

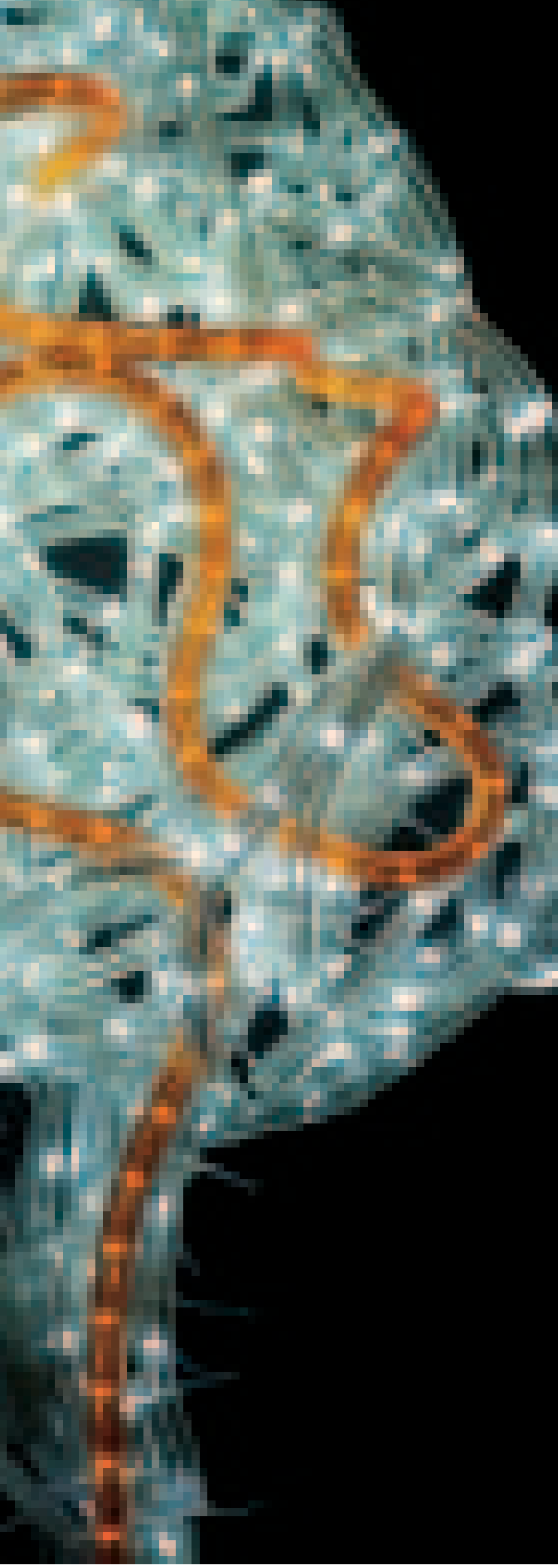


THE MARRIAGE OF OPTICS AND GENETICS:

A promising young union // that controls the firing of particular neurons // which have been reengineered to be activated by light // **uncovering the brain's mysteries with a flash**

Age of Enlightenment


■ BY CATHRYN DELUDE // PHOTOGRAPHS BY SAM KAPLAN



Much of what science knows about the human brain has come through deduction. If a stroke or trauma has destroyed a particular area, researchers can look at what that person can no longer do—talk, move the left pinky, do math—and infer that the affected region is linked to that behavior. In animal models, researchers often produce lesions artificially, or they inject a drug to inhibit or excite neural activity in a specific area. Yet as important as this approach has been, there are many things it can't accomplish. Chief among those is pinpointing which of the many kinds of cells in a given brain region are the ones that matter.

As a result, when it comes to autism, Alzheimer's disease and a long list of mental illnesses, what we do understand is dwarfed by all that we can only imagine. Treatments, too, are often a matter of trial and error. To try to prevent intractable epileptic seizures, for example, surgeons may destroy a part of the brain they believe is implicated. Often it works; sometimes it doesn't. Understanding aberrant behavior, obsessive thoughts, learning disabilities, depression, anxiety, aggression—for all of those, the learning curve remains very steep.

