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Optogenetics: Controlling the Brain with Light [Extended Version]

In this web exclusive, the author offers a longer version of his December 2010 *Scientific American* article on how researchers can probe how the nervous system works in unprecedented detail, using a technique called optogenetics

By Karl Deisseroth | Wednesday, October 20, 2010 | 4 comments

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.

Improving on nature

The number of optogenetic tools, along with the diversity of their capabilities, has since expanded rapidly because of a remarkable convergence of ecology and engineering. Investigators are adding new opsins to their tool kits by scouring the natural world for novel ones; they are also applying molecular engineering to tweak the known opsins to make them even more useful for diverse experiments in a wider range of organisms.

In 2008, for instance, our genome searches led by Feng Zhang on a different algal species, *Volvox carteri*, revealed a third channelrhodopsin (VChR1), which responds to yellow light instead of blue as we showed together with Peter Hegemann. Using VChR1 and the other channelrhodopsins together, we can simultaneously control mixed populations of cells, with yellow light exerting one type of control over some of them and blue light sending a different command to others. And we now have found that the most potent channelrhodopsin of all is actually a hybrid of VChR1 and ChR1 (with no contribution from ChR2 at all). Our other modified opsins (created with Ofer Yizhar, Lief Fenno, Lisa Gunaydin and Hegemann and his students) now include "fast" and "slow" channelrhodopsin mutants that offer exquisite control over the timing and duration of action potentials: the former can drive action potentials more than 200 times per second, whereas the latter can push cells into or out of stable excitable states with single pulses of light. Our newest opsins can also now respond to deep red light that borders on the infrared, which stays more sharply focused, penetrates tissues more easily and is very well tolerated by subjects. Many groups are now also pushing opsin engineering forward, including those of Hiromu Yawo in Japan, Ernst Bamberg in Frankfurt and Roger Tsien in San Diego.

Many of the natural opsin genes now being discovered in various non-animal genomes encode proteins that mammalian cells do not make well. But Viviana Gradinaru in my group has developed a number of general-purpose strategies for improving their delivery and expression. For example, pieces of "trafficking" DNA can be bundled with the opsin genes to act as "zip codes" to ensure the genes are transported to the correct compartments within mammalian cells and translated properly into functional proteins. This generalizable approach has served to unlock the broad ecological repertoire of microbial opsin genes.

Molecular engineering has also extended optogenetic control beyond cells' electrical behaviors, to well-defined biochemical events. A large fraction of all approved medical drugs act on a family of membrane proteins called G-protein coupled receptors. These proteins sense extracellular signaling chemicals, such as epinephrine, and respond by changing the levels of intracellular biochemical signals, such as calcium ions, and thus the activity of the cells. By adding the light-sensing domain from a rhodopsin molecule to G-protein

coupled receptors, early in 2009 Raag Airan and others in my laboratory published a set of receptors called optoXRs that respond rapidly to green light. When viruses are used to insert the single-component optoXR genes into the brains of lab rodents, the first cell type–specific fast optical control over defined biochemical pathways was enabled, working even in freely moving mammals. Optical control over defined biochemical events is now also being explored in many laboratories, and opens the door to optogenetics in essentially every cell and tissue in biology.

With fiber-optic tools we developed and published in 2006 and 2007, investigators can now deliver light for optogenetic control to any area of the brain—whether surface or deep—in freely moving mammals. And to enable simultaneous readouts of the dynamic electrical signals elicited by optogenetic control, we also have published millisecond-scale instruments that are integrated hybrids of fiber optics and electrodes ("optrodes"). A long-sought synergy can emerge between optical stimulation and electrical recording because the two can be set up to not interfere with each other. We can now, for instance, directly observe the changing electrical activity in the neural circuits involved in motor control at the same time that we are optically controlling those circuits with microbial opsins. The more rich and complex that both our optogenetic inputs and the electrical-output measures of neural circuits become, the more powerfully we can infer the computational and informational roles of neural circuits from how they transform our signals.

The round-trip back to psychiatry

The importance of optogenetics as a research tool, particularly in conjunction with other technologies, continues to grow rapidly. In addition to distributing these diverse engineered opsin genes to more than 700 laboratories worldwide (http://www.optogenetics.org), my students have worked hard over the past few years to develop and deliver optogenetics instruction. We have found that despite the unusual combination of technologies required for optogenetics, the fundamentals can be taught in focused hands-on courses in the laboratory, which accelerate the benefits of the technology. Scientists from all over the world come to practice optogenetics and return to their home institutions, where they serve as local sources of knowledge and wisdom, resulting in application to diverse settings and challenges across the globe.

