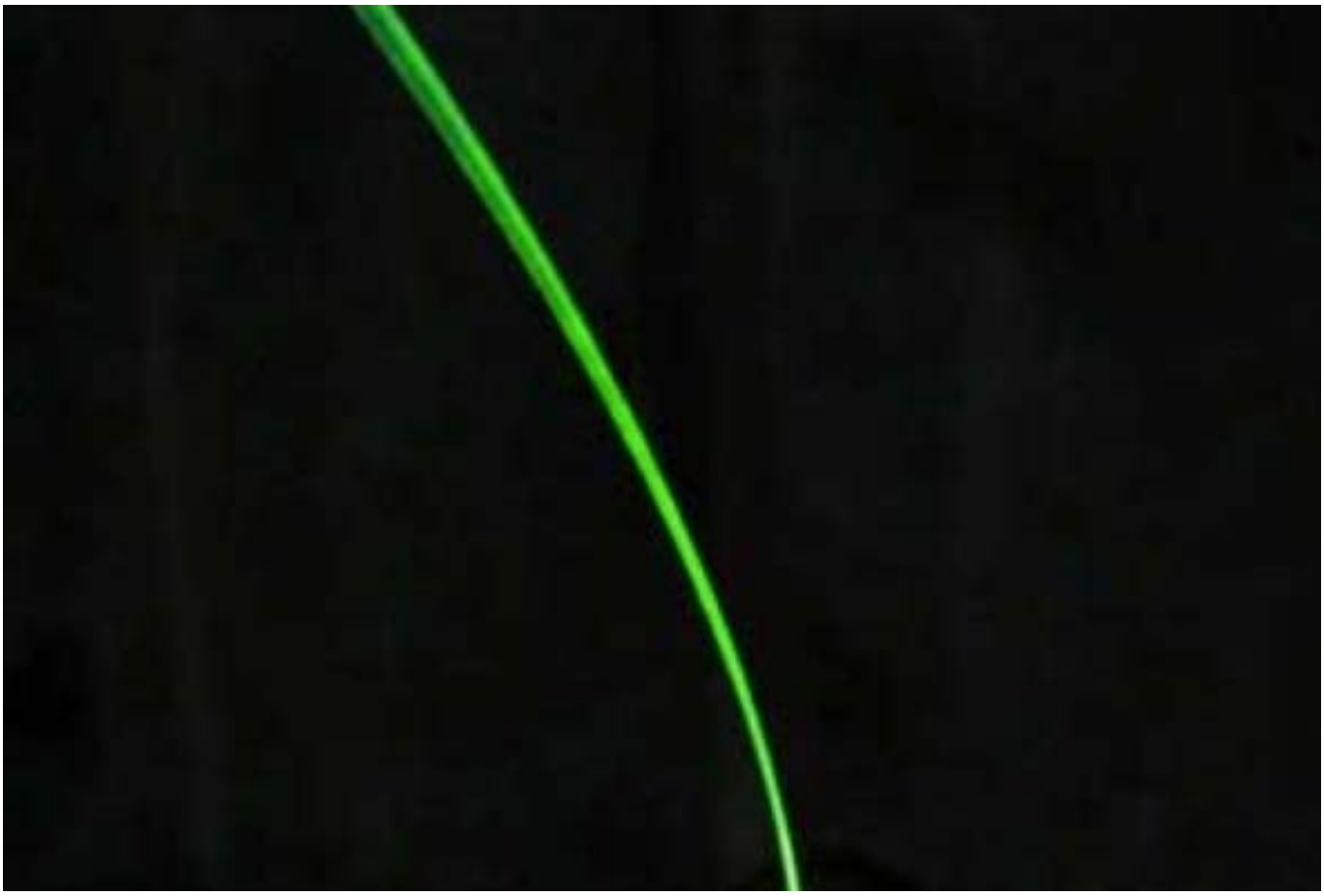
MIND

Breakthrough Prize for Illuminating the Brain's Secret Code

A revolutionary technique that switches brain circuits on and off has taken neuroscience by storm and is now undergoing a new round of innovation

By Simon Makin on November 8, 2015 **Véalo en español**



Light piped into the brain of a genetically engineered rodent switches on a set of neurons

Light piped into the brain of a genetically engineered rodent switches on a set of neurons.
Image Source: Deisseroth Lab, Stanford University/HHMI.

