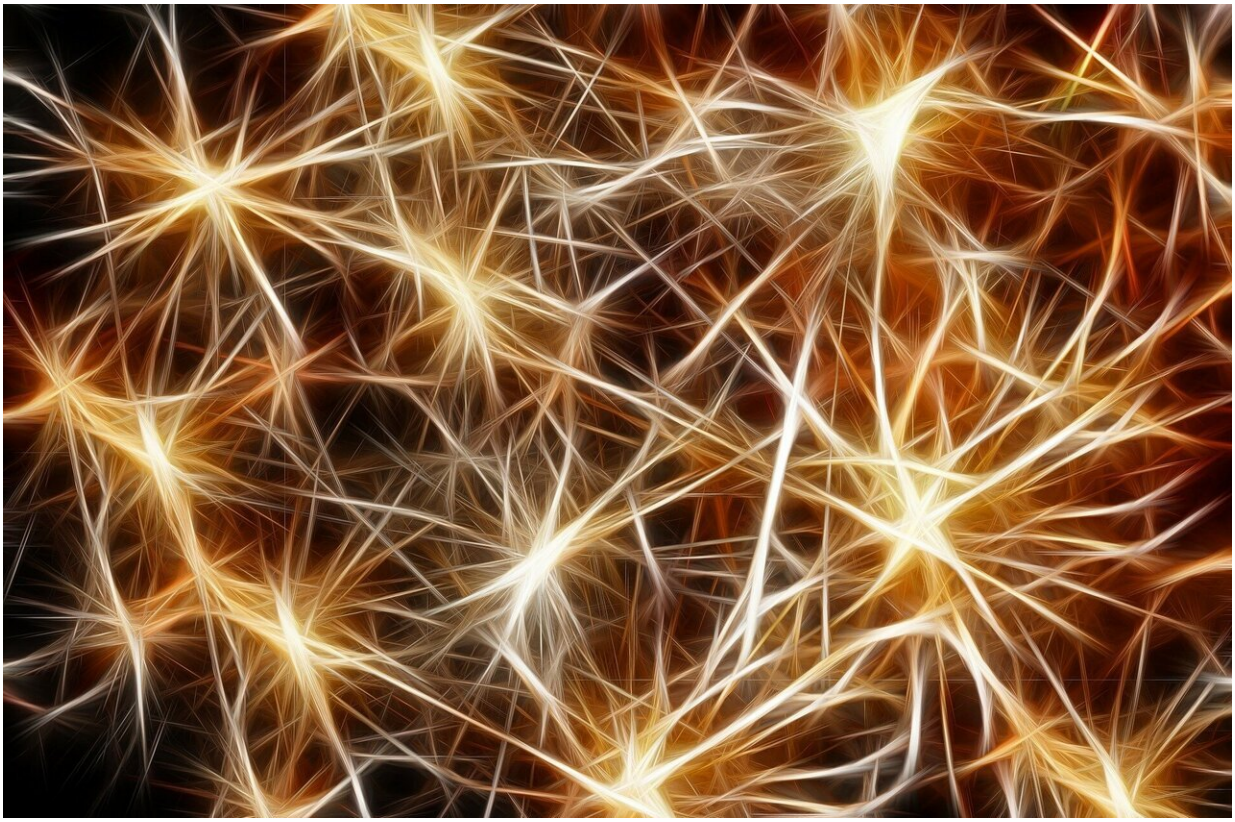

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Since the 1950s at least, researchers have speculated that the brain is a kind of computer in which neurons make up complex circuits that perform untold numbers of calculations every second. Decades later,

neuroscientists know that these brain circuits exist, yet technical limitations have kept most details of their computations out of reach.

Now, neuroscientists reported December 12 in *Cell*, they may finally be able to reveal what circuits deep in the brain are up to, thanks in large part to a molecule that lights up brighter than ever before in response to subtle electrical changes that neurons use to perform their compuations.

Currently, one of the best ways to track neurons' electrical activity is with molecules that light up in the presence of calcium ions, a proxy for a neuron spike, the moment when one neuron passes an electrical signal to another. But calcium flows too slowly to catch all the details of a neuron spike, and it doesn't respond at all to the subtle electrical changes that lead up to a spike. (One alternative is to implant electrodes, but those implants ultimately damage neurons, and it isn't practical to place electrodes in more than a handful of neurons at once in living animals.)

To solve those problems, researchers led by Michael Lin, an associate professor of neurobiology and of bioengineering and a member of the Wu Tsai Neurosciences Institute, and Stéphane Dieudonné, an INSERM research director at the École Normale Supérieure in Paris, focused on fluorescent molecules whose brightness responds directly to voltage changes in neurons, an idea Lin and his team had been working on for years.

Still, those molecules had a problem of their own: Their brightness hasn't always been that responsive to voltage, so Lin and his team at Stanford turned to a well-known method in biology called electroporation. In that technique, researchers use electrical probes to zap holes in cell membranes, with the side effect that their voltage drops rapidly to zero like a punctured battery. By zapping a library of candidate molecules, Lin and colleagues could then select those whose brightness was most responsive to the voltage shift. The resulting molecule, called ASAP3, is

the most responsive voltage indicator to date, Lin said.

Dieudonné and his lab focused on another problem: how to scan neurons deep in the brain more efficiently. To make fluorescent molecules such as ASAP3 light up deep in the brain, researchers often use a technique called two-photon imaging, which employs infrared laser beams that can penetrate through tissue. Then, in order to scan multiple neurons fast enough to see a spike, which itself lasts only about a thousandth of a second, researchers must move the laser spot quickly from neuron to neuron—something hard to do reliably in moving animals. The solution, Dieudonné and colleagues found, was a new algorithm called ultrafast local volume excitation, or ULoVE, in which a laser rapidly scans several points in the volume around a neuron, all at once.

"Such strategies, where each laser pulse is shaped and sent to the right volume within the tissue, constitute the optimal use of light power and will hopefully allow us to record and stimulate millions of locations in the brain each second," Dieudonné said.

Putting those techniques together, the researchers showed in mice that they could track fine details of brain activity in much of the mouse cortex, the top layers of the brain that control movement, decision making and other higher cognitive functions.

"You can now look at neurons in living mouse brains with very high accuracy, and you can track that for long periods of time," Lin said. Among other things, that opens the door to studying not only how neurons process signals from other neurons and how they decide, so to speak, when to spike, but also how neurons' calculations change over time.

In the meantime, Lin and colleagues are focused on further improving on their methods. "ASAP3 is very usable now, but we're confident there

will be an ASAP4 and ASAP5," he said.

More information: Vincent Villette et al, Ultrafast Two-Photon Imaging of a High-Gain Voltage Indicator in Awake Behaving Mice, *Cell* (2019). DOI: 10.1016/j.cell.2019.11.004