Optogenetics: Controlling the Brain with Light

[Extended Version]

By Karl Deisseroth

Despite the enormous efforts of clinicians and researchers, our limited insight into psychiatric disease (the worldwide-leading cause of years of life lost to death or disability) hinders the search for cures and contributes to stigmatization. Clearly, we need new answers in psychiatry. But as philosopher of science Karl Popper might have said, before we can find the answers, we need the power to ask new questions. In other words, we need new technology.

Developing appropriate techniques is difficult, however, because the mammalian brain is beyond compare in its complexity. It is an intricate system in which tens of billions of intertwined neurons—with multitudinous distinct characteristics and wiring patterns—compute with precisely timed, millisecond-scale electrical signals, as well as with a rich diversity of biochemical messengers. Because of that complexity, neuroscientists lack a deep grasp of what the brain is really doing—of how specific activity patterns within specific brain cells ultimately give rise to thoughts, feelings and memories. By extension, we also do not know how the brain's physical failures produce distinct psychiatric disorders such as depression or schizophrenia. The ruling paradigm of psychiatric disorders—casting them in terms of chemical imbalances and altered levels of neurotransmitters—does not do justice to the brain's high-speed electrical neural circuitry. And psychiatric treatments have historically been largely serendipitous: helpful for many but rarely illuminating, and suffering from the same challenges as basic neuroscience.

In a 1979 *Scientific American* article Nobel laureate Francis Crick suggested that the major challenge facing neuroscience was the need to control one type of cell in the brain while leaving others unaltered. Electrical stimuli cannot meet this challenge because electrodes are too crude a tool: they stimulate all the circuitry at their insertion site without distinguishing between different cell types, and their signals cannot turn off neurons with precision. Drugs are not specific enough either, and they are much slower than the natural operating speed of the brain. Crick later speculated in lectures that light might have the properties to serve as a control tool because it could be delivered in precisely timed pulses, but at the time no one had a strategy to make specific cells responsive to light.

Meanwhile, in a realm of biology as distant from the study of the mammalian brain as might seem possible, researchers were working on microorganisms that would only much later turn out to be relevant. At least 40 years ago biologists knew that some microorganisms produce proteins that directly regulate the flow of electric charge across cell membranes in response to visible light. These proteins, which are produced by a characteristic set of "opsin" genes, help to extract energy and information from the light in the microbes' environments. In 1971 Walther Stoeckenius and Dieter Oesterhelt, both then at the University of California, San Francisco, discovered that one of these proteins, bacteriorhodopsin, acts as a single-component ion pump that can be briefly activated by photons of green light—a remarkable all-in-one molecular machine. Later identification of other members of this family of proteins—the halorhodopsins in 1977 and the channelrhodopsins in 2002—continued this original theme from 1971 of single-gene, all-in-one control.

In 20/20 hindsight, the solution to Crick's challenge—a potential strategy to dramatically advance brain research—was latent in the scientific literature even before he articulated the challenge. Yet it took more than 30 years, until the summer of 2005, for these fields to come together in a new technology (optogenetics) based on microbial opsin genes.

Optogenetics is the combination of genetics and optics to control well-defined events within specific cells of living tissue. It includes the discovery and insertion into cells of genes that confer light responsiveness; it also includes the associated technologies for delivering light deep into organisms as complex as freely moving mammals, for targeting light-sensitivity to cells of interest, and for assessing specific readouts, or effects, of this optical control.

What excites neuroscientists about optogenetics is control over defined events within defined cell types at defined times—a level of precision that is most likely crucial to biological understanding even beyond neuroscience. The significance of any event in a cell has full meaning only in the context of the other events occurring around it in the rest of the tissue, the whole organism or even the larger environment. Even a shift of a few milliseconds in the timing of a neuron's firing, for example, can sometimes completely reverse the effect of its signal on the rest of the nervous system. And millisecond-scale timing precision within behaving mammals has been essential for key insights into both normal brain function and into clinical problems such as parkinsonism.

Optogenetics, medicine and psychiatry

Work from the World Health Organization has shown that psychiatric disease is the leading source of disability worldwide in terms of years of life lost to death or disability. Even a single psychiatric disease, major depression, is the leading cause of disability worldwide in women aged 15 to 44. But much stigma remains (which may relate to why hearing about this epidemiology is so surprising to many people). Why the stigma? A major reason is our collective lack of understanding. Just as a cancer diagnosis once carried more stigma than it does now (perhaps because of confusion over what cancer really "is," over concerns for contagion or even over blame for the cancer on personality features of the patient), so too does lack of insight into psychiatric disease contribute

to stigmatization, further slowing progress in this enormous problem for global human health. This lack of insight, sadly, is universal: throughout the global community, from members of the general public to the most influential and advanced psychiatrists, we don't know what psychiatric disease "is" at a fundamental level.

As one example: What is depression? Unlike the case with heart failure, for example, we don't have good models for what organ dysfunction depression represents. The heart is a pump, and its dysfunction (to a first-order approximation) relates to its pumping, which can be readily understood, measured, modeled and tuned. But we lack deep understanding of what the brain is really doing, which of course means that we don't understand its failure modes.

I come face-to-face with this challenge continually. In addition to running a research laboratory in a bioengineering department, I am also a practicing psychiatrist, and I treat patients regularly using combinations of medication, therapy, and electrical or magnetic brain stimulation. After my undergraduate years at Harvard University, I had obtained my MD and PhD degrees at Stanford University, focusing on synaptic electrophysiology and optical studies of mammalian neural circuitry. I then completed my psychiatry residency and postdoctoral fellowships at Stanford, where I developed as a physician and developed skills in the study of animal behavior. Although as a physician I employ modern tools (such as transcranial magnetic stimulation), these tools are still not good enough and, most important, do not provide deep insight into the diseases, only highlighting (as do the patients) our limitations. I remember a brilliant young college student suffering from psychotic depression and terrified by the incomprehensible voices and uncontrollable bizarre ideas in his mind. I remember a retired woman so severely depressed that she was unable to smile, barely able to eat and unresponsive to her grandchildren. My inability to explain these changes in a scientific way and the unfortunately failed responses to treatments these patients experienced have never left my mind.

