

Optical excitation yin and yang

A halorhodopsin can function as the yin to channelrhodopsin-2's yang for photoinducible control of neuronal activity.

Chinese teachings say that two opposing but complementary principles yin (passive) and yang (active) can be found in all non-static objects and processes. Several optical methods have been developed to stimulate neurons. But so far none can selectively stimulate and inhibit a single neuron, by depolarization and hyperpolarization respectively, in a rapid and precise manner. This is now possible, however, thanks to the development of a yin and yang pair of photoreceptors, halorhodopsin from *Natronomonas pharaonis* (NpHR) and channelrhodopsin-2 (ChR2).

ChR2 previously was shown to be a useful tool for reliable induction of spiking in neurons through collaboration between Georg Nagel at the University of Wuerzburg, who has been studying such proteins for years, and Karl Deisseroth from Stanford University (Boyden *et al.*, 2005). The idea of pairing this with NpHR was also a collaborative event. Nagel recalls how last year Deisseroth was presenting this work in Frankfurt, Germany, and they discussed ways to inhibit neurons, ideally at a wavelength distinct from ChR2. "I brought up bacteriorhodopsin, which is a proton pump, but he did not like this because it also changes pH," says Nagel, "and there is also halorhodopsin, a Cl⁻ pump." Nagel had studied halorhodopsin (HR) and other rhodopsins extensively in collaboration with Ernst Bamberg, and knew that HR expressed well, can hyperpolarize oocytes and has an action spectrum maximum around 580 nm in contrast to ChR2's maximum of ~460 nm. There are many varieties of HR, but Nagel recommended NpHR, in particular, even though it was less well characterized, because it was more stable than HR from *Halobacterium salinarum* (HsHR).

Knowing which HR to use was the key insight and Deisseroth's previous experience with ChR2 gave their work impetus. HRs use all-*trans* retinal like ChR2, so they knew they would not have to add this molecule to mammalian brain tissue. The distinct action spectrum was an added bonus. With the help of Alexander Gottschalk, they verified that NpHR was superior to HsHR. Application of NpHR to neurons was greatly facilitated by Deisseroth's graduate student Feng Zhang,

who had also contributed to the ChR2 work.

They expressed NpHR and ChR2 together in neurons in culture and in acute brain slices, and showed that photoactivation of ChR2 produced voltage spikes as they previously showed with ChR2 alone. Hyperpolarization produced by selective photoactivation of NpHR, however, prevented ChR2-induced spiking. They could even inhibit single spikes by activating NpHR at the same time the spike was triggered by either ChR2 activation or current pulses in clamped neurons. Finally, they demonstrated that they could alter the behavior of transgenic worms expressing the two proteins in muscle cells or motoneurons in a light-specific manner, and published their results in *Nature* (Zhang *et al.*, 2007). A paper presenting similar but more limited work has been published online for open peer review at PLoS ONE by one of Deisseroth's former postdocs (Han & Boyden, 2007).

Deisseroth believes that expressing ChR2 and NpHR in the same cell has a huge application: "You can test the necessity of endogenous activity in neurons by shutting them down, and in the same experiment you can test sufficiency of a candidate proposed activity pattern by providing it using ChR2." This makes it possible to eliminate sources of experimental variation and this idea of necessity and sufficiency is fundamental to scientific proof. Deisseroth adds, "I think that is not just a parlor trick; it is really the core utility of the technique."

A big remaining challenge is to get the method to work with two-photon excitation which does not activate enough channels or pumps. Deisseroth is hopeful that clever optical techniques will overcome this hurdle. He is mostly interested in the potential of the method for clinical applications, however, and seems happy to let other researchers help find ways to optimize these opposing but complementary tools.

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RESEARCH PAPERS

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Han, X. & Boyden, E.S. Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *PLoS ONE* **2**, e299 (2007).

Zhang, F. *et al.* Multimodal fast optical interrogation of neural circuitry. *Nature* **446**, 633–639 (2007).