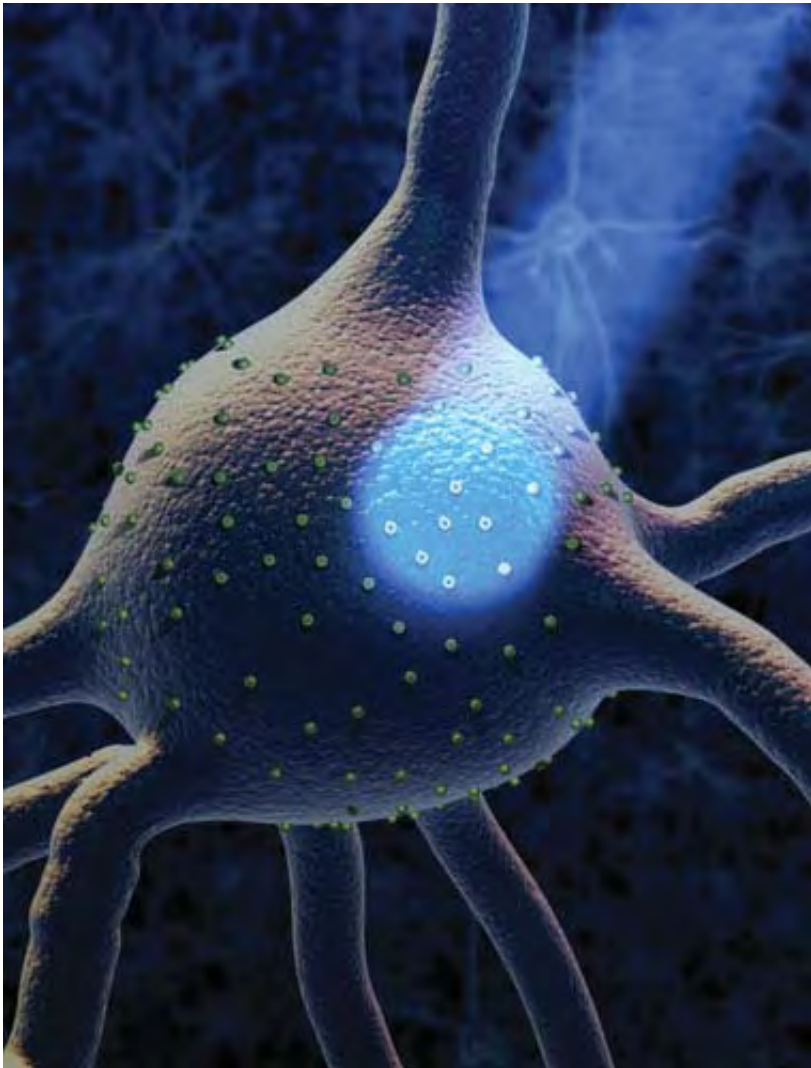


The Birth of Optogenetics

An account of the path to realizing tools for controlling brain circuits with light

By Edward S. Boyden | July 1, 2011



Blue light hits a neuron engineered to express opsin molecules on its surface, opening a channel through which ions pass into the cell—activating the neuron. MIT McGovern Institute, Julie Pryor, Charles Jennings, Sputnik Animation, Ed Boyden

For a few years now, I've taught a course at MIT called "Principles of Neuroengineering." The idea of the class is to get students thinking about how to create neurotechnology innovations—new inventions that can solve outstanding scientific questions or address unmet clinical needs. Designing neurotechnologies is difficult because of the complex properties of the brain: its inaccessibility, heterogeneity, fragility, anatomical richness, and high speed of operation. To illustrate the process, I decided to write a case study about the birth and development of an innovation with which I have been intimately involved: optogenetics—a toolset of genetically encoded molecules that, when targeted to specific neurons in the brain, allow the activity of those neurons to be driven or silenced by light.

