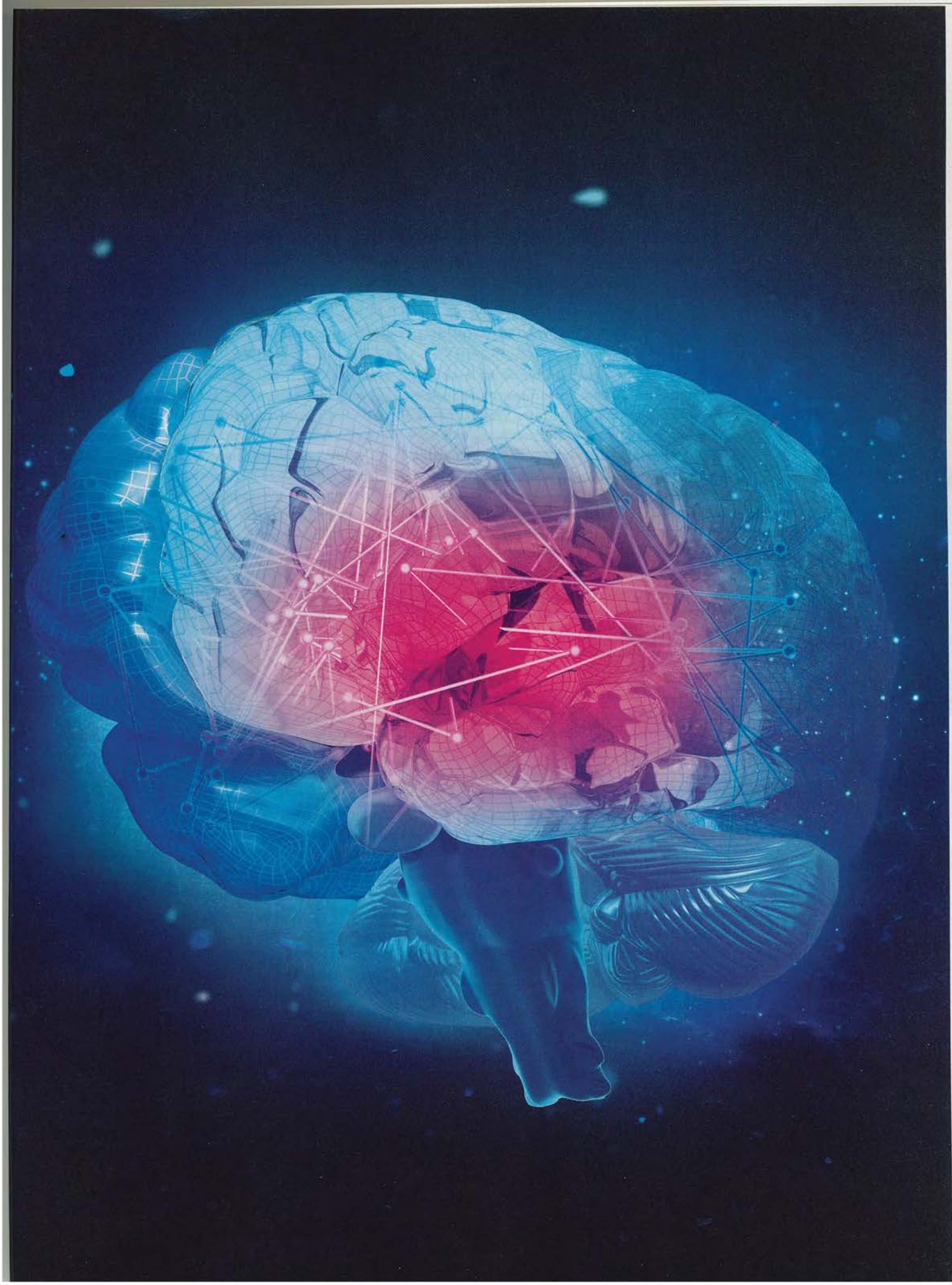
LET THERE BE LIGHT

By engineering brain cells to switch on or off in response to light, scientists are unlocking the mysteries of the mind and crafting new remedies for brain disorders

By Edward S. Boyden

Illustration by **VAULT 49**



In a neuroscience laboratory in Boston, a mouse explores a plastic box, poking its nose into this corner and that. The behavior is normal, but the rodent bears a novel prosthesis: a thin glass optical fiber extends from its head and connects to a laser that can generate brief pulses of blue light. The fiber is directed at a small cluster of cells deep in the brain that manufacture the neurotransmitter dopamine.

