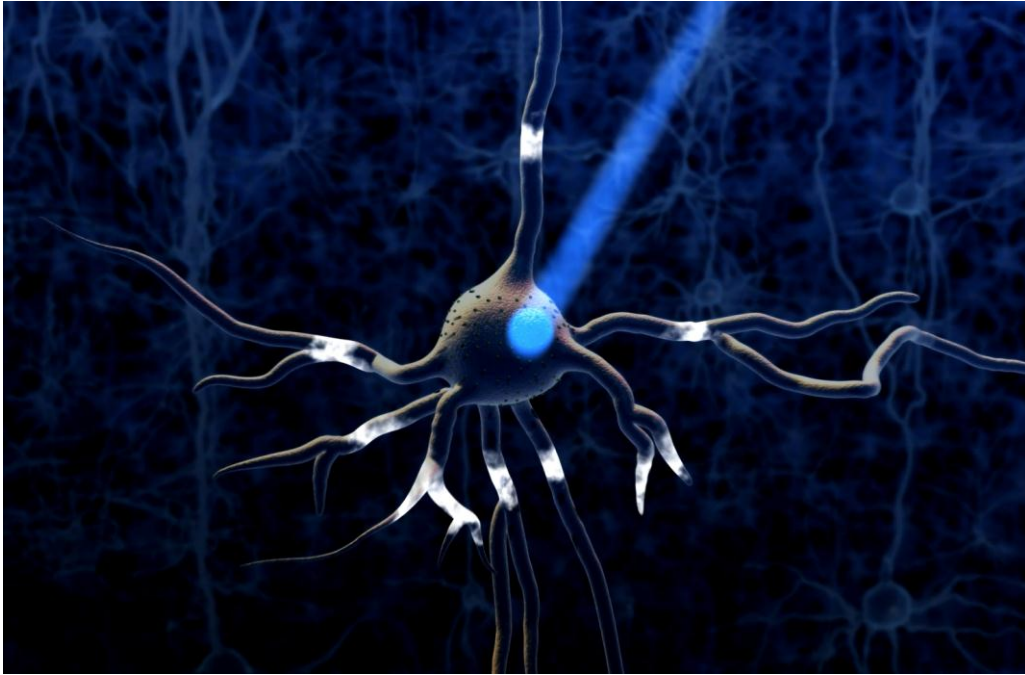# Optogenetics: Using Light to Control the Brain

## By Edward S. Boyden, Ph.D.



Courtesy of the MIT McGovern Institute, Julie Pryor, Charles Jennings, Sputnik Animation, and Ed Boyden.

*Editor's note: The brain is densely packed with interconnected neurons, but until about six years ago, it was difficult for researchers to isolate neurons and neuron types to determine their individual roles in brain processes. In 2004 however, scientists, including author Edward S. Boyden, Ph.D., found that the neural expression of a protein, channelrhodopsin-2 (ChR2), allowed light to activate or silence brain cells. This technology, now known as optogenetics, is helping scientists determine the functions of specific neurons in the brain, and could play a significant role in treating medical issues as diverse as sleep disorders and vision impairment.*

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The brain is an incredibly densely wired computational circuit, made out of an enormous number of interconnected cells called neurons, which compute using electrical signals. These neurons are heterogeneous, falling into many different classes that vary in their shapes, molecular compositions, wiring patterns, and the ways in which they change in disease states. It is difficult to analyze how these different classes of neurons work together in the intact brain to mediate the complex computations that support sensations, emotions, decisions, and movements—and how flaws in specific neuron classes result in brain disorders. Ideally, one would study the brain using a technology that would enable the control of the electrical activity of just one type of neuron, embedded within a neural circuit, in order to determine the role that that type of neuron plays in the computations and functions of the brain. Silencing a neuron would reveal what computations or pathologies it was necessary or critical for; activating a neuron would reveal which ones it was capable of driving or sustaining.

Such a cell-targetable neural-control technology would open up a number of new frontiers in treating brain disorders. For example, by revealing the role that a given kind of neuron plays in a brain disorder state, or in overcoming a brain-disorder state, such technologies could reveal neurons in the brain that could serve as targets for more efficacious, reduced-side-effect drugs for treating brain disorders. Pinpointing the parts of a circuit that mediate a disorder could help neurosurgeons target electrodes to those areas for improved electrical neuromodulation. This could reveal better targets for disorders treated through deep brain electrical stimulation, such as Parkinson's disease. And, if researchers could precisely enter information into specific cells in the brain, then such a technology might enable new kinds of prosthetics for the direct repair of complex brain disorders that are not treatable with any existing technologies.