A strategy: controlling the brain with light

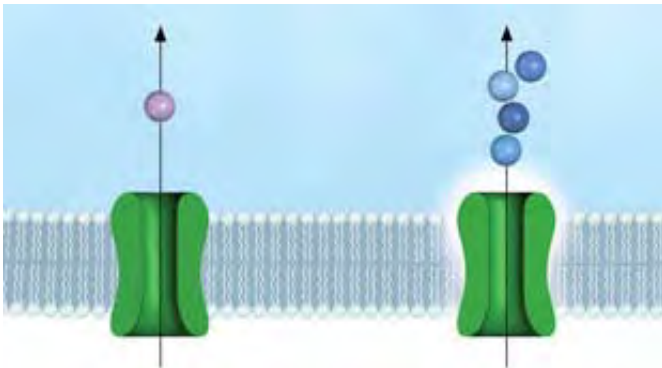
As an undergraduate at MIT, I studied physics and electrical engineering and got a good deal of firsthand experience in designing methods to control complex systems. By the time I graduated, I had become quite interested in developing strategies for understanding and engineering the brain. After graduating in 1999, I traveled to Stanford to begin a PhD in neuroscience, setting up a

home base in Richard Tsien's lab. In my first year at Stanford I was fortunate enough to meet many nearby

biologists willing to do collaborative experiments, ranging from attempting the assembly of complex neural circuits in vitro to behavioral experiments with rhesus macaques. For my thesis work, I joined the labs of Richard Tsien and of Jennifer Raymond in spring 2000, to study how neural circuits adapt in order to control movements of the body as the circumstances in the surrounding world change.

In parallel, I started thinking about new technologies for controlling the electrical activity of specific neuron types embedded within intact brain circuits. That spring, I discussed this problem—during brainstorming sessions that often ran late into the night—with Karl Deisseroth, then a Stanford MD-PhD student also doing research in Tsien’s lab. We started to think about delivering stretch-sensitive ion channels to specific neurons, and then tethering magnetic beads selectively to the channels, so that applying an appropriate magnetic field would result in the bead’s moving and opening the ion channel, thus activating the targeted neurons.

By late spring 2000, however, I had become fascinated by a simpler and potentially easier-to-implement approach: using naturally occurring microbial opsins, which would pump ions into or out of neurons in response to light. Opsins had been studied since the 1970s because of their fascinating biophysical properties, and for the evolutionary insights they offer into how life forms use light as an energy source or sensory cue.¹ These membrane-spanning microbial molecules—proteins with seven helical domains—react to light by transporting ions across the lipid membranes of cells in which they are genetically expressed. (See the illustration above.) For this strategy to work, an opsin would have to be expressed in the neuron’s lipid membrane and, once in place, efficiently perform this ion-transport function. One reason for optimism was that bacteriorhodopsin had successfully been expressed in eukaryotic cell membranes—including those of yeast cells and frog oocytes—and had pumped ions in response to light in these heterologous expression systems. And in 1999, researchers had shown that, although many halorhodopsins might work best in the high salinity environments in which their host archaea naturally live (i.e., in very high chloride concentrations), a halorhodopsin from *Natronomonas pharaonis* (Halo/NpHR) functioned best at chloride levels comparable to those in the mammalian brain.²



Infographic: OPSINS: Tools of the Trade
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I was intrigued by this, and in May 2000 I e-mailed the opsin pioneer Janos Lanyi, asking for a clone of the *N. pharaonis* halorhodopsin, for the purpose of actively controlling neurons with light. Janos kindly asked his collaborator Richard Needleman to send it to me. But the reality of graduate school was setting in: unfortunately, I had already left Stanford for the summer to take a neuroscience class at the Marine Biology Laboratory in Woods Hole. I asked Richard to send the clone to Karl. When I returned to Stanford in the fall, I was so busy learning all the skills I would need for my thesis work on motor control that the opsin project took a backseat for a while.