Drugs of abuse increase dopamine levels in the brain, suggesting that the neurotransmitter conveys reward or pleasure, sometimes to a detrimental end—but no one knows the precise role of dopamine-making cells in addiction. By stimulating these cells specifically, my group, working with neuroscientist Chris Fiorillo of the Korea Advanced Institute of Science and Technology, hoped to find out.

Perhaps out of curiosity, the mouse pokes its nose into a corner of the box equipped with a movement sensor, triggering a flash of blue light from the laser, which courses down the fiber and activates the dopamine neurons near its tip. The mouse pauses for a second and then jabs its nose again into the sensor, earning another pulse of light. Over and over, it repeats this behavior, working for light. The results, published in 2012, show that activating just this small cluster of dopamine cells, even briefly, can make an animal do more of what it was just doing. In this way, dopamine neurons could directly drive a pattern of behavior reminiscent of addiction.

Of course, dopamine cells are not normally activated by light. We make them light-sensitive using a technology we developed known as optogenetics. We endow neurons with molecules that act as miniature solar panels, which enable them to convert illumination into electrical signals, the currency of computation in the brain. By issuing these tiny solar panels to one type of neuron of the many thousands in the brain, we can determine the precise role of those cells in behavior, sensory processing or even disease.

For a century neuroscientists have used electricity to trigger neuronal activity, sending current down a conducting wire. In the 1950s neurosurgeon Wilder Penfield, for example, found that stimulating certain sites in the brains of epileptic patients could cause them to recall childhood memories. Others have found they could create visual perceptions, make ordinary things seem funny or even partially arouse people from a coma. Stimulating the brain can prove that neurons in a specific region drive complex emergent behaviors in a way that simply observing the brain cannot.

But because the brain packs approximately 100,000 neurons and a billion synaptic connections in every cubic millimeter of tissue, electrically stimulating even a tiny location in the brain will excite a very large number of intermeshed cells of different kinds. Thus, electricity cannot elucidate exactly which cells drive what behaviors. In contrast, if cells of a single type, such as dopamine neurons, are equipped with light-sensitive molecules, illumination will excite those cells exclusively. Over the past decade hundreds of research groups have used optogenetics to learn how various networks of neurons contribute to behavior, perception and cognition. For example, they have identified one set of neurons that triggers aggression, another that can drive memory recall and a third that can augment perception of detail. In recent years we have expanded our molecular toolbox to include not only molecules that enliven neurons but also those that silence them. We have found proteins that respond to different wavelengths of light, allowing scientists to modulate multiple sets of interacting neurons at once. The result is ever more precise manipulation of brain circuits.

Optogenetics is also revealing potential targets for the treatment of brain disorders, which affect more than a billion people worldwide. By revealing how neural circuits work, the technology could point to better targets in the brain for both drugs and

FAST FACTS

SOLAR-POWERED THOUGHT

1. Using optogenetics, scientists endow neurons with molecules that act as miniature solar panels, enabling the cells to convert illumination into electrical signals, the currency of computation in the brain.
2. By delivering the tiny solar panels to a single type of neuron of the many thousands in the brain, researchers can use light to determine the precise role of those neurons in behavior, sensory processing or even disease.
3. Over the past decade hundreds of research groups have used optogenetics to identify, among other things, a set of neurons in mice that triggers aggression, another that can drive memory recall and a third that can augment perception of visual details.

With my training in electrical engineering and physics, I hoped to develop tools to analyze the brain as if it were a computer circuit.

implanted electrical stimulators. If optogenetics could be adapted to humans, a process that would require advanced gene therapy techniques and is likely years away, brain dynamics could be sculpted with extreme precision. One might be able to instantly turn down the aberrant activity during an epileptic seizure or enhance motor function in patients with Parkinson's disease. Making visual cells responsive to light might even yield a treatment for blindness.