Optogenetics is probably the biggest buzzword in neuroscience today. It refers to techniques that use genetic modification of cells so they can be manipulated with light. The net result is a switch that can turn brain cells off and on like a bedside lamp.

The technique has enabled neuroscientists to achieve previously unimagined feats and two of its inventors—Karl Deisseroth of Stanford University and the Howard Hughes Medical Institute and Ed Boyden of Massachusetts Institute of Technology—received a Breakthrough Prize in the life sciences on November 8 in recognition of their efforts. The technology is able to remotely control motor circuits—one example is having an animal run in circles at the flick of a switch. It can even label and alter memories that form as a mouse explores different environments. These types of studies allow researchers to firmly establish a cause-and-effect relationship between electrical activity in specific neural circuits and various aspects of behavior and cognition, making optogenetics one of the most widely used methods in neuroscience today.

As its popularity soars, new tricks are continually added to the optogenetic arsenal. The latest breakthroughs, promise to deliver the biggest step forward for the technology since its inception. Researchers have devised ways of broadening optogenetics to enter into a dynamic dialogue with the signals moving about inside functioning brains.

The term optogenetics usually refers to the *control* of neurons. Researchers insert a gene for a light-sensitive protein into cells. The cells then produce that protein on their surfaces. When these cells are later exposed to light, channels open and charged particles

nding an electrical signal to other cells. The most commonly used proteins are "channelrhodopsins," originally discovered in algae, but there is also a protein from a bacterium found in Egyptian salt lakes that has the opposite effect. Negative, rather than positive (chlorine) ions, rush into the cell, which prevents it from firing. Researchers can

thus use these two actuator proteins to switch neurons on and off, using light. This can be achieved via fiber-optic cables, so researchers can manipulate neurons in freely moving animals and observe the effects on behavior.

The genes are delivered by various forms of genetic manipulation. Different genes are turned on, or expressed in different types of cell, so the gene is accompanied by a special genetic sequence, called a promoter, which is only active in specific cell types, thus ensuring the protein is only produced in the desired target.

More generally, optogenetics refers to any method for communicating with cells using genetics plus optics—and that can mean observing cell activity, not just turning a neuron on or off. Nongenetic approaches, such as fluorescent dyes that increase cell illumination in response to activity, have been used previously, but lack the precision of targeting a particular type of cell.

A new way to watch what's happening with cells utilizes the same genetic targeting methods used to switch circuits off and on, Except now indicator proteins are integrated into selected cells via genetic tweaks. The indicators generally consist of a protein sensitive to cell activity, linked to a fluorescent protein, so they light up in response to a cell's firing. Combining these genetically targeted optical readouts with the standard armamentarium of tools for controlling cell activity unlocks the full potential of optogenetics. The combined technique allows researchers to hold two-way conversations with populations of selected neurons, using only pulses of light. Once various technical difficulties are overcome, researchers will be able to carry out this dialogue with single neurons, in real-time, allowing a level of interaction with awake, functioning brains, that has not been possible before now.

At the recent Society for Neuroscience meeting in Chicago, several leading researchers spoke about "all-optical interrogation of neural circuits" and co-wrote an accompanying review in *The Journal of Neuroscience*. They outlined the challenges involved and described pioneering work to overcome these obstacles. The approach has the potential to shed light, in literal terms, on the relationship between brain activity, on one hand, and cognition, behavior and emotion, on the other. This goal fits well with the U.S. BRAIN initiative, which aims to encourage the development of new tools for exploring the link between neural signals and cognition.

One of the speakers and co-authors was neuroscientist and practicing psychiatrist Deisseroth of Stanford University, whose work developing the original channelrhodopsin-based photosensitive actuator cells together with colleague Boyden was what earned the pair their recent Breakthrough Prize**.** Deisseroth's research has always been conducted with an eye on psychiatry.

