OPTOGENETICS: Shining New Light on Neural Circuits
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Emerging methods that combine genetics and optics have neuroscientists glowing about the possibilities

Shining New Light on Neural Circuits

WHEN RESEARCHERS FROM YALE university reported last year that they’d used a laser to activate neurons in fruit flies and in turn control the insects’ behavior, even Jay Leno thought it was cool. In a skit, the Tonight Show host pretended to use a remote-controlled fly to harass President George W. Bush during a speech. “I thought it was actually quite funny,” says Gero Miesenböck, the neuroscientist who led the study. A video clip of Leno’s skit elicited chuckles when Miesenböck played it during a presentation at October’s meeting of the Society for Neuroscience in Atlanta, Georgia.

But the neuroscientists who packed the crowded lecture hall hadn’t come for laughs. Miesenböck’s talk was part of a symposium on “optogenetics,” an emerging field that combines tools from optics and genetics to visualize and stimulate the nervous system. Several of the new methods, such as the one Miesenböck developed for the fly experiments, use genetic manipulations to confer light sensitivity on specific groups of neurons, making it possible to control their activity with pulses of light. Many neuroscientists say such stimulation methods represent powerful new tools for investigating neural circuits. “I think it’s really exciting,” says Liqun Luo, a neurobiologist at Stanford University in Palo Alto, California, who attended the symposium. “It’s at the cutting edge.”

“All you have to do is express this one protein, and now you can control the activity of the neurons with light.”

—Edward Callaway, Salk Institute for Biological Studies

Currently, several new photostimulation methods are in various stages of development, and scientists are just beginning to use them to address questions about brain function. But down the road—way down the road—some researchers envision exciting clinical applications. One idea is to replace the metal electrodes used for deep-brain stimulation in patients with Parkinson’s disease and other disorders with fiber-optic probes that carry light deep inside the brain to boost the activity of only those neurons that need it.

Lighting up

Using light to manipulate the nervous system is not a new idea. In the past 20 years, researchers have done many experiments with neurotransmitters bound to molecules that change shape in response to light. Such “caged” neurotransmitters are inactive, but a pulse of laser light sets them free to activate their usual receptors. Glutamate, the brain’s chief excitatory neurotransmitter, has become a particularly popular tool in caging experiments. Glutamate uncaged with lasers can stimulate synapses with precise temporal and spatial control; a team from Princeton University reported last year in Nature Methods that they had done this at up to 20,000 different locations in an excised slice of brain tissue. There are drawbacks, however. Because almost all neurons respond to glutamate, it’s virtually impossible to target only neurons of a particular type. And the precise spatial control requires a stationary target—a nonstarter for researchers who want to study behavior in intact animals.

Miesenböck’s fly experiments circumvent these problems. Together with graduate student Susana Lima, Miesenböck inserted a rat gene that encodes an ion channel into flies. Fly neurons normally don’t make this cell membrane portal, which opens in response to ATP, the energy-storage molecule involved in cell metabolism. Using standard genetic engineering tools, Lima and Miesenböck created several fly strains that...
expressed the ATP-gated channel only in specific classes of neurons. Then they injected caged ATP into the flies. When liberated by a flash of light, the ATP opened the channels in the modified neurons and allowed sodium and calcium ions to rush in, thereby prompting the neurons to fire a burst of electrical impulses. Because only neurons made to express the channel could respond to light, precise aim wasn’t necessary; a fly-sized spotlight did the trick.

In one strain of fruit flies, Lima and Miesenböck put the ATP-gated channel in just two neurons out of the roughly 100,000 in the fly’s nervous system, the so-called giant fiber neurons that control the fly’s escape reflex. A brief flash of light made these insects jump and frantically flap their wings. In another strain, the researchers restricted the channel to neurons that make the neurotransmitter dopamine. Stimulating these neurons with light made the flies more active and increased the time they spent exploring their enclosure, Lima and Miesenböck reported in the 8 April 2005 issue of Cell; it was this study that inspired Lenos’s skill.

These fly findings are consistent with the idea, suggested by many earlier studies, that dopamine helps animals predict rewards and punishments, Miesenböck says. One possibility, he explains, is that activating dopaminergic neurons increases exploratory behavior because flies interpret the dopamine burst as a signal that something good—or bad—is nearby. His team is now working on ways to target the ATP-gated channel to different subsets of the fly’s 150 or so dopaminergic neurons so that their roles in exploratory and other types of behaviors can be investigated.