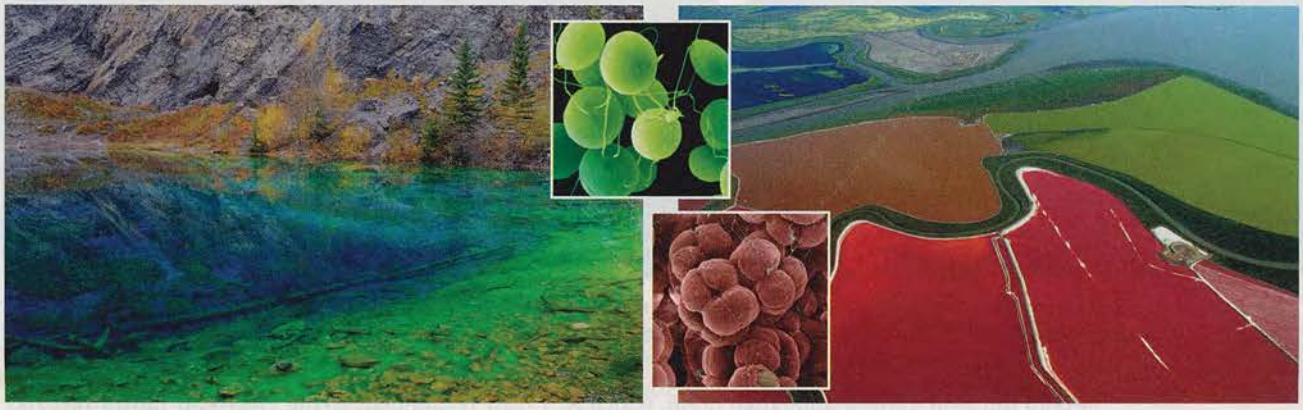
Adoptions from Algae

Organisms scattered all over the tree of life contain molecules that capture light and use it to drive important electrical or chemical processes. In photosynthesis, these molecules translate light into energy-rich chemical compounds; other life-forms engage these molecules in a primitive kind of vision. In 1971 the late biologist Walther Stoeckenius of the University of California, San Francisco, and biologist Dieter Oesterhelt, now at the Max Planck Institute of Biochemistry in Martinsried, Germany, discovered that a class of single-celled organisms that live in very salty water are endowed with light-driven proteins that sit in cell membranes. On illumination, the proteins transport protons (positively charged ions) from one side of the membrane to the other. These proteins, known as bacteriorhodopsins, support cellular metabolism and may help bacteria thrive in harsh environments where energy sources other than light are scarce.

In the late 1970s scientists identified light-driven chloride pumps known as halorhodopsins in the same organisms. And in 2002 a third class of molecules, light-gated ion channels known as channelrhodopsins, were discovered in green algae; they convert light into molecular signals that control the algae's flagella so that the microbes can steer toward the surface of ponds to capture sunlight. For decades biophysicists, microbiologists and other scientists studied these molecules to better understand how organisms interact with light.

As an engineer, I became fascinated with the potential utility of these tiny light-driven actuators. In the spring of 2000, as a graduate student at Stanford University, I was brainstorming with my fellow student Karl Deisseroth about technologies we could develop to radically accelerate the study of the brain. With my training in electrical engineering and physics, I hoped to develop tools to analyze the brain as if it were a computer circuit. I began to imagine inserting these microbial ion pumps into the membranes of brain cells so that shining a light on them would move negative ions into the cells to shut them down—or, potentially, to convey positive ions into the cells and boost their activity. Using either manipulation, we could determine the role of just those neurons in specific behaviors or pathological states.

The fact that living things made these proteins meant we could likely engineer them into other organisms, such as mice, and maybe someday into humans. In living creatures, genes specify the production of proteins, so if we delivered the genes for



To turn on or off specific neurons in the brain, scientists have borrowed light-sensitive proteins from microbes. One such protein comes from the green alga *Chlamydomonas* (*left inset*), whose habitats include freshwater lakes (*left*). Halobacteria (*right inset*), which produce several light-sensitive proteins, populate very salty water, including the salt ponds of San Francisco Bay (*right*).