One example of an unexpected class of application involves brain imaging. In recent years, neuroscience has made many advances based on the brain-scanning technique called functional magnetic resonance imaging (fMRI). These scans are usually billed as providing detailed maps of neural activity in response to various stimuli. Yet strictly speaking, fMRI shows only changes in blood-oxygen levels in different areas of the brain; those changes are just a proxy for actual neural activity. Some nagging uncertainty has therefore always surrounded the question of whether these complex fMRI signals can be triggered by increases in local excitatory neural activity. This past May, however, my laboratory used a combination of optogenetics and fMRI (dubbed ofMRI) to verify that the firing of local excitatory neurons is fully sufficient to trigger the complex signals detected by fMRI scanners. In addition, ofMRI can map working neural circuits with an exactness and completeness not previously possible with electrodes or drugs. Optogenetics is thereby helping to validate and advance a wealth of scientific literature in neuroscience and psychiatry.

Optogenetics has also been employed to control a kind of neuron (the hypocretin cells) thought to be involved in the sleep disorder narcolepsy, in the first application of optogenetics to a freely moving mammal. Specific types of electrical activity in those neurons, we have found, set off the complex transition of awakening. Optogenetics has also been employed to help determine how dopamine-making neurons may give rise to feelings of reward and pleasure. In this work with Hsing-chen Tsai, Feng Zhang, Antonello Bonci, Garrett Stuber and Luis de Lecea, we optogenetically drove well-defined dopamine neurons in the mouse in different temporal patterns during free behavior, and found parameters that were sufficient to drive reinforced behavior (for example, in the absence of any other cue or reward, healthy animals simply chose to spend more time in places where they had received particular kinds of optogenetic bursting activity in dopamine neurons). This work is relevant to hedonic (pleasure-related) pathologies involved in depression (as in my depressed patient who could no longer even enjoy seeing her grandchildren, otherwise one of the most rewarding experiences known to humankind) and in substance abuse, as well as in healthy reward processes.

The optogenetic approach has also improved our understanding of Parkinson's disease, which involves a disturbance of information processing in certain motor-control circuits of the brain. Since the 1990s some Parkinson's patients have received relief via a therapy called deep-brain stimulation, in which an implanted device similar to a pacemaker applies carefully timed, oscillating electric stimuli to certain areas far inside the brain, such as the subthalamic nucleus. Yet the promise of this technique for Parkinson's (and indeed for a variety of other conditions) is partially limited because electrodes stimulate nearby brain cells unselectively and medical understanding of what stimuli to apply is woefully incomplete. Recently, however, we have used optogenetics to study animal models of Parkinson's and gained fundamental insight into the nature of the diseased circuitry and the mechanisms of action of therapeutic interventions. For example, we have found that deep-brain stimulation may be most effective when it targets not cells but rather the connections between cells—affecting the flow of activity between brain regions. And we have worked with Anatol Kreitzer of U.C.S.F. who has functionally mapped two pathways in brain movement circuitry: one that slows movements and one that speeds them up and can counteract the parkinsonian state.

We have also learned how to prod one kind of cell, neocortical parvalbumin neurons, to modulate 40-cycles-per-second rhythms in brain activity called gamma oscillations. Science has known for some time that schizophrenic patients have altered parvalbumin cells

and that gamma oscillations are abnormal in both schizophrenia and autism—but the causal meaning of these correlations (if any) was not known. Using optogenetics, Vikaas Sohal and Feng Zhang in my group (along with Li-Huei Tsai and Chris Moore at M.I.T. and our other collaborators) showed that parvalbumin cells, in cooperation with other cell types, serve to modulate gamma waves. Those waves in turn enhance the flow of information through cortical circuits. In my patients with schizophrenia I see what clearly appear to be information-processing problems, in which mundane random events are incorrectly viewed as parts of larger themes or patterns (an informational problem perhaps giving rise to paranoia and delusions). These patients may suffer from some failure of an internal "notification" mechanism that informs us when thoughts that are self-generated (an informational problem perhaps underlying the frightening phenomenon of "hearing voices"). Conversely in my patients with autism spectrum disease, rather than inappropriately broad linkages in information, I see overly restricted information processing: they miss the big picture by focusing too narrowly on just parts of objects, people, conversations and so on. These failures of information processing may lead to failures in communication and social behavior, and better understanding of brain rhythms has provided insights into these complex diseases.

