NEUROSCIENCE

New tools to enlighten brain function

The pioneers of the first generation of widely used optogenetic tools now describe new variants that open further possibilities for studying neural function.

The transmission of electrical signals is the essence of neuronal function. For a long time neuroscientists have cherished the idea of controling the switch that operates these currents in order to study how neurons functionally relate to each other. Thanks to the booming field of optogenetics, this is now possible. In optogenetics, light-gated ion channels derived from microbial organisms called opsins are expressed in cells of interest, where they modulate electrical activity. Opsins such as channelrhodopsin-2 (ChR2) and halorhodopsin have 'colonized' neurobiology laboratories as the preferred systems to activate (depolarize) or inhibit (hyperpolarize) a set of genetically targeted neurons through pulses of blue or red light, respectively. But like any biological tool, these molecules are not perfect, and now the researchers who first implemented their use are leading the discovery of the next generation of 'opto-tools'.

Karl Deisseroth of Stanford University led a team including Ed Boyden, Feng Zhang and Georg Nagel that first applied ChR2 for light-mediated control of spiking in neurons in 2005. In 2007, Boyden, now running his own lab at the Massachusetts Institute of Technology, headed one of the two groups that first used halorhodopsin to turn off neurons. Not satisfied with the current size and recovery time of halorhodopsin, he set out to search for new opsin variants in other ecological niches. His group now reports a set of molecules, the light-gated proton pumps, that can serve as alternative off-switches for nerve cells (Chow *et al.*, 2010). "These molecules pump positive charge in the form of protons out of cells, instead of negative charge into the cell as halorhodopsin does, provoking the same effect but using a different strategy," explains Boyden.

His recent work focuses on the two proton pumps 'Arch' from *Halorubrum sodomense* and 'Mac' from *Leptosphaeria maculans*, which have three main advantages over previous neural inhibitors: higher currents, spontaneous recovery and different 'color flavors'—Arch responds to yellow light and Mac to blue light. This opens the possibility of inactivating neighboring neurons using different light spectra, as Boyden and colleagues demonstrate by combining halorhodopsin and Mac. Importantly, introducing these proteins in neurons does not affect the intracellular pH, as neural cells have self-limiting mechanisms to prevent excessive proton-mediated swings in voltage. Boyden's group is interested in applying these tools to study basic mechanisms of brain function but is also engaged in translational work. "We are expressing these directly in nonhuman primates to see if we can use them as potential neuro-silencing therapies," he says.

The image illustrates the intersection of genomic search and molecular engineering to achieve new optogenetic tools. Image courtesy of Feng Zhang, Peter Hegemann and Karl Deisseroth.

Another way of expanding the optogenetic toolbox is by tailoring known variants through targeted mutagenesis, as shown by Peter Hegemann, at Humboldt University in Berlin, and Karl Deisseroth. This team reports a new variant of ChR2, 'ChETA', engineered to reduce extra spikes and artifact signals (Gunaydin *et al.*, 2010). One of the most interesting aspects of ChETA is that it can be used to spike neurons at frequencies above 40 Hz (also called gamma oscillations) that could not be studied using the original ChR2. As Deisseroth points out, "There is an emerging understanding that higher-frequency oscillations might be important for brain function, [and ChETA] opens up that whole signaling band for testing hypotheses with causal control." The authors targeted ChETA to fast-spiking cortical parvalbumin interneurons and showed that ChETA potently improved the responses to two-millisecond light pulses over a broad range of frequencies in these cells.

Deisseroth's motivation for getting these microbial proteins to work better for neuroscientists is to understand not just basic science but also disease. As a psychiatrist, he sees a lot of potential in these tools for studying circuit function that might lead to pharmacological treatments. Meanwhile Hegemann, a biophysicist who has studied opsins for decades, is interested in extending the use of optogenetic tools to other biological fields. "We would like to extend this concept to enzymes," he says. "Nature gives examples like the phototropins—light-activated kinases from plants or bacteria—or we can imagine light-activated transcription factors to control gene expression and then we can expand optogenetics to other fields like cell biology or development."

The collaborative work of these two groups shows the great potential of symbiotic relationships in science. "Our biophysics knowledge complements Deisseroth's demands for new tools and this goes back and forward smoothly," says Hegemann. The potential of these new molecules is enormous, as they are enticing people to ask more and more ambitious questions relating neural circuitry to behavior and disease. Improved optogenetic tools will keep coming in future years, and their use will surely extend to researchers studying other excitable—and exciting—cells such as heart cells.

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RESEARCH PAPERS

Chow, B.Y. *et al.* High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature* **463**, 98–102 (2010).

Gunaydin, L.A. *et al.* Ultrafast optogenetic control. *Nat. Neurosci.* **13**, 387–392 (2010).