AGE FOTOSTOCK (*lake*); GETTY IMAGES (*salt ponds and green algae Chlamydomonas*); SCIENCE SOURCE (*halobacteria*)

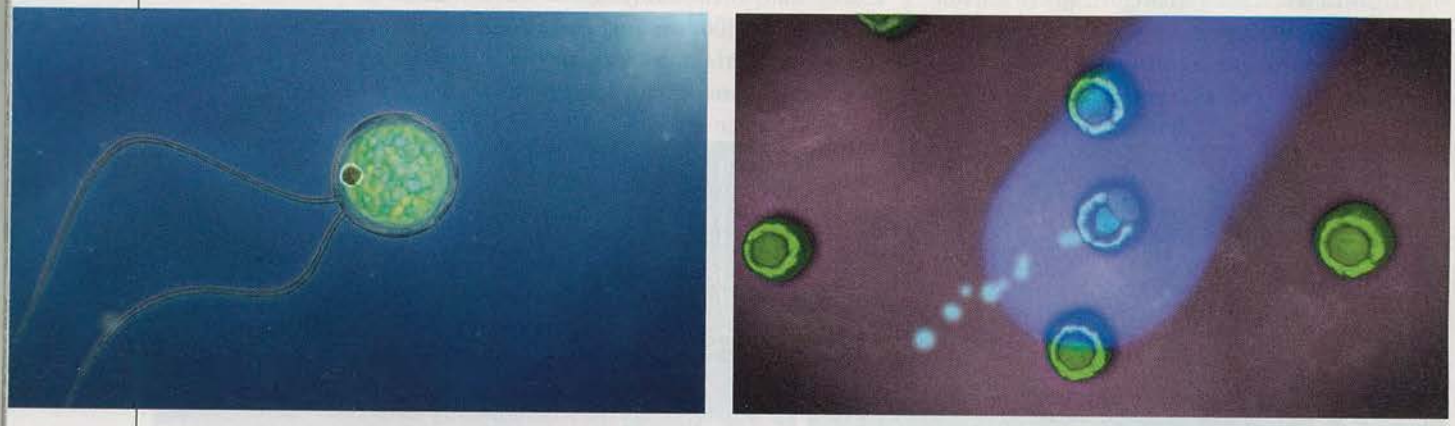
these light-sensitive proteins into brain cells, the cells could, in theory, do the rest. Current genetic engineering techniques do not allow us to systematically direct genes to specific cell types, such as dopamine neurons. We can, however, tailor a gene so that its protein is made, or expressed, only in certain cells. Because different types of cells turn on different sets of genes, we can take DNA sequences that only, say, dopamine neurons switch on and link those pieces to the gene we want to insert. Then when we deliver the gene to a mouse, only the dopamine cells will produce its protein.

I asked several colleagues to send me pieces of DNA encoding light-activated ion pumps so that I could start trying to get them into neurons. One challenge was finding light-driven pumps that would function in the mammalian brain, which is quite different from an extreme saltwater environment. In late 2003 I read a paper published earlier that year by biophysicist Georg Nagel of the University of Würzburg in Germany and his colleagues. Nagel's team had successfully inserted the gene for a light-gated ion channel from a green alga into cultured mammalian cells. The cells produced a functional protein, channelrhodopsin-2, that transported positively charged ions into the cells in response to light.

Deisseroth, then a postdoctoral scholar, and I asked Nagel to send us his gene. Deisseroth bathed cultured neurons with the gene, and at 1 A.M. on August 4, 2004, I exposed the first neuron to flashes of blue light from a lamp on a microscope. The experiment was a bit of a long shot. We had no idea whether an ion channel that evolved to help algae move toward light would have any effect on neurons. The channel might, for example, open at the wrong speed or generate too little current to alter neuronal signaling. The inserted protein could also prove toxic to the neuron for any number of reasons.

To my amazement, however, the first test neuron responded, firing electrical pulses just like those that neurons naturally generate. What is more, the neuron looked none the worse for wear. It easily survived the insertion of this protein adopted from a plant. We had used light to reliably stimulate electrical activity and neurotransmitter release in cultured neurons; we described these results in a 2005 paper.