Much of the problem stems from the brain's sheer complexity. "It's made of 100 billion interconnected cells, which fall into many distinct classes—differing by shape, molecular composition and function—and which change in different ways in different brain disorders," says Edward Boyden, a neuroscientist and biological engineer in the MIT Media Lab, whose goal is to develop technologies for "fixing the broken brain." But it's not just the number of neurons that makes the brain so challenging; it's also that they fire in essentially arbitrary numbers of patterns depending on what we are doing, perceiving and thinking, how we feel emotionally and whether we are well or sick. Moreover, some neurons excite other neurons, some inhibit others, and some fine-tune how sensitive other neurons are to being excited or inhibited. Neurons within the same brain region may connect to others locally or



use long arms called axons to reach remote parts of the brain. Further complicating attempts to make sense of all this, diverse types of neurons are likely to be tightly intermingled in tiny volumes of brain tissue.

In 1979, Francis Crick, who helped discover the structure of DNA in the 1950s and later turned to neuroscience, cautioned that scientists could never understand the brain unless they could precisely control discrete sets of neurons to see what they do. “Without that level of understanding, I realized we cannot fully help patients with depression, schizophrenia and autism,” says Karl Deisseroth, a psychiatrist and neuroscience researcher at Stanford University.

But a new technology, optogenetics, which Deisseroth and Boyden helped develop at Stanford, along with Feng Zhang, a graduate student in Deisseroth’s lab, and two other researchers from Germany, Georg Nagel and Ernst Bamberg, could finally begin to map the brain’s vast frontiers. Combining optics and genetics, optogenetics allows scientists to control individual classes of neurons distributed among many other cell types—and with a flash of light.

Introduced in 2005, optogenetics remains a young technology. Yet like a child prodigy, it has garnered rave reviews. It has been called transformative, exquisite and a great leap forward. Researchers around the world are using it to illuminate—literally and figuratively—the mysterious brain and to investigate links between brain circuits and specific behaviors with a precision that could only have been imagined less than a decade ago.

For years before optogenetics was invented, reports had circulated about microorganisms that produce proteins called opsins. An opsin spans the cell membrane and

contains both a light-absorbing component—retinal, a vitamin A derivative—and a channel pore that is closed when the opsin is not illuminated. When the opsin’s retinal absorbs light, the protein changes form and temporarily opens up a pore that lets ions through. In effect, these naturally occurring opsins convert light into changes in cellular voltage. And because a voltage change is what causes neurons to fire, in 2000 Deisseroth and Boyden began brainstorming ways they might harness the special proteins to control neurons. In 2004, the team decided to focus on Channelrhodopsin-2 (ChR2), a protein from a one-celled alga that has an “eye” to guide the organism toward the daylight at the surface of a murky pond for photosynthesis. When exposed to blue light, a pore in the cell opens and positively charged ions flow in.

Deisseroth and Zhang, who is now a professor at MIT, used genetic engineering techniques to deliver the gene for ChR2 into the nucleus of rodent neurons they grew in cell cultures; the neurons then successfully produced the opsin. Boyden, who has an MIT degree in engineering and physics, rigged a system for directing blue light on those novel neurons.

Amazingly, the neurons fired instantly when illuminated. Also wonderful: The neurons immediately returned to their normal state when the light pulsed off, giving an unprecedented precision in timing neural activity. The process was perfectly simple, requiring only a single gene.

The team published its work in August 2005 in *Nature Neuroscience*, and they and other scientists adapted the system to try in living mice. Researchers insert the gene for ChR2 into

One researcher's optogenetics and electrophysiology studies in mice show that neurons in aggression and mating are intermingled.

impulses from neurons in the hypothalamus as male mice fought potential male competitors and as they mated with females. “We were surprised that the nerve cells involved in both aggression and sex are closely intermingled in the same neighborhood,” Anderson says.