As a physician, I find this work thrilling because we are bringing engineering principles and quantitative technology to bear on seemingly "fuzzy" but devastating and intractable psychiatric diseases. Optogenetics is thus helping to move psychiatry toward a network-engineering approach, in which the complex functions of the brain (and the behaviors it produces) are interpreted as properties of the neural system that emerge from the electrochemical dynamics of the component cells and circuits. It thus fundamentally changes our understanding of how electrically excitable tissues function in health and disease. We have come a long way from bacteriorhodopsin, indeed.

Bounty of the unexpected

Scientists spend a great deal of time thinking about not just their own laboratories and their own fields, but also more general questions about how science is conducted. At large and diverse scientific gatherings (such as the Society for Neuroscience annual meeting, with more than 30,000 attendees), I have occasionally heard colleagues overwhelmed by the diversity of the field take the devil's advocate position and suggest that it would be more efficient to focus tens of thousands of scientists on one massive and urgent project at a time—for example, eliminating Alzheimer's disease. Indeed, a common topic of conversation and policymaking in science considers the extent to which either diverse exploration or massively focused efforts should predominate. Even for small focused efforts, how much should funding sources and scientific organizations set the scientific agenda and guide investigators? There is no question that both directed and exploratory approaches have value, and as a practicing psychiatrist I fully appreciate the urgency for directed efforts. As they did for me, clinical need can and should drive and inspire basic science and engineering.

But how exactly should this inspiration be implemented? The story of optogenetics makes a strong argument. Even envisaging the concept of optical control of targeted neurons, as Crick did, did not predict the need for decades of pure basic research on microbial membranes. The bottom line is that there is no possible way one could have predicted the impact of archaeal or algal biology on our understanding of Parkinson's disease and its treatment. It is still too early in the exhilarating history of humanity's march toward understanding of the natural world.

The more directed and targeted research becomes, the more likely we are to slow our progress, and the more certain it is that the distant and untraveled realms, where truly disruptive ideas can arise, will be utterly cut off from our common scientific journey. Purely directed funding will adversely affect most seriously the fields most distant from human health (such as archaeal biology), but will even stultify fields clearly relevant to human health and disease, such as neuroscience. In a supreme irony, even if 30,000 scientists could be directed toward a common goal, the very act of focusing on that goal could ensure that the goal would not be reached.

The importance and urgency of these goals should not be underestimated. The benefits to our society and to global health of preserving a healthy community of undirected research will show up in many ways, ranging from insight into psychiatric disease to protections for the environment. Consider the importance of basic science—which could be so vulnerable to criticism when misrepresented—in the case of optogenetics, which could be cast in an unflattering light as money spent studying genes from pond scum. Consider the importance of preserving biodiversity, as in the harsh, barren and otherwise quite useless Saharan salt lakes from which some of the most useful opsins originated. Consider again the stigma of brain disease—so deeply tied to our lack of understanding.

In classic microbial opsin work, we find meaning for the modern world—not just for science, but also for medicine and psychiatry—that makes a strong and clear statement for environmental protection, for preservation of biodiversity and for the pure quest for understanding. And the journey of optogenetics shows that hidden within the ground we have already traveled over or passed by, there may reside the essential tools, shouldered aside by modernity, that will allow us to map our way forward. Sometimes these neglected or archaic tools are those that are most needed—the old, the rare, the small and the weak.

Some years ago (during a period of undirected reading) in the *Annual Review of Biochemistry*, I stumbled across Harvard biochemist Eugene Kennedy's reflections on his career—and the tension between past and future, between old age and renewal, and between the mortality of the human body and the immortality of art and science. Kennedy concluded, "The anonymity that is the fate of nearly every scientist as the work of one generation blends almost without a trace into that of the next is a small price to pay for its unending

progress, the great long-march of human reason. To feel that one has contributed to this splendid enterprise, on however small a scale, is reward enough for labor at the end of a day." I would add to that magnificent sentiment only that the story of optogenetics recounted above sends a strong, clear message. In our quest to move the enterprise of science forward we should never forget that we do not know where this long march is taking us or what we will need to get there.

Related links:

Optogenetics Resource Center
www.stanford.edu/group/dlab/optogenetics/index.html

Minimally Invasive Brain Stimulation
www.nature.com/nature/journal/v466/n7310_supp/box/466S15a_BX2.html

Optogenetics News
www.forbes.com/forbes/2010/0719/opinions-lasers-algae-bioengineering-ideas-opinions.html
www.stanford.edu/group/dlab/news.html

www.hfsp.org/PDF_Files/Press%20release%20-%20Nakasone%20Award%202010%20to%20Karl%20Deisseroth_final.pdf

Lectures on Microbial Opsin Optogenetics
www.stanford.edu/group/dlab/karlsfntalk.html
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