**Ball and chain**

Richard Kramer of the University of California (UC), Berkeley, has been investigating ways to make neurons sensitive to light by modifying other ion channels. In 2004, Kramer, neuroscientist Ehud Isacoff, and chemist Dirk Trauner, both also at UC Berkeley, described modified potassium channels that open and close when exposed to different wavelengths of light. Their approach makes use of an unusual feature of a molecule called azobenzene. In visible light, an azobenzene molecule is relatively straight and measures about 17 angstroms from end to end. When illuminated by ultraviolet light (UV), however, it folds in the middle, shortening the distance between the two ends to about 10 angstroms.

To take advantage of this shape change, Kramer and colleagues incorporated azobenzene into a molecular ball and chain that attaches to the extracellular side of a common variety of potassium channel. First, they tweaked the potassium channel gene to create a favorable binding site and expressed the altered channels in transgenic mice. Then they bathed slices of brain tissue from these mice in a solution containing the ball and chain, which has three components. The ball is a quaternary ammonium ion that can fit snugly into the channel’s pore and prevent the flow of potassium ions. Next comes an azobenzene molecule and then a compound called maleimide that links the azobenzene to the potassium channel. When azobenzene is in its long state, the chain is just long enough to allow the ammonium ball to plug the pore. But when a pulse of UV light converts azobenzene to its shorter, bent configuration, the ammonium plug is pulled from the pore and potassium can flow freely into the neurons.

Opening potassium channels typically inhibits neural firing, so in the 2004 work, UV light acted something like an off switch on neurons equipped with the azobenzene-modified channels. Kramer and company recently created a light-controlled switch for neurons by further tweaking the potassium channel gene so that the protein admits sodium ions as well. When these modified channels are lit up with UV light, sodium rushes into neurons and excites them, the researchers reported in the November 2006 issue of the Journal of Neurophysiology.

The UC Berkeley group has also added an azobenzene photoswitch to glutamate receptors, ubiquitous ion channels in neurons that normally open in response to glutamate.

**Enlightening.** In a fly with light-sensitive neurons (top), a flash of light (middle) triggers an escape response, flapping wings (bottom).

They described the receptors in the January 2006 issue of *Nature Chemical Biology*. Kramer, Isacoff, and Trauner will be part of a newly announced center for studying the optical control of biological function. Funded by the National Institutes of Health and run jointly by UC Berkeley and Lawrence Berkeley National Laboratory, the center is part of NIH’s nanomedicine initiative.

**Help from algae**

Across San Francisco Bay, Karl Deisseroth and colleagues at Stanford have developed yet another optogenetics approach, based on a light-sensitive ion channel found in a unicellular green alga. Called channelrhodopsin-2 (ChR2), the channel opens in response to light, allowing positively charged ions to pass through its pore. The photosynthetic algae use ChR2 to orient to light. Expressing the gene for ChR2 in neurons makes them fire when exposed to light, Deisseroth and colleagues first reported in the September 2005 issue of *Nature Neuroscience*.

With this approach, there’s no need for extra steps such as adding caged ATP or azobenzene to neurons. “It’s a very simple system because all you have to do is express this one protein, and now you can control the activity of the neurons with light,” says Edward Callaway, a neuroscientist at the Salk Institute for Biological Studies in San Diego, California. And unlike the ATP-gated channels and the azobenzene photoswitch, the ChR2 system can trigger neural firing within just a few milliseconds of being hit by a laser beam. That makes it possible to deliver light pulses that drive the neurons in precisely controlled patterns that mimic the normal chatter of neural activity, explains Edward Callaway.
Gary Westbrook, a neuroscientist at Oregon Health & Science University in Portland. “I think the biggest advantage of channelrhodopsin is the ability to stimulate with such high time resolution,” he says.

Westbrook’s lab intends to express ChR2 in newborn neurons in the mouse hippocampus to study how these cells communicate with mature neurons as they integrate themselves into preexisting neural circuits (Science, 17 February, p. 938). “We don’t know anything about the output of that population of cells,” Westbrook says.