The seeds of this idea were in the air around the time that I started graduate school at Stanford University, with several groups demonstrating pioneering methods for driving the electrical activity of neurons with light. For example, in 2002 the lab of Gero Miesenbock, then at Memorial Sloan-Kettering Cancer Center, demonstrated the use of the light-sensitive protein signaling cascade found in *Drosophila* photoreceptors to enable neurons to be made light-sensitive over timescales of seconds.[1] In 2003 the same group reported that by expressing, in neurons, receptor proteins that are activated by specific small molecules, and then delivering chemically "caged"[2-7] versions of the small molecules that are activated by light, illumination of the neural circuits would result in activation of the "caged" chemicals.

These chemicals would then bind the receptors and selectively activate the neurons that express the receptors.[8, 9] And in 2004 three labs working together (the Trauner, Isacoff, and Kramer labs, at Berkeley) developed a method by which engineered ion channels would be expressed in targeted neurons, with ion channel agonists or antagonists tethered directly to the channels via a light-activated chemical linker.[10]

Over the last decade a specific toolset, which has come to be known as optogenetics, has emerged—the set of microbial opsins, naturally occurring membrane proteins, that directly convert light into changes in electrical potential across the cell membranes into which they are inserted. These molecules change the electrical potential of cells in which they are expressed in response to light, perhaps not unlike the way that a solar cell might be used to charge a battery. These reagents are genetically encoded and in many species do not require chemical supplementation for operation, which makes them easy to use. They also possess a very high speed of operation, responding to pulses of light with voltage changes that are precise to the millisecond. Microbial opsins respond to light by translocating ions across the membranes of the cells in which they are genetically expressed, making the neurons in which they are expressed sensitive to being activated or silenced by light.

These opsins, which in their native species assist with the sensation of light or the production of energy using light, were discovered in the 1970s. They were subsequently studied for their biophysical and signaling functions. Because they move ions across the membranes of cells in which they are expressed, they change the electrical potential of these cells when illuminated. And since neurons are electrical devices, this means that the electrical activity of opsin-expressing neurons can be controlled by light. The opsin channelrhodopsin-2 (ChR2) from the green alga *C. reinhardtii*, for example, translocates positively charged ions into cells when illuminated. We found that neurons expressing ChR2 become electrically activatable by blue light (figure 1). When exposed to light, the opsins halorhodopsin and archaerhodopsin pump chloride into, and protons out of, cells, respectively. We found that these molecules make the neurons electrically silenceable by green or yellow light (figure 2).

**Figure 1**
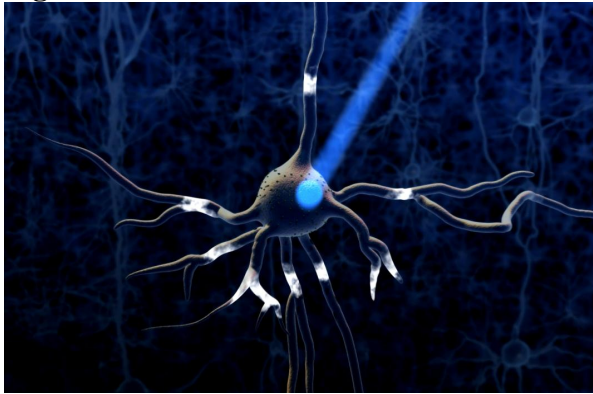


3-D rendering of a neuron expressing the light-gated cation channel channelrhodopsin-2 (dots on the neuron surface), and being illuminated by a beam of blue light, causing it to fire an electrical spike or action potential (white waves propagating away from cell body). Credit: MIT McGovern Institute, Julie Pryor, Charles Jennings, Sputnik Animation, and Ed Boyden.

**Figure 2**
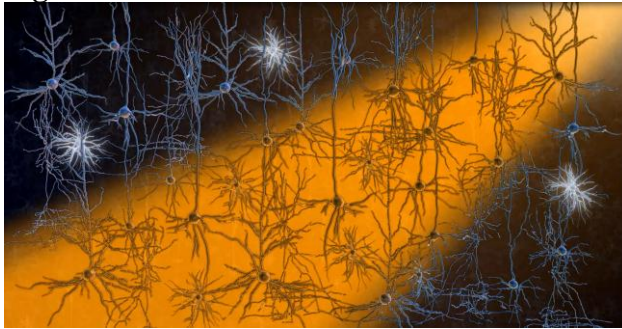


Rendering of a neural network in the brain, containing neurons that express a light-driven neural silencer (like a halorhodopsin or archaerhodopsin), so that when the neural network is illuminated with orange light, the illuminated part of the network is quieted (neurons are shaded dark). Credit: MIT McGovern Institute, Julie Pryor, Charles Jennings, Sputnik Animation, and Ed Boyden.