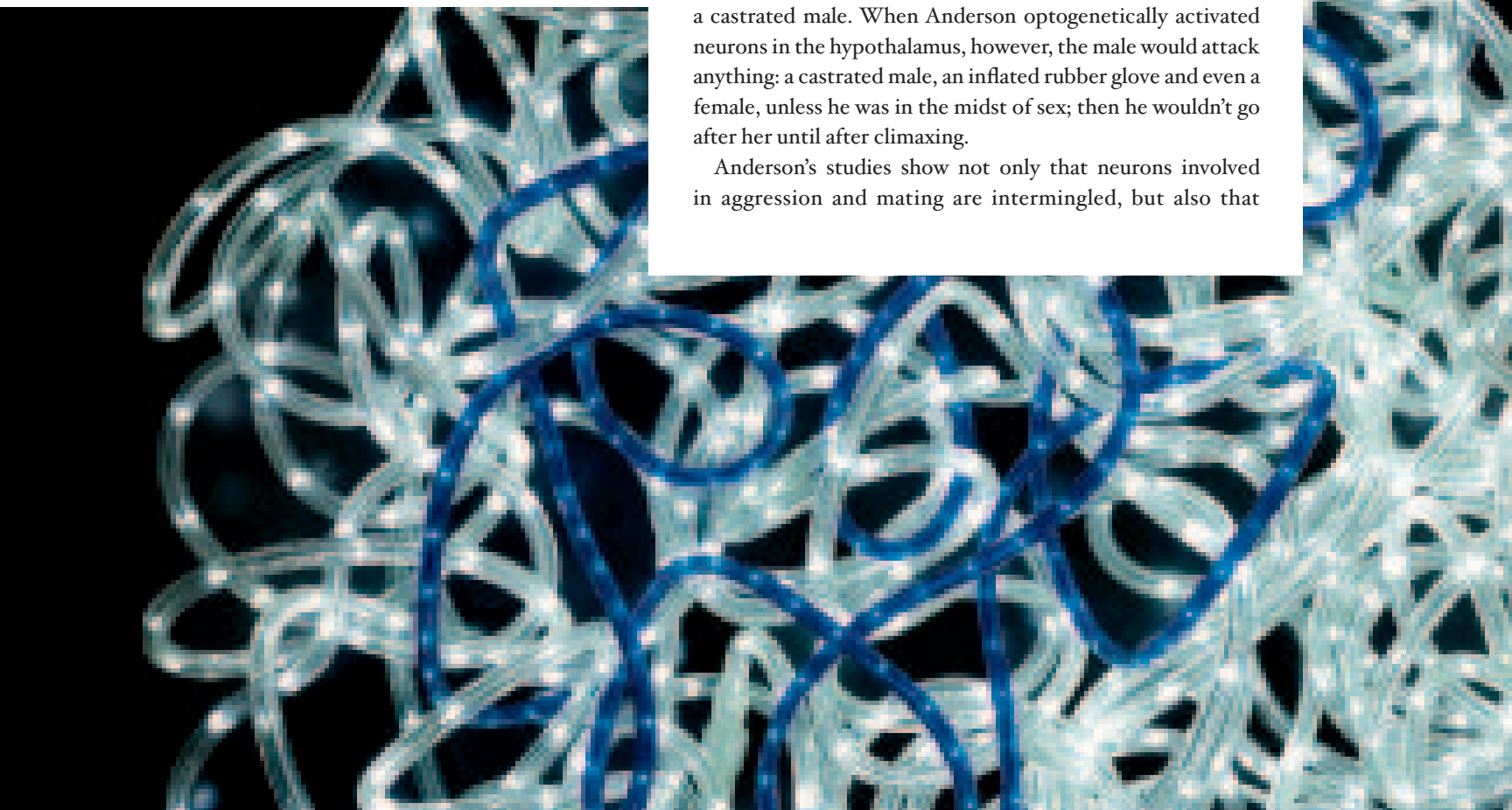
Normally, within a split second of an intruder male mouse entering a cage, the resident male streaks toward it and bites its neck. But the resident mouse will not attack a female—or a castrated male. When Anderson optogenetically activated neurons in the hypothalamus, however, the male would attack anything: a castrated male, an inflated rubber glove and even a female, unless he was in the midst of sex; then he wouldn't go after her until after climaxing.

Anderson's studies show not only that neurons involved in aggression and mating are intermingled, but also that

a harmless virus, which is injected into a region of the mouse's brain and “infects” the neurons there with the gene. The neurons then produce the light-activated protein all over their cell membranes. Next, implanted optic wires threaded to that brain region direct light there. When the light pulses on, only those cells bearing the opsin fire.