This new work focuses on overcoming limitations of existing technical methods. Two main types of genetically inserted indicators are commonly used for these all-optical apparatuses. Calcium indicators exploit the fact that when neurons fire, calcium channels on cells open, causing levels of calcium to rise. Indicators make use of this to deform a calcium-sensitive protein, which is linked to a fluorescent protein that emits light. The main problem is speed. “Calcium signals are slow, they last one second or so, and the brain is a little faster than that,” says Thomas Knöpfel, chair of Optogenetics and Circuit Neurosciences at Imperial College London, who didn't speak at the session. Furthermore, calcium levels can also change without neurons firing, and some important changes that don't actually cause neurons to fire don't alter calcium levels. This is because calcium is a proxy for the signal researchers are really interested in—voltage.

Knöpfel has been developing genetically encoded voltage indicators (GEVIs) for 20 years. The main problem, compared with calcium indicators, is the signals are weaker and are harder to detect. The problems are only exacerbated by the faster signals, which require shorter exposure times. The signals also tend to be noisier.

Another challenge is observing or stimulating cells deep inside the brain. Traditional one-photon microscopy suffers from poor depth penetration and image quality—photons are absorbed and scattered by tissue. Two-photon microscopy overcomes this using near-infrared light. The longer wavelength light penetrates tissue, but because the photons have less energy, two must strike a protein to excite it, hence the name. This has the advantage that only proteins in the tiny focus-spot of the beam are stimulated, but it also means that, when trying to stimulate a neuron, few channels are activated, which may not be enough to trigger a spike.

There are two ways to solve this issue: One is by using scanning lasers, which quickly scan a laser-beam across a target (whether one or multiple cells), activating many channels

a laser beam across a target (whether one or multiple cells), activating many channels sequentially. The other involves parallel approaches, which use holographic techniques to shape a beam into the required pattern, illuminating the whole target at once. This method can even produce three-dimensional illumination patterns that stimulate cells at different depths. The main advantage though, is speed. “Applications that require precise control over spike timing are better using parallel approaches,” says Valentina Emiliani of the neurophotonics laboratory at Paris Descartes University, lead review author who presented her group's holographic work at the conference.

The biggest obstacle though, is that both stimulating and recording activity with light causes problems if wavelengths overlap. This is especially challenging because the proteins used as indicators need to be excited by light in order to emit light. “The compounds used for imaging and photo-stimulation have very overlapping spectra,” Emiliani says. “It's difficult to illuminate your preparation to do imaging, while making sure you don't also photo-stimulate.” Likewise, researchers must be careful the indicator signals they record are not corrupted by wavelengths used for stimulation.

Much work in the field is therefore now focused on finding proteins whose wavelengths don't overlap. For instance, Harvard University biophysicist Adam Cohen and his team presented work, together with Ed Boyden's group at M.I.T., which combines a channelrhodopsin shifted toward blue wavelengths with a voltage-indicator that emits near-infrared. The groups have used the indicator called QuasAr in live mice, and stimulated neurons with blue light while monitoring in red, in stem-cell cultures derived from people with Lou Gehrig's disease. They plan to test the combination in live animals next.

Once these challenges are overcome, all-optical techniques could revolutionize neuroscience by allowing researchers to simultaneously control and monitor precisely even single spikes from either single neurons or large ensembles of neurons as experimental animals move freely about. "This approach will open a whole new range of experiments," says Michael Hausser, a neuroscientist at University College London, who co-chaired the conference session alongside Emiliani. “Unlocking the full potential of optogenetics requires going beyond targeting genetically-defined cell-types, to targeting cells according to functional properties, rather than just genetic identity.”

In other words, rather than just passively monitoring, or observing the results of an

experiment in which the level of stimulation of neurons must be carefully planned beforehand, these innovations will enable researchers to adapt how they stimulate cells depending on how cells are behaving. “If you can tailor stimulation to activity patterns, you can do the manipulation on the fly,” Hausser explains. “For instance, during a decision-making experiment, if you can track the activity of ensembles of neurons in real time, you can influence behavior more effectively by manipulating ensembles as they form.”

“These are two game-changing advances, compared to what we could do before: targeting functional ensembles and manipulating activity in real time,” Hausser says. “Ultimately these approaches could help define the neural codes used in the brain to drive behavior.”

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