Here, I describe how the innovation of optogenetics emerged over the past decade. These tools are now in widespread use in neuroscience and in other biological and bioengineering fields, where they are used to turn different neurons and pathways on and off with light in order to study how those neurons and pathways contribute to neural computations. These tools can easily be utilized in a wide variety of animals via gene-delivery technologies, such as viral gene delivery, physical or chemical gene delivery, or direct insertion of the gene into the genome of the organism.

As you will see, the path of innovation was nonlinear and often advanced by brief moments of insight or luck amid long-standing persistent effort. In this sense, optogenetics is like many other innovations in biology and bioengineering in which success is often a result of the ability to optimize one's luck. Some aspects of this history may be instructive for thinking about how to go about developing neuroengineering innovations: how to combine

information in multidisciplinary ways toward a tool-invention goal; how to orchestrate pilot studies so as to reduce neurotechnology risk; and how to approach a problem from many angles simultaneously to improve feasibility and augment impact.

There are also aspects of the history that may point toward the kinds of new technologies that will emerge in the years to come. Specifically, technologies that are impactful and that spread rapidly typically not only solve big problems in a clear way, but yield easily interpretable data, are easy for scientists to use, and are supported by parallel trends in technology development. Optogenetics, for example, spread rapidly in part because of the then-increasing popularity of optics and viruses in neuroscience.

Now to describe the path of innovation—why don't we start the story at the moment of experimental discovery?

**The Light-Activated Neurons**

It was around one in the morning on August 4, 2004, in Richard Tsien's neurophysiology lab at Stanford University. At the time, I was a Ph.D. student working on the mechanisms of motor learning—how the brain learns to control movements to adapt to changes in the body or the environment—as a graduate student in the labs of Dr. Tsien and Jennifer Raymond. I sat in the corner of the room by a small optics table, on which stood a microscope equipped with electrical neural recording equipment and, importantly, a fast-switching light source, designed originally for delivering brief pulses of light to neurons in order to image their electrical activity. I placed into the microscope a small, thin piece of glass, on which grew cultured mammalian neurons. Earlier, Karl Deisseroth, then a postdoctoral researcher in Robert Malenka's lab at Stanford, had delivered to these neurons a piece of DNA containing the gene that encoded for the protein ChR2, the light-activated protein from green algae. Karl had worked to optimize the methods for expressing this protein in neurons, and found evidence of good expression. If this protein had the right properties to work in neurons—that is, if it safely expressed in neurons; if it inserted into the membrane in high enough quantities to mediate neurally relevant voltage changes; if its function and speed of operation in neurons could match the natural millisecond-timescale operation of neurons; if it didn't require the addition of chemicals to supplement its function—then we could potentially drive electrical impulses in neurons with pulses of light (figure 1) and thus control the neural codes of specific neurons in the brain with great precision.

I could see which neurons expressed channelrhodopsin-2 because one of the discoverers of this protein's function, Georg Nagel, then at the Max Planck Institute of Biophysics, had sent us this gene in March already fused with the gene for yellow fluorescent protein, and so the neurons of interest were glowing brightly. Georg Nagel, Ernst Bamberg, and Peter Hegemann had earlier published the discovery of this remarkable molecule in a paper in which they also demonstrated functional expression in a cell line, and commented on its possible utility in altering cellular membrane potential.[11] I lowered a thin glass electrode onto a glowing neuron in order to record the electrical activity of the neuron, and instructed the computer to activate the fast-switching light source so that it would deliver pulses of blue light to the neuron. To my amazement, the very first cell that I recorded from proved that the molecule exceeded our highest expectations—a true stroke of serendipity, to be sure. Upon receiving a brief train of light pulses, the neuron fired off a series of electrical impulses, or action potentials (figure 3). The speed and kinetics of the molecule were excellent. Even complexly timed sequences of light pulses resulted in precision sequences of electrical impulses, mimicking those found in the living brain. The molecule was clearly expressing at high levels, the neurons appeared healthy, and the molecule appeared functional, even after extended illumination.