By 2007, Boyden—who by then had his own lab at MIT—Zhang, and Deisseroth independently showed that another naturally occurring opsin, halorhodopsin, responds to yellow or green light and inhibits neurons, preventing them from firing. Halorhodopsin allows researchers to zero in on particular kinds of neurons and control when they fire—in live, conscious animals. “People had been dreaming of this ability for decades,” says Wim Vanduffel, a neurophysiologist and radiologist at Massachusetts General Hospital who has begun using the technology in his research.

Optogenetics has clear advantages over electrophysiology, a technology that uses implanted electrodes to force neurons to fire. But a combination of the technologies can be particularly powerful. Electrophysiology can record how neurons fire both during normal activity and when optogenetically controlled. David Anderson, a neurobiologist at the California Institute of Technology, used this approach to explore aggression and sex in mice. With electrophysiology, he recorded electrical

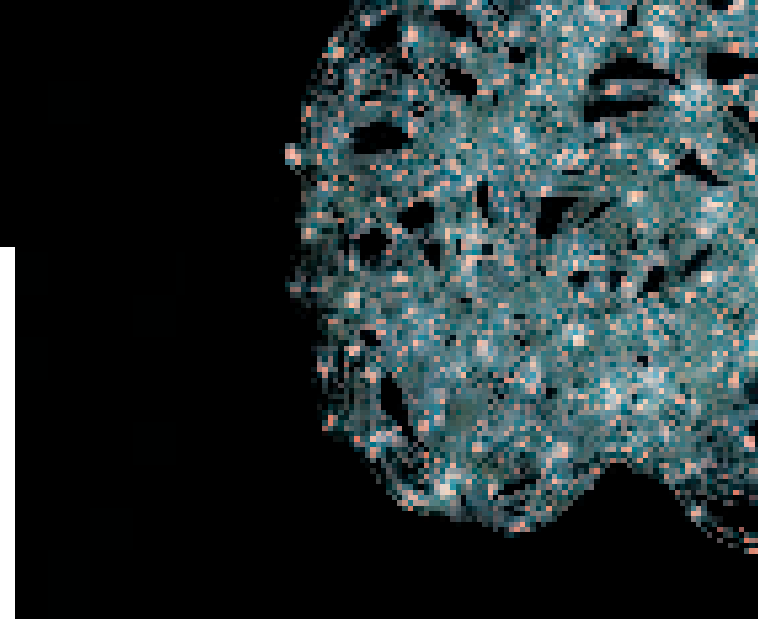


interactions with females inhibit the neurons normally active in male aggression. “If this holds for humans, perhaps we’ll learn that something goes wrong with this inhibition in sexual pathologies when circuits get miswired,” he says. He’s planning experiments to trace that circuitry.

Karl Deisseroth believes optogenetics is well suited to studying neuropsychiatric disorders, and many of his experiments in rodents aim to parse different neural circuits that account for varied aspects of depression and its diverse causes. Recently, he and his collaborators have concentrated on learning how neurons in one brain region may connect to others within the same region and also to other regions.

For example, Deisseroth and Kay Tye, a principal investigator in neuroscience at MIT, who was previously a postdoctoral researcher in Deisseroth’s lab, wanted to manipulate a “microcircuit” of connections within the amygdala, which numerous previous studies have implicated in stress and anxiety. They wanted to control signals running from the basal lateral amygdala (BLA) to the central amygdala (CeA), and see whether exciting BLA neurons increased anxious behaviors and whether inhibiting those neurons ameliorated the anxiety.

