NEUROSCIENCE

Two photons as exciting as one

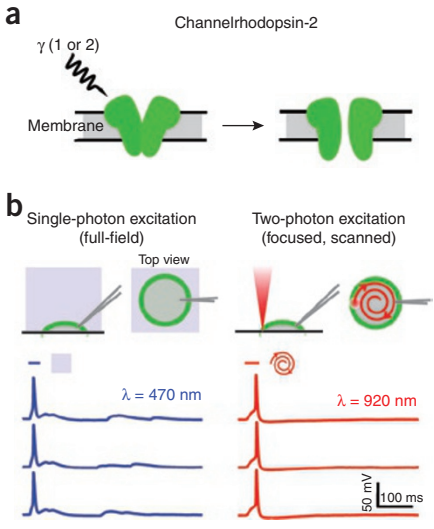
Channelrhodopsin-2 can be efficiently activated by infrared two-photon excitation light and stimulates action potentials in cultured neurons.

To understand how the brain works, it is necessary to study neural circuitry. Which neurons, in other words, connect to which other neurons? What are the patterns of activity and communication between them? And how does this relate to the behavior and biology of the organism? To tease out such relationships, it is necessary to image neurons at high resolution and to stimulate their activity with precision. Ideally, this would be done within the intact animal.

Optical activation of channelrhodopsin-2 (ChR2), a light-activated cation channel, is emerging as a powerful tool for neuronal stimulation. ChR2 is typically activated by excitation with blue light, upon which it allows entry of cations into the cell and consequent depolarization, leading, in excitable cells, to action potentials. It has been used both in brain slices as well as in live animals for the dissection of neural circuitry. However, ChR2 applications in neuroscience typically use single-photon excitation light.

For precise, high-resolution stimulation of single cells in thick tissue, two-photon excitation with longer wavelength light is desirable. Thick biological samples, such as intact brains, effectively scatter light, limiting the experiments that can be performed with single-cell precision using one-photon excitation. Although this can be mitigated to some extent by cell type–specific expression of ChR2, this approach rarely achieves unique expression in single cells, at least in the mouse. Now, John Rickgauer and David Tank at Princeton University set the stage for experiments that use infrared two-photon light to stimulate neurons expressing ChR2.

There has been a general impression in the field, says Tank, that neuronal stimulation using ChR2 and two photon excitation is not effective. He and Rickgauer now show that this view is incorrect. Moreover, their biophysical model of the process provides a possible explanation for why efficient neuronal stimulation using two-photon activation of ChR2 has not been achieved in the past.



Channelrhodopsin-2 excitation. (**a**) Infrared two-photon excitation, like visible-light single-photon excitation, can activate channelrhodopsin-2 photocurrents. (**b**) Alternate optical excitation modes used to stimulate action potentials in cultured neurons. Image courtesy of David Tank.

Working with tissue culture cells expressing the channel, the researchers found that ChR2 is in fact very efficiently activated by infrared two-photon excitation. With strong light, at power levels typical for two-photon microscopy, all of the proteins in an illuminated patch of membrane are very rapidly excited. But what this saturation or ground-state depletion means, as Tank explains, is that "you don't get any more current from that patch of membrane, and since it is a tiny fraction of the surface area of the cell, you can't stimulate a neuron that way."

Even scanning the beam over the cell surface at speeds typical for laser scanning microscopy is insufficient for effective activation. But as Rickgauer and Tank show, if the beam is scanned much more rapidly over the surface of the cell—in the best case using spiral scans covering the entire cell membrane in under 20 milliseconds—the activated ChR2 currents in the cell can achieve peak amplitudes 52–84% of those achieved under whole-cell, single-photon, blue-light illumination. "By scanning over more of the cell membrane in a smaller time window, a larger fraction of the transiently activated molecules simultaneously contribute to total current," explains Tank. This is possible, in part, because ChR2 is readily excited by two-photon absorption; the beam can thus move very rapidly over the cell and does not need to stay in one place for very long to excite molecules ar relatively low intensities. They also showed that, in neurons, rapid scanning of the two-photon beam efficiently stimulated the cells to fire action potentials.

For high-resolution measurements, the intensity of the excitation light is also a critical parameter. "At high light intensities," says Tank, "you actually get appreciable currents from excitation out of focus." This would be problematic for experiments that need single-cell resolution, although it could benefit other applications. "We really see this work as a foundation for optimizing experiments with two-photon excitation of ChR2," emphasizes Tank.

Where next, with this tool in hand? The goal, says Tank, is to apply it to high-resolution neuronal stimulation in awake and unanaesthetized rodents. A prospect that is undoubtedly 'exciting'.

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RESEARCH PAPERS
Rickgauer, J.P. & Tank, D.W. Two-photon excitation of channelrhodopsin-2 at saturation. *Proc. Natl. Acad. Sci. USA* advance online publication (14 August 2009).