**Figure 3**
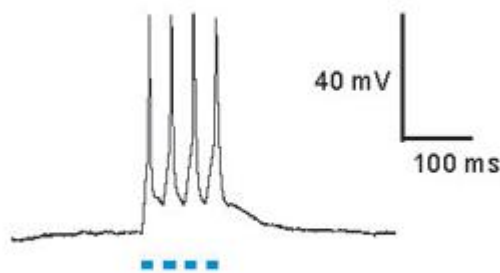


Raw electrical recording of light-activated spikes (driven by 15 ms blue light pulses) acquired from the first channelrhodopsin-2-expressing neuron recorded, in the study that culminated in our first paper on controlling the activity of neurons with light. The recording was performed on a cultured hippocampal neuron with the whole-cell patch clamp method, in current-clamp mode.

That night, I collected data that demonstrated the core principles of the paper that we eventually published a year later, announcing that you could control the activity of neurons expressing this gene just by using light. Given how finicky and fragile neurons are, it was amazing that this molecule appeared to be useful and safe without needing any painstaking molecular optimization or alteration. It was an exhilarating moment to see those first light-driven spikes.

In January 2005, Karl transitioned from being a postdoctoral researcher to becoming an assistant professor at Stanford. He had recruited a Stanford chemistry graduate student, Feng Zhang, to make benign viral vectors that could be used to deliver the gene for ChR2 to neurons. This facilitated data collection, and I continued to perform experiments on ChR2 in the Tsien lab, where I was still a Ph.D. student. I owe Richard Tsien a debt of gratitude for providing an environment in which new ideas could be pursued, and I regret that we did not acknowledge in our first paper on optogenetics that many of the key experiments had been done in his lab. Around the end of March, I started doing experiments in Karl's new lab, as he had acquired hardware appropriate for optical neural control, and by early May 2005 we had submitted our paper describing the discovery that ChR2 worked perfectly in neurons to the journal *Nature Neuroscience*. The paper was quickly accepted and appeared in publication online in August 2005.[12]

Clearly the idea of using ChR2 in neurons was in the air. In the months following the appearance of our paper, several other groups published papers describing the use of this molecule in different kinds of neurons.[13-16] The Yawo lab at the Tohoku University Graduate School of Life Sciences showed it working in intact mammalian brain slices, the Herlitze and Landmesser labs at Case Western Reserve University showed it working in the chick spinal cord, the Pan lab at Wayne State University showed it working in the retina of the living mouse, and the Gottschalk lab at Goethe-University Frankfurt, also working with Nagel, showed it working in freely behaving worms. These papers nicely explained that opsins could work in intact brain tissue, enabling specific cells bearing ChR2 to be activated by light, while neighboring cells not expressing ChR2 would not be directly affected by light (figure 4). Furthermore, as anticipated, no chemicals needed to be added to supplement the function of the opsin *in vivo*. In October 2005 I finished my Ph.D. work and went on the faculty job market while starting a postdoctoral position with Karl and with Mark Schnitzer, also at Stanford. A year later I started my neuroengineering group at the MIT Media Lab.

**Figure 4**



Rendering of a neural network in the brain, containing many kinds of neurons, in which one kind of neuron, the basket cell is selectively expressing channelrhodopsin-2, so that when the neural network is illuminated with blue light, just the basket cells fire (indicated by white highlighting). Credit: MIT McGovern Institute, Julie Pryor, Charles Jennings, Sputnik Animation, and Ed Boyden.

### The Seeds and Future of the Idea

How did different lines of thought converge to bring us to the 2004–2005 collaboration described above? A few different sources of momentum contributed. First, I had trained as an engineer and physicist at MIT before starting neuroscience graduate school. Much of my research had focused on the control of complex systems, so thinking about the brain as a computer system to be engineered felt natural. Second, when I was a first-year graduate student at Stanford, I met Karl, who was then a graduate student finishing his M.D./Ph.D. (also working in the Tsien lab at the time), and we started brainstorming ways to control the brain (indeed, we acquired the clone for the first halorhodopsin eventually used in neurons back in 2000, based on a paper that showed that this particular halorhodopsin could operate in a ionic milieu similar to that found in the brain). Although we did not pursue the project right away, the seeds of possibility and the avenues we eventually pursued, took time to scope out, and it was good to begin early. Third, of course, was serendipity—these molecules worked well in neurons on the first try, but that was not necessarily guaranteed.