Other labs are also using the ChR2 system. At the recent neuroscience meeting, Guoping Feng of Duke University in Durham, North Carolina, presented preliminary work he’s done in collaboration with Deisseroth and George Augustine at Duke. Feng and colleagues have created two strains of transgenic mice, one that expresses ChR2 in the output cells in a specific layer of the cerebral cortex and another that expresses the light-sensitive channel in mitral cells in the olfactory bulb. These efforts have convinced Feng that the method “works really well in vivo.” Ultimately, he hopes to use the ChR2 system to investigate neural circuits involved in addiction and compulsive behavior. “Many neurological diseases are diseases of specific subtypes of neurons,” he says. “This will give us a way to target specific neurons to understand their function in the circuitry of the brain.”

At the neuroscience meeting, Deisseroth presented preliminary work that further illustrates the potential. He used a virus to put ChR2 into neurons in a slice of mouse hippocampus, genetically tagged the same neurons with a fluorescent dye so that they were visible under a microscope, and used a fluorescent indicator of calcium flux to monitor their activity. The ability to simultaneously see, stimulate, and record the activity of neurons with light is a powerful combination for investigating the connectivity of neural circuits, he and others say.

Clinical vision?
The new optogenetics techniques should provide more sophisticated options for exploring the neural underpinnings of behavior, Miesenböck says. Although neuroscientists have long used metal electrodes to manipulate neural activity, it’s nearly impossible to use electrodes to stimulate a distributed population of neurons simultaneously, he explains. “The dopamine experiment in the fly would have been impossible with electrodes because you have about 150 cells arranged in different clusters,” Miesenböck says.

Not that there are no obstacles. The main one at present, several researchers say, is the ability to deliver the required genes to specific classes of neurons. “All these methods rely on the ability to direct gene expression to a particular cell type,” says Callaway. “I think 5 or 10 years ago, we all thought that was going to be really easy, but it hasn’t proven so easy to do.” Another hurdle is getting light to deep-lying parts of the nervous system; so far, the techniques have only been used in slices of brain tissue and areas close to the surface of the brain in live animals.

This last obstacle may not be insurmountable, however. Deisseroth’s lab has been experimenting with using flexible optical fibers to stimulate ChR2-bearing neurons deep in the brains of awake, behaving mice. Off-the-shelf fiber optics are sufficient for stimulation alone, Deisseroth says, but more elaborate experiments that combine stimulation with recording and imaging—such as the ones he has described in brain slices—may also be possible in live animals before long. And at the neuroscience meeting, Stanford applied physicist Mark Schnitzer showed off a microendoscope small enough to fit on the head of a freely moving mouse. The thumbnail-sized device weighs less than 4 grams, and its fiber-optic probes can reach any structure in the mouse brain. So far, Schnitzer’s group has been using the device for imaging cells labeled with fluorescent dyes, but he says there’s no reason it couldn’t also be used to stimulate light-sensitive neurons.

Far in the future, it’s conceivable that fiber-optic light stimulation could replace deep brain stimulation via electrodes, a method currently under investigation for Parkinson’s disease, depression, epilepsy, and other disorders. “An electrode stimulates all the cell types” that happen to be near its tip, Deisseroth says. “It’s generally understood that that will contribute to side effects and reduce the efficacy.” A better solution, he says, would be to target the stimulation to certain classes of cells. But Deisseroth cautions that “a lot of things have to fall into place for this to happen,” not the least of which is resolving the serious safety concerns about gene therapy in humans.

Another potential clinical application is restoring sight in people with retinal degeneration. In the 6 April 2006 issue of Neuron, researchers led by a team at Wayne State University in Detroit, Michigan, reported encouraging results from an experiment in which they used a virus to deliver the ChR2 gene to retinal ganglion cells in mice whose retinas lack photoreceptor cells. Retinal ganglion cells are normally insensitive to light. But adding ChR2 made them respond to light and made the animals’ visual cortices responsive to visual stimuli. (Kramer’s team has been experimenting with ways to add a photoswitch to these cell’s natural ion channels by chemical means alone rather than introducing foreign genes.)

There’s no reason the future clinical applications of photostimulation methods would have to be limited to the nervous system, Kramer adds. He can imagine doctors one day using fiber-optic probes to examine the heart and other organs—using light to perturb a few specific cells that had been temporarily made light-sensitive by genetic or other means and recording a physiological response. Kramer is quick to add that any such clinical payoffs are a long way off: “It’s so far out there who knows if it will ever happen,” he says. But when it comes to basic neuroscience research, he and others are confident that the new optogenetics methods have a bright future.

—GREG MILLER