As a principal investigator and psychiatrist at Stanford in 2004 (and supported by a new grant from the National Institute of Mental Health), I was able to put together and launch a research team to address the technological challenge of precise neural control. And as so often happens in science, our collective need for new ideas has helped drive the development of new technology. Being asked to reflect on our optogenetics work here also provides an opportunity to consider broader implications of the scientific process.

Casting light on life

Biology has a tradition of using light to intervene in living systems. Researchers have long employed a light-based method called CALI to destroy, and thus inhibit, selected proteins; lasers have also been used to destroy specific cells, for example, in the worm Caenorhabditis elegans. Conversely, Richard L. Fork of Bell Laboratories (in the 1970s) and Rafael Yuste of Columbia University (in 2002) reported ways to stimulate neurons with lasers that partially disrupted cell membranes. More recently, the laboratories of Gero Miesenböck, then at Memorial Sloan–Kettering Cancer Center, and of Ehud Isacoff, Richard H. Kramer and Dirk Trauner, then all at the University of California, Berkeley, employed multicomponent systems for modulating targeted cells with light. They introduced, for example, both a protein that regulates neurons and a chemical that would spur the protein into action when triggered by ultraviolet light.

Yet destroying proteins or cells of interest obviously limits one's experimental options; and methods that depend on multiple components, although elegant and useful, entail practical challenges and have not experienced broad applicability or utility in mammals. A fundamental strategic shift to a single-component strategy was necessary. As it turned out, this single-component strategy was not able to build on any of the parts or methods from earlier approaches, but instead employed the remarkable all-in-one light-activated proteins from microbes: proteins now called bacteriorhodopsins, halorhodopsins and channelrhodopsins.

Well after bacteriorhodopsin and halorhodopsin had become known to science, in 2000 the Kazusa DNA Research Institute in Japan posted online thousands of new gene sequences from the green algae *Chlamydomonas reinhardtii*. While reviewing them, Peter Hegemann, then at the University of Regensburg in Germany, who had predicted that *Chlamydomonas* would have a light-activated ion channel, noticed two long sequences similar to those for bacteriorhodopsin. He obtained copies of them from Kazusa and asked Georg Nagel (then a principal investigator in Frankfurt) to test if they indeed coded for ion channels. In 2002 Hegemann and Nagel described their finding that one of these sequences encoded a single-protein membrane channel responsive to blue light: when hit by blue photons, it regulated the flow of positively charged ions. The protein was consequently dubbed channelrhodopsin-1, or ChR1. The following year Nagel and Hegemann (along with their colleagues, including Ernst Bamberg in Frankfurt) explored the other sequence and named the encoded protein "channelrhodopsin-2," or ChR2. Almost simultaneously, John L. Spudich in Houston provided evidence that those genes were important to the light-dependent responses of *Chlamydomonas*. But these channelrhodopsins—a third type of single-component light-activated ion-conductance protein—did not immediately translate into an advance in neuroscience any more than the discoveries of bacteriorhodopsins and halorhodopsins in previous decades had. Several years passed uneventfully after 2002, as they had since 1971.

A number of scientists have confided to me

that they had considered inserting bacterial or algal opsin genes into neurons and trying to control the altered cells with light but had abandoned the idea. Indeed, anything is possible in biology, but what can actually be made to work is another story indeed. With challenges in funding, the need for low-risk projects to support trainee careers, and other issues there is a very high threshold for risk-taking in modern academic science. Animal cells were unlikely to manufacture these microbial membrane proteins efficiently or safely, and the proteins were virtually certain to be too slow and weak to be effective. Furthermore, to function, the proteins would require an additional cofactor—a vitamin A–related compound called all-*trans* retinal to absorb the photons. The risk of wasting time and money was far too great.

Nevertheless, for the bioengineering research team I had assembled at Stanford University, the motivation to improve our understanding of the brain in psychiatric disease states was more than enough to justify the extremely high risk of failure. As a principal investigator at Stanford beginning in 2004, I formed a team that included the extraordinarily talented graduate students Edward Boyden and Feng Zhang (both now assistant professors at the Massachusetts Institute of Technology) to address this challenge. I introduced channelrhodopsin-2 into mammalian neurons in culture by the well-established techniques of transfection—that is, by splicing the gene for ChR2 and a specific kind of on switch, or promoter, into a vector (like a benign virus) that ferried the added genetic material into the cells. Promoters can ensure that only selected kinds of neurons (such as those able to secrete the neurotransmitter glutamate) will express, or make, the encoded proteins.

Against all odds, the experiments worked shockingly well. Using nothing more than safe pulses of visible light, we attained reliable, millisecond-precision control over the cells' patterns of firing of action potentials—the voltage blips, or impulses, that enable one neuron to convey information to another. In August 2005 my team published the first report that by introducing a microbial opsin gene, we could make neurons precisely responsive to light. Channelrhodopsins (and, eventually as we found, the bacteriorhodopsin from 1971 and the halorhodopsins, too) all proved able to turn neurons on or off, efficiently and safely in response to light. They worked in part because mammalian tissues contain naturally robust quantities of all-trans retinal—the one chemical cofactor essential for photons to activate microbial opsins—so nothing beyond an opsin gene needs to be added to targeted neurons. Microbial opsin genes provided the long-sought single-component strategy.