Over the last several years, our group at MIT, and many others, have demonstrated the use of a number of other molecules that enable the manipulation of neural electrical activity with light. We find these molecules by mining genomes of different species of archaea, bacteria, fungi, and plants. In 2007 we published a paper describing the use of Halo/NpHR, a yellow light-driven inward chloride pump, to quiet neural activity (figure 2).[17] In 2010 we published a paper on a more powerful neural silencer, Arch, which could mediate complete silencing of neural activity in the awake mouse.[18] In that paper, we also reported on

a fungal opsin, Mac, which was driven by blue light, enabling multicolor perturbation alongside the older molecules that could be driven by redder colors of light.

Researchers continue to look for ever-more-powerful molecules in nature and to cause mutations in the molecules to improve their functions. Several other groups have published a number of novel light-driven neural activators. They found these chiefly in two ways. The first was through either the examination of genomes of different organisms for gene sequences that appeared to encode for light-activated ion channels or pumps. The second was by genetic alteration of previously described genes to optimize them for some specific feature, such as the amplitude or kinetics of the physiological currents. In the future, the principles and tools of biotechnology—high-throughput screening and improved genomic techniques—will certainly play a role in improving these opsins further still.

**How Scientists Are Using the Technology**

Many labs are now using these optogenetic tools to drive or silence specific neurons within the brain, to figure out how they contribute to behavior, or to determine how they contribute to symptoms of neural disorders or ways of overcoming neural disorders. Working with nonprofit distributors of viruses, DNA, and transgenic mice, our group at MIT has now distributed these reagents to approximately 500 research groups around the world. As one of many examples, groups have used these tools to activate and silence a small cluster of neurons, the hypocretin/orexin neurons in the hypothalamus, deep in the brain of living mice, showing that when driven, they result in awakening of mice,[19] and when silenced, they result in mice falling asleep.[20] This kind of study enables scientists to pinpoint the causal role that specific neurons play in the brain, revealing principles of how the brain works. In addition, the technology identifies cells that could serve as specific drug targets for pharmaceutical development. For example, it is possible that drugs that selectively modulate this population of cells could be of use in the sleep-disorder therapy field. Over the last few years, studies have been published pinpointing neurons or pathways involved with reward, anxiety, spinal-cord injury, and many other behaviors and brain disorders, revealing both principles of basic brain operation as well as new targets for drugs or electrodes to be used in the treatment of brain disorders.

Some groups have also proposed that such technologies could be used directly as therapeutic technologies. For example, many forms of human blindness involve loss of photoreceptors, light-sensitive cells in the retina (the image-receiving and -processing neural

component of the eye). No classical small-molecule drug can replace the lost photoreceptors. However, by taking these light-sensitive proteins and delivering them to the spared, remaining cells of the eye using a gene therapy,[15, 21] it is possible to enable other cells to recapitulate the lost image-receiving function normally performed by the photoreceptors. Previously blind mice, when treated with such gene-therapy vectors, can make cognitive use of visual information, even solving mazes with their restored sense of vision.[22] The recent safe demonstration of the use of these molecules in the non-human primate brain may, if borne out by continued safety testing, bode well for therapies based on optogenetic tools.[23] Already groups have begun to explore the use of temporally precise optical control to treat, in animal models, other disease states such as diabetes, Parkinson's, and chronic pain.

The ability to precisely enter information into specific cells embedded within the nervous system is opening up the ability to precisely determine which cells make critical contributions to behavioral and disorder-related processes. This knowledge set will reveal new targets for clinical treatment of brain disorders, and the technology itself may eventually find use as a building block of a new set of ultra-precise neural control prosthetics.

**Edward S. Boyden, Ph.D.,** is the Benesse Career Development Professor and associate professor of biological engineering and brain and cognitive sciences at the MIT Media Lab and MIT McGovern Institute. He leads the synthetic neurobiology group, which develops tools for controlling and observing the dynamic circuits of the brain. The tools his group has invented include a suite of optogenetic tools for activating and silencing neurons with light. Dr. Boyden has launched an award-winning series of classes at MIT that teach principles of neuroengineering, starting with basic principles of how to control and observe neural functions, and culminating with strategies for launching companies in the nascent neurotechnology space. He was named to the "Top 35 Innovators Under the Age of 35" by *Technology Review* and to the "Top 20 Brains Under Age 40" by *Discover Magazine*, and has received the NIH Director's New Innovator Award, the Society for Neuroscience Research Award for Innovation in Neuroscience, the Paul Allen Distinguished Investigator Award, and the New York Stem Cell Foundation Robertson Investigator Award. Dr. Boyden received his Ph.D. in neurosciences from Stanford University and three degrees in electrical engineering and physics from MIT.

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