The channelrhodopsin collaboration

In 2002 a pioneering paper from the lab of Gero Miesenböck showed that genetic expression of a three-gene *Drosophila* phototransduction cascade in neurons allowed the neurons to be excited by light, and suggested that the ability to activate specific neurons with light could serve as a tool for analyzing neural circuits.³ But the light-driven currents mediated by this system were slow, and this technical issue may have been a factor that limited adoption of the tool.

This paper was fresh in my mind when, in fall 2003, Karl e-mailed me to express interest in revisiting the magnetic-bead stimulation idea as a potential project that we could pursue together later—when he had his own lab, and I had finished my PhD and could join his lab as a postdoc. Karl was then a postdoctoral researcher in Robert Malenka's lab (also at Stanford), and I was about halfway through my PhD. We explored the magnetic-bead idea between October 2003 and February 2004. Around that time I read a just-published paper by Georg Nagel, Ernst Bamberg, Peter Hegemann, and colleagues, announcing the discovery of channelrhodopsin-2 (ChR2), a light-gated cation channel and noting that the protein could be used as a tool to depolarize cultured mammalian cells in response to light.⁴

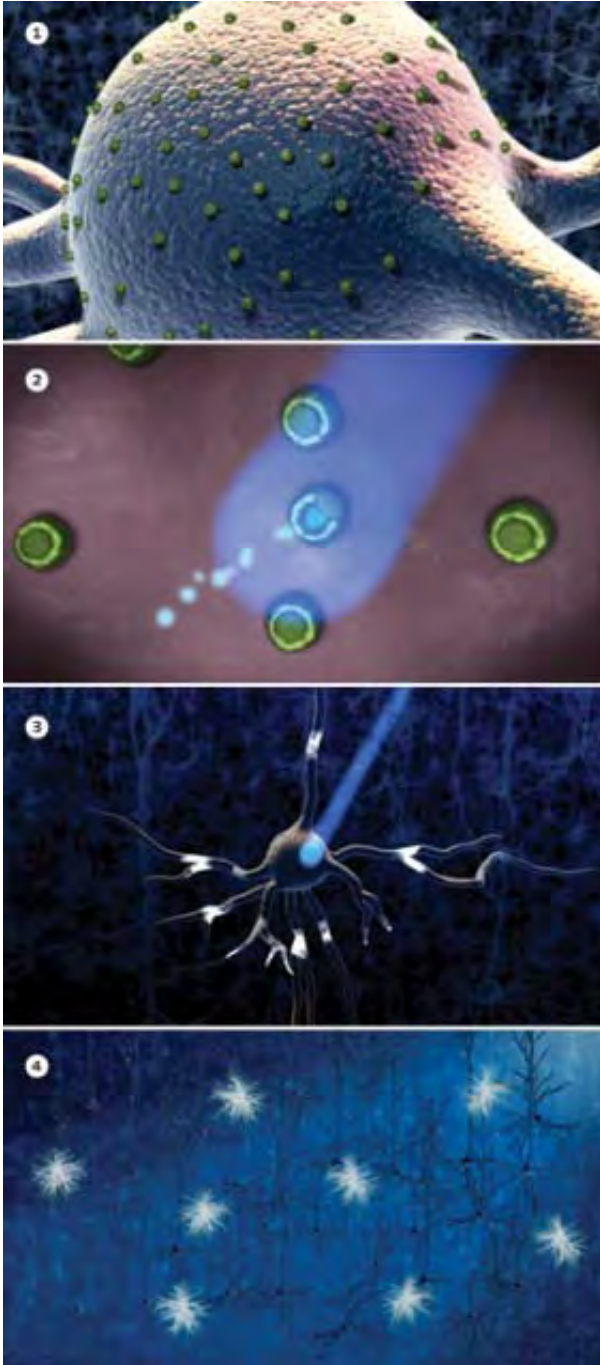
In February 2004, I proposed to Karl that we contact Georg to see if they had constructs they were willing to distribute. Karl got in touch with Georg in March, obtained the construct, and inserted the gene into a neural expression vector. Georg had made several further advances by then: he had created fusion proteins of ChR2 and yellow fluorescent protein, in order to monitor ChR2 expression, and had also found a ChR2 mutant with improved kinetics. Furthermore, Georg commented that in cell culture, ChR2 appeared to require little or no chemical supplementation in order to operate (in microbial opsins, the chemical chromophore all-*trans*-retinal must be attached to the protein to serve as the light absorber; it appeared to exist at sufficient levels in cell culture).