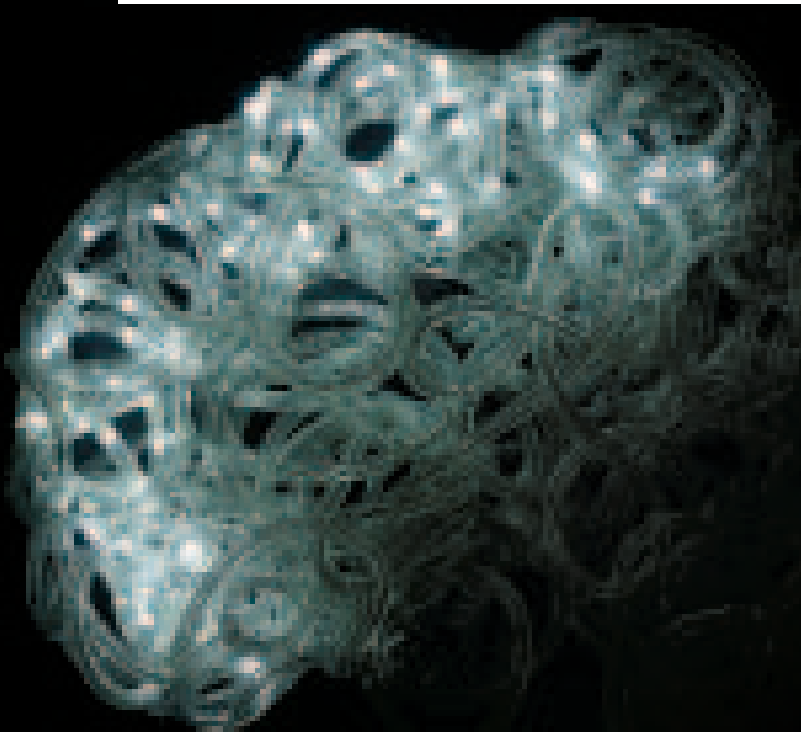
But directing light at the neural cell bodies in the BLA did

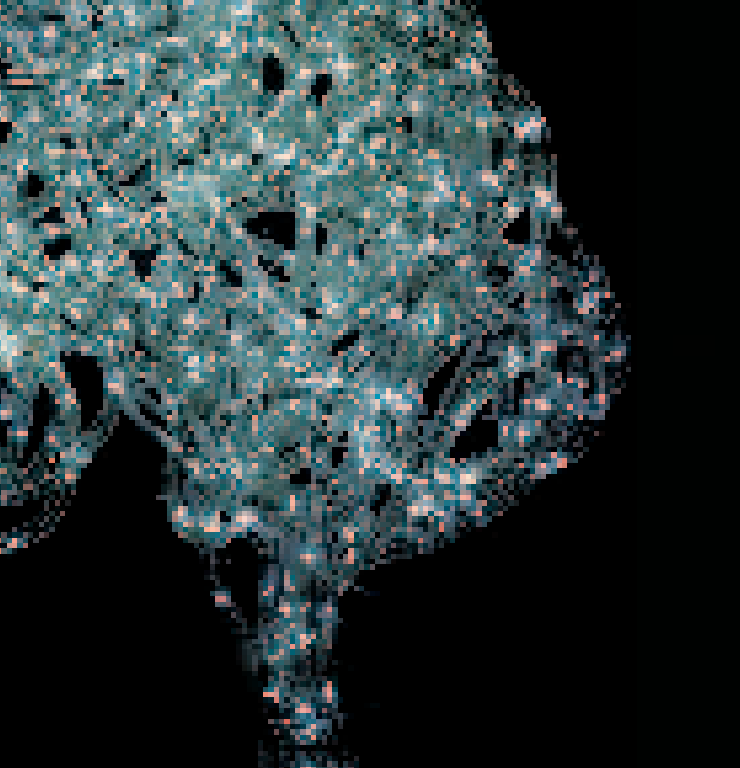


not have the anticipated effect on anxious behavior—likely because the neurons project along pathways that can have opposing functions and counteract the anxiety circuit. Tye wanted to selectively activate or inhibit just the BLA cells that projected to the CeA, so she exploited the fact that when neurons express the gene for an opsin, the light-activated protein appears all over the neuron’s membrane, including on the axon fibers that connect to other neurons. The BLA axons reaching the CeA had the light-activated proteins, but none of the neurons residing in the CeA had them. As a result, shining the light on the CeA just controlled the BLA neurons projecting into the CeA; it didn’t affect the CeA neurons themselves or any BLA neurons projecting to other regions that counteract the anxiety circuit. And indeed, as reported in a March 2011 paper in *Nature*, this more targeted control allowed her to dial anxiety up and down in mice.

In a December 2012 *Nature* study, Tye and Deisseroth used a similar method to regulate two different manifestations of depression in rodents—lack of pleasure and lack of motivation—by controlling the circuit leading from the brain’s reward processing center (the ventral tegmental area) to its pleasure center (the nucleus accumbens). They illuminated projections from neurons producing dopamine, a neurotransmitter normally associated not with depression but with habits, addiction and movement disorders. Current antidepressants and deep brain stimulation treatments for major depression do not target the dopamine circuit. But the knowledge generated by such optogenetic studies could help tweak these therapies to be more effective.