I then began working with colleagues to expand our neural control toolbox. In a paper published in 2007 while I was an assistant professor at the Massachusetts Institute of Technology, neuroengineer Xue Han, now at Boston University, and I showed that on insertion into neurons, a halorhodopsin would pump in chloride ions (which are negatively charged) and silence neural signaling. In the next two years neuroengineer Brian Chow, now at the University of Pennsylvania, Han and I found light-driven proton pumps that could suppress neural activity by pumping positively charged ions out of cells in response to light.



Some algae have an eyespot (*left*) that enables them to swim toward light. When light hits the eyespot, protein channels open to let charged particles into it (*right*). This flow of charge fuels molecular motors that power the alga's whiplike flagella, which propel it.

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EDWARD S. BOYDEN is an associate professor of biological engineering and brain and cognitive sciences at the M.I.T. Media Lab and the McGovern Institute for Brain Research at M.I.T. He recently shared the Grete Lundbeck European Brain Research Prize, the largest brain research prize in the world, for his work in optogenetics. He is interested in radically improving the human condition through new technologies for mapping and fixing the brain.

A few years ago our then graduate student Nathan Klapoetke discovered, through mining a database of gene sequences from algae, light-driven ion channels that can respond rapidly enough to produce the fastest electrical pulses seen in the brain. Also from this database, Klapoetke identified ion channels that activate neurons in response to red light, whose longer wavelengths can penetrate deeper into tissue than blue light can and thus can activate neurons from farther away. A graduate student in our lab, Amy Chuong, also found an ion pump that can silence neurons in response to red light, even deep in the brain. Together these molecules opened the door to turning up or down the volume on multiple populations of neurons using different colors of light, enabling scientists to draw sophisticated connections between various brain circuits and behavior. For instance, activating dopamine neurons while stimulating a sensory pathway might show us how patterns of activity from sensory neurons—such as those associated with drug-related objects or odors—drive behavior.

When scientists delivered light through the fiber, activating these neurons, the mice immediately attacked other mice. They also assaulted objects such as gloves.

Attack Neurons

We have distributed our newfound tools to well over 1,000 research groups worldwide. Using these microbial machines to control neurons, researchers are identifying the neural networks that drive particular behaviors. A few years ago neuroscientists Dayu Lin, now at New York University, and David J. Anderson of the California Institute of Technology wanted to know which cells in the brain could trigger violence. In work published in 2011 they and their colleagues searched for a molecular sign of recent neural activity in the brains of mice that had just been in a fight. They found this sign in a cluster of cells in the hypothalamus, a small, deeply embedded brain region. The activation of these neurons during aggressive behavior, however, did not mean they contribute to violence, because their activity could have been a by-product of computations elsewhere in the brain. So the researchers injected a virus carrying the gene for channelrhodopsin-2 into this part of the hypothalamus, where they also implanted an optical fiber. When they delivered light through the fiber, activating these neurons, the mice immediately attacked other mice. They even assaulted inanimate objects such as gloves, underscoring the cells' powerful influence over this complex behavior. Defining such circuits may lead us to an improved understanding of the causes of aggression and suggest new ways to help people control their actions.

Researchers have also used optogenetics to hunt down the neural basis of memory recall. In a 2012 study neuroscientists Xu Liu and Susumu Tonegawa, both at M.I.T., and their colleagues created transgenic mice that carried the gene for channelrhodopsin-2 but would only express the protein in neurons that had recently been active. The mice then heard a tone before receiving a shock. Over time the mice learned to associate the tone with the shock, and they froze from fear whenever they heard it. After a mouse learned this link, the researchers found that neurons in its dentate gyrus, a part of the hippocampus known to be involved in memory formation, were expressing channelrhodopsin-2, suggesting that these neurons were involved in creating the fearful memories. Delivering light to this region reactivated the neurons, and the animals would freeze again even in the absence of a tone, showing that these neurons can independently trigger the recall of specific memories. In this way, optogenetics can reveal how complex information is stored in the brain.