Finally, we were getting the ball rolling on targetable control of specific neural types. Karl optimized the gene expression conditions, and found that neurons could indeed tolerate ChR2 expression. Throughout July, working in off-hours, I debugged the optics of the Tsien-lab rig that I had often used in the past. Late at night, around 1 a.m. on August 4, 2004, I went into the lab, put a dish of cultured neurons expressing ChR2 into the microscope, patch-clamped a glowing neuron, and triggered the program that I had written to pulse blue light at the neurons. To my amazement, the very first neuron I patched fired precise action potentials in response to blue light. That night I collected data that demonstrated all the core principles we would publish a year later in *Nature Neuroscience*, announcing that ChR2 could be used to depolarize neurons.⁵ During that long, exciting first night of experimentation in 2004, I determined that ChR2 was safely expressed and physiologically functional in neurons. The neurons tolerated expression levels of the protein that were high enough to mediate strong neural depolarizations. Even with brief pulses of blue light, lasting just a few milliseconds, the magnitude of expressed-ChR2 photocurrents was large enough to mediate single action potentials in neurons, thus enabling temporally precise driving of spike trains. Serendipity had struck—the molecule was good enough in its wild-type form to be used in neurons right away. I e-mailed Karl, “Tired, but excited.” He shot back, “This is great!!!!”

Transitions and optical neural silencers

In January 2005, Karl finished his postdoc and became an assistant professor of bioengineering and psychiatry at Stanford. Feng Zhang, then a first-year graduate student in chemistry (and now an assistant professor at MIT and at the Broad Institute), joined Karl's new lab, where he cloned ChR2 into a lentiviral vector, and produced lentivirus that greatly increased the reliability of ChR2 expression in neurons. I was still working on my PhD, and

continued to perform ChR2 experiments in the Tsien lab. Indeed, about half the ChR2 experiments in our first optogenetics paper were done in Richard Tsien's lab, and I owe him a debt of gratitude for providing an environment in which new ideas could be pursued. I regret that, in our first optogenetics paper, we did not acknowledge that many of the key experiments had been done there. When I started working in Karl's lab in late March 2005, we carried out experiments to flesh out all the figures for our paper, which appeared in *Nature Neuroscience* in August 2005, a year after that exhilarating first discovery that the technique worked.



CHANNELRHODOPSINS IN ACTION

A neuron expresses the light-gated cation channel channelrhodopsin-2 (green dots on the cell body) in its cell membrane (1). The neuron is illuminated by a brief pulse of blue light a few milliseconds long, which opens the

channelrhodopsin-2 molecules (2), allowing positively charged ions to enter the cells, and causing the neuron to fire an electrical pulse (3). A neural network containing different kinds of cells (pyramidal cell, basket cell, etc.), with the basket cells (small star-shaped cells) selectively sensitized to light activation. When blue light hits the neural network, the basket cells fire electrical pulses (white highlights), while the surrounding neurons are not directly affected by the light (4). The basket cells, once activated, can, however, modulate the activity in the rest of the network.

[Watch Video](#) MIT McGovern Institute, Julie Pryor, Charles Jennings, Sputnik Animation, Ed Boyden

Around that same time, Guoping Feng, then leading a lab at Duke University (and now a professor at MIT), began to make the first transgenic mice expressing ChR2 in neurons.⁶ Several other groups, including the Yawo, Herlitze, Landmesser, Nagel, Gottschalk, and Pan labs, rapidly published papers demonstrating the use of ChR2 in neurons in the months following.^{7,8,9,10} Clearly, the idea had been in the air, with many groups chasing the use of channelrhodopsin in neurons. These papers showed, among many other groundbreaking results, that no chemicals were needed to supplement ChR2 function in the living mammalian brain.