Such studies show that antidepressant treatments could potentially focus on circuits linked to a particular psychological symptom, and they could help determine an appropriate





Raising the Voltage //

Electrophysiology, a precursor to optogenetics, lets researchers stimulate brain activity. But there's a lot it can't do.

In the 1920s, a Swiss scientist named Walter Rudolf Hess conducted one of the first experiments to test the notion that neural activity in particular parts of the brain might cause specific actions. He focused on aggressive behavior in cats, and his work showcased what was then a brand-new technology—electrophysiology, an approach that in some ways foreshadowed optogenetics.

Electrophysiology uses electrodes implanted in the brain to either detect electrical activity or directly activate neurons, which are essentially electrical devices. A neuron normally allows positively and negatively charged ions to flow in and out of the cell, and when the voltage rises past a certain threshold, the neuron fires an electrical pulse. That current races down the axon, which reaches out to other nerve cells and affects their activity. With implanted wire electrodes, electrical current can raise the voltage in nearby neurons so that they fire without the normal signals from other parts of the brain.

In Hess's cat experiments, he stimulated the hypothalamus to investigate its role in the nervous system's fight-or-flight response. An electrical surge turned a placid kitty cat into a hissing, spitting beast, while turning off the electrode returned the cat to its docile self. This directly implicated the activity of neurons in the hypothalamus in aggression and the "fight" aspect of the stress response.

In the many decades since then, detecting the brain's electrical signals and creating new ones have produced remarkable insights about the functions of various brain regions. But it can't answer many essential questions. What exactly is being stimulated by the electrodes? Which types of neurons are being activated? There's also the possibility that an electrode in one area of the brain may provoke a response in other regions via long axon fibers.

"Electrophysiology can show correlations between brain activity and a change in behavior," says neuroscientist Christopher Moore at Brown University. "But it cannot prove that a specific neuron, brain region or circuit causes the behavior."

treatment for an individual. "We won't have one antidepressant drug that works for everybody," says Tye, "but it might be possible to have treatments tailored to each individual patient."

To extend this work further, optogenetic researchers hope to determine not just which brain regions the axon projections extend to but also the precise kinds of neurons the axons connect to. Such information could enable scientists to explore feedback loops within a circuit, including those implicated in epilepsy.

One type of epilepsy results from a traumatic head injury that causes a stroke in the cerebral cortex—the brain's outer layer in which higher-order processing occurs. But intervening there with surgery—the typical approach to controlling seizures that drugs can't prevent—risks worse damage.

Studies have suggested that the injured cortex communicates with the remote thalamus during epileptic seizures with back-and-forth signals that become self-perpetuating. By inducing this type of seizure in rats and then examining the brain tissue, Stanford neurologist John Huguenard surmised that although the seizure originates in the cortex, it's the thalamus that becomes hyperexcited and propagates the seizure. In collaboration with Deisseroth's lab, he used halorhodopsin, the light-activating protein that silences neurons, to dampen the thalamus's excitability when a seizure began. Upon illumination, the seizures immediately stopped, the



oscillations between the thalamus and the cortex returned to normal, and the rat again behaved normally.

The researchers also engineered a method for detecting and then silencing seizures as they occurred. They coupled the optical device that delivers the light with an electrode that could detect abnormal firing in the thalamus and trigger the light to pulse on. That illumination inhibited the neurons with the halorhodopsin, and it prevented the seizures. The study, published in the January 2013 issue of *Nature Neuroscience*, proved that the thalamus was necessary to maintain the seizure—the first evidence that it plays a part in epilepsy—and suggested that targeting the thalamus, rather than the cortex, could successfully treat this type of seizure.

“This is a great illustration of how a structure remote from primary brain damage but connected to it by long-range projections can be involved in abnormal brain network activity, and that it can be targeted therapeutically,” says Bruce Rosen, director of the Martinos Center for Biomedical Imaging at MGH, who was not involved in the study. “It shows us a new site for deep brain stimulation that we didn’t know before.”