Optogenetic tools can also help scientists better understand the function of specific types of neurons within known brain circuits. In a study published in 2012 neuroscientists Seung-Hee Lee, now at the Korea Advanced Institute of Science and Technology, Yang Dan of the University of California, Berkeley, and their colleagues engineered mice to express optogenetic activators in star-shaped neurons in the visual cortex, at the back of the brain. When they flipped the light switch, selectively activating these neurons, the mice more reliably licked a spout when the scientists showed them an array of lines that signaled the availability of water from the spout. Acti-

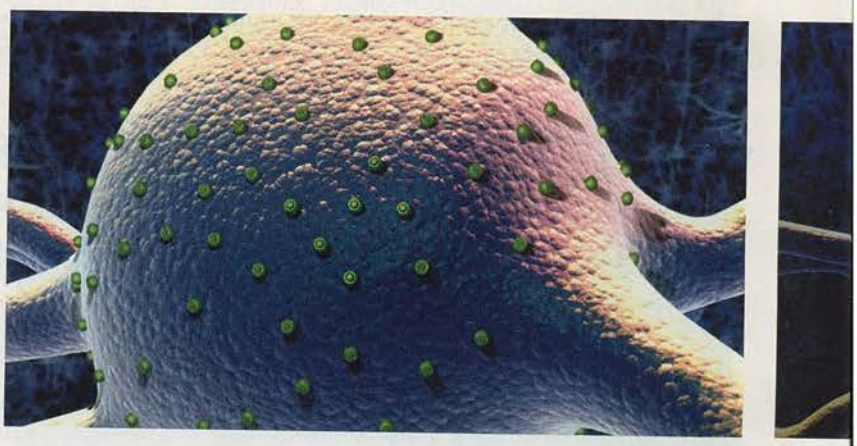
vating these star-shaped neurons seemed to help the mice distinguish arrays of different orientations, perhaps sharpening their vision. As a result, they were more confident when the stimulus that signified the availability of water was present. The results indicate that this set of neurons can enhance the brain's ability to discriminate visual features in the environment.

Making a Move

These technologies can also be used to identify—and potentially target—specific circuits in the brain that underlie various neurological disorders. Patients with temporal lobe epilepsy (one of the most common types in adults) who do not respond to medication sometimes opt for surgical removal of the part of the brain generating the seizures. Depending on which tissue is removed, the treatment can cause permanent impairment in critical functions, such as speech or movement. The ability to reset the aberrant neural circuits with light could represent a safer option for these patients. In work published in 2013 neuroscientists Esther Krook-Magnuson and Ivan Soltesz and their colleagues at the University of California, Irvine, took a step in this direction in mice. These mice had been given a drug that made them prone to epileptic seizures but were also engineered with an antidote: they expressed the gene for a halorhodopsin in excitatory neurons in the forebrain. The scientists found that shining light on these cells as soon as a seizure began could, in many cases, halt the seizure within seconds.

In other cases, results from optogenetics experiments in animals have suggested safer ways of delivering existing treatments. For example, tens of thousands of patients with Parkinson's and other movement disorders have had electrodes implanted in their brains to facilitate movement and reduce tremors. The stimulation is often aimed at a deep-seated structure called the subthalamic nucleus because surgeons have serendipitously found that electrodes in that location have therapeutic effects. In an experiment published in 2009 Viviana Gradinaru, now a neuroscientist at Caltech, Deisseroth and their colleagues tested a less intrusive Parkinson's therapy in mice using optogenetics. Because of the influence of a drug, these mice were hobbled on one side of the body by a slow gait, among other characteristics of Parkinson's, causing them to walk in circles. When the researchers used light to activate certain cells in the motor cortex that made channelrhodopsin-2, the gait of these mice straightened out and their movements became more symmetrical. Because the motor cortex is on the brain's surface, this finding suggests that Parkinson's patients might benefit from electrodes placed more superficially in the brain or even outside on the scalp.

In another application of optogenetics, making visual cells responsive to light might one day become a remedy for some types of blindness. In the disorder retinitis pigmentosa, photoreceptor (light-sensitive) cells in the eye atrophy or die off as the result of any of more than 100 mutations in various genes, leading to blindness. Without photoreceptor



Scientists use genetic engineering techniques to insert copies of a light-sensitive ion channel from a microbe into the membrane of a neuron (*left*). When blue light shines on the neuron, the channels open; positive ions flow into the cell, activating it (*center*). At the far right, orange illumination silences a set of neurons engineered to carry light-sensitive ion pumps that admit negative ions.