Almost immediately after I finished my PhD in October 2005, two months after our ChR2 paper came out, I began the faculty job search process. At the same time, I started a position as a postdoctoral researcher with Karl and with Mark Schnitzer at Stanford. The job-search process ended up consuming much of my time, and being on the road, I began doing bioengineering invention consulting in order to learn about other new technology areas that could be brought to bear on neuroscience. I accepted a faculty job offer from the MIT Media Lab in September 2006, and began the process of setting up a neuroengineering research group there.

Around that time, I began a collaboration with Xue Han, my then girlfriend (and a postdoctoral researcher in the lab of Richard Tsien), to revisit the original idea of using the *N. pharaonis* halorhodopsin to mediate optical neural silencing. Back in 2000, Karl and I had planned to pursue this jointly; there was now the potential for competition, since we were working separately. Xue and I ordered the gene to be synthesized in codon-optimized form by a DNA synthesis company, and, using the same Tsien-lab rig that had supported the channelrhodopsin paper, Xue acquired data showing that this halorhodopsin could indeed silence neural activity. Our paper¹¹ appeared in the March 2007 issue of *PLoS ONE*; Karl's group, working in parallel, published a paper in *Nature* a few weeks later, independently showing that this halorhodopsin could support light-driven silencing of neurons, and also including an impressive demonstration that it could be used to manipulate behavior in *Caenorhabditis elegans*.¹² Later, both our groups teamed up to file a joint patent on the use of this halorhodopsin to silence neural activity. As a testament to the unanticipated side effects of following innovation where it leads you, Xue and I got married in 2009 (and she is now an assistant professor at Boston University).

I continued to survey a wide variety of microorganisms for better silencing opsins: the inexpensiveness of gene synthesis meant that it was possible to rapidly obtain genes codon-optimized for mammalian expression, and to

screen them for new and interesting light-drivable neural functions. Brian Chow (now an assistant professor at the University of Pennsylvania) joined my lab at MIT as a postdoctoral researcher, and began collaborating with Xue. In 2008 they identified a new class of neural silencer, the archaerhodopsins, which were not only capable of high-amplitude neural silencing—the first such opsin that could support 100 percent shutdown of neurons in the awake, behaving animal—but also were capable of rapid recovery after having been illuminated for extended durations, unlike halorhodopsins, which took minutes to recover after long-duration illumination.¹³ Interestingly, the archaerhodopsins are light-driven outward pumps, similar to bacteriorhodopsin—they hyperpolarize neurons by pumping protons out of the cells. However, the resultant pH changes are as small as those produced by channelrhodopsins (which have proton conductances a million times greater than their sodium conductances), and well within the safe range of neuronal operation. Intriguingly, we discovered that the *H. salinarum* bacteriorhodopsin, the very first opsin characterized in the early 1970s, was able to mediate decent optical neural silencing, suggesting that perhaps opsins could have been applied to neuroscience decades ago.

Beyond luck: systematic discovery and engineering of optogenetic tools

An essential aspect of furthering this work is the free and open distribution of these optogenetic tools, even prior to publication. To facilitate teaching people how to use these tools, our lab regularly posts white papers on our website* with details on reagents and optical hardware (a complete optogenetics setup costs as little as a few thousand dollars for all required hardware and consumables), and we have also partnered with nonprofit organizations such as Addgene and the University of North Carolina Gene Therapy Center Vector Core to distribute DNA and viruses, respectively. We regularly host visitors to observe experiments being done in our lab, seeking to encourage the community building that has been central to the development of optogenetics from the beginning.