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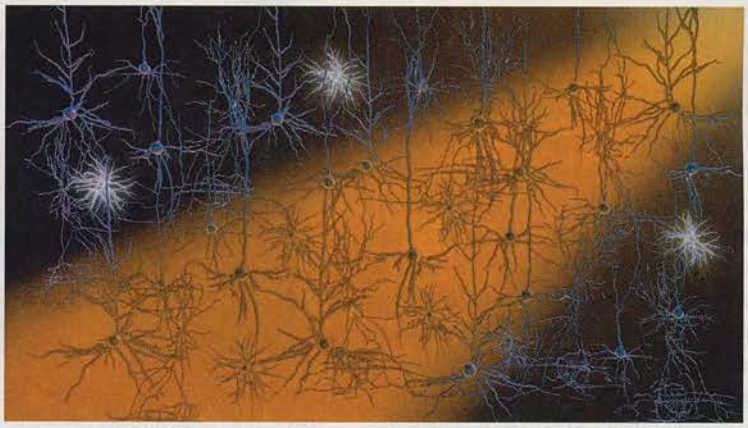
cells, the eye cannot convert light into neural signals that the brain can interpret. Various research teams are exploring whether electrical stimulators connected to a camera can convey visual information from the captured image to spared visual cells. Because electricity spreads, however, this technique produces only low-resolution vision: people see points of light or heavily pixelated images. An alternative strategy is to genetically deliver one of the proteins that converts light into neural signals to a set of surviving cells. Such a technique has restored some vision in blind mice. Several teams, some of them at biotech companies, are now exploring whether this genetic modification could be a cure for blindness in people.



Engineering the Human Brain?

To use optogenetics to treat patients, however, would require several significant advances. By precisely manipulating the DNA of mice and other creatures very early in their development, we can insert a new protein into specific cells of their bodies. In humans, in contrast, making cells responsive to light would require inserting a new gene into a fully formed individual, and gene therapy techniques that can accomplish that feat cannot reliably restrict the expression of that gene to a specific cell type. Such a therapy—including the new, foreign proteins—would also have to be proved safe over the long term. In addition, we would need to develop implantable optical devices that could illuminate neurons over an extended period.

In the meantime, and perhaps more important, optogenetic tools will help us greatly refine our maps of the brain, which will then point the way toward strategies for fixing it. To make the most progress in this endeavor, we need to invent new ways of recording neural activity that show us how perturbation of a defined set of cells influences brain dynamics. In a step in this direction, we worked with physicist and neuroscientist Alipasha Vaziri of the University of Vienna in Austria and his colleagues earlier this year to develop a microscope optimized for imaging neural activity, in 3-D, throughout entire organisms.

The Obama administration's BRAIN Initiative, launched in 2013, is aimed at stimulating the development of technologies for revealing how the brain works. The discoveries in optogenetics underscore the importance of looking in unlikely places for those tools, from desert salt lakes to mountain ponds. The extraordinary story that began with curiosity about microbial proteins that had no obvious practical use is leading, half a century later, to the unraveling of such fundamental brain processes as thought and emotion and to the discovery of safer, more effective treatments for brain disorders. M

FURTHER READING

- **Optical Deconstruction of Parkinsonian Neural Circuitry.** Viviana Gradinaru et al. in *Science,* Vol. 324, pages 354–359; April 17, 2009.
- **A Light Switch for Neurons.** TED talk by Ed Boyden; March 2011. **www.ted.com/talks/ed_boyden**
- **A History of Optogenetics: The Development of Tools for Controlling Brain Circuits with Light.** Edward S. Boyden in *F1000 Biology Reports,* Vol. 3, Article No. 11; May 3, 2011.
- **Optogenetic Stimulation of a Hippocampal Engram Activates Fear Memory Recall.** Xu Liu et al. in *Nature,* Vol. 484, pages 381–385; April 19, 2012.
- **On-Demand Optogenetic Control of Spontaneous Seizures in Temporal Lobe Epilepsy.** Esther Krook-Magnuson et al. in *Nature Communications,* Vol. 4, Article No. 1376; January 22, 2013.

From Our Archives

- **Controlling the Brain with Light.** Karl Deisseroth; *Scientific American,* November 2010.