As a case study, the birth of optogenetics offers a number of interesting insights into the blend of factors that can lead to the creation of a neurotechnological innovation. The original optogenetic tools were identified partly through serendipity, guided by a multidisciplinary convergence and a neuroscience-driven knowledge of what might make a good tool. Clearly, the original serendipity that fostered the formation of this concept, and that accompanied the initial quick try to see if it would work in nerve cells, has now given way to the systematized luck of bioengineering, with its machines and algorithms designed to optimize the chances of finding something new. Many labs, driven by genomic mining and mutagenesis, are reporting the discovery of new opsins with improved light and color sensitivities and new ionic properties. It is to be hoped, of course, that as this systematized luck accelerates, we will stumble upon more innovations that can aid in dissecting the enormous complexity of the brain—beginning the cycle of invention again.

Putting the toolbox to work

These optogenetic tools are now in use by many hundreds of neuroscience and biology labs around the world. Opsins have been used to study how neurons contribute to information processing and behavior in organisms including *C. elegans*, *Drosophila*, zebrafish, mouse, rat, and nonhuman primate. Light sources such as conventional mercury and xenon lamps, light-emitting diodes, scanning lasers, femtosecond lasers, and other common microscopy equipment suffice for in vitro use.

In vivo mammalian use of these optogenetic reagents has been greatly facilitated by the availability of inexpensive lasers with optical-fiber outputs; the free end of the optical fiber is simply inserted into the brain of the live animal when needed,¹⁴ or coupled at the time of experimentation to an implanted optical fiber.

For mammalian systems, viruses bearing genes encoding for opsins have proven popular in experimental use, due

to their ease of creation and use. These viruses achieve their specificity either by infecting only specific neurons, or by containing regulatory promoters that constrain opsin expression to certain kinds of neurons.

An increasing number of transgenic mouse lines are also now being created, in which an opsin is expressed in a given neuron type through transgenic methodologies. One popular hybrid strategy is to inject a virus that contains a Cre-activated genetic cassette encoding for the opsin into one of the burgeoning number of mice that express Cre recombinase in specific neuron types, so that the opsin will only be produced in Cre recombinase-expressing neurons. [15](#)

In 2009, in collaboration with the labs of Robert Desimone and Ann Graybiel at MIT, we published the first use of channelrhodopsin-2 in the nonhuman primate brain, showing that it could safely and effectively mediate neuron type-specific activation in the rhesus macaque without provoking neuron death or functional immune reactions. [16](#) This paper opened up a possibility of translating the technique of optical neural stimulation into the clinic as a treatment modality, although clearly much more work is required to understand this potential application of optogenetics.

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OPSINS: TOOLS OF THE TRADE

The optogenetic toolset is composed of genetically encoded molecules that, when targeted to specific neurons in the brain, enable the electrical activity of those neurons to be driven or silenced by light. When these opsins are expressed in the lipid membranes of specific neurons

and subsequently illuminated, the resulting ion transport changes the electrical potential of the neurons, and results in the targeted neurons being either activated or silenced in response to light, depending upon the identity of the ion being transported.

LIGHT-ACTIVATED ION CHANNELS

Bacteriorhodopsin, discovered in the early 1970s in the archaeon *Halo-bacterium salinarum*, pumps protons out of cells in response to green light, supporting energy production in this organism ①. In the late 1970s, the related molecule halorhodopsin, an orange-light-driven inward chloride pump ②, was discovered in the same organism. And in the early 2000s, the opsins that drive phototaxis in the green alga *Chlamydomonas reinhardtii*, channelrhodopsins, were found to be light-gated cation

channels that, when illuminated, let positively charged ions (such as H^+ and Na^+) pass into cells ③. When heterologously expressed in neurons, light-driven outward proton pumps and light-driven chloride pumps enable optical silencing of neural electrical activity, and light-driven cation channels enable optical activation of neural activity—just what is needed to achieve precision control, using light, of the electrical activity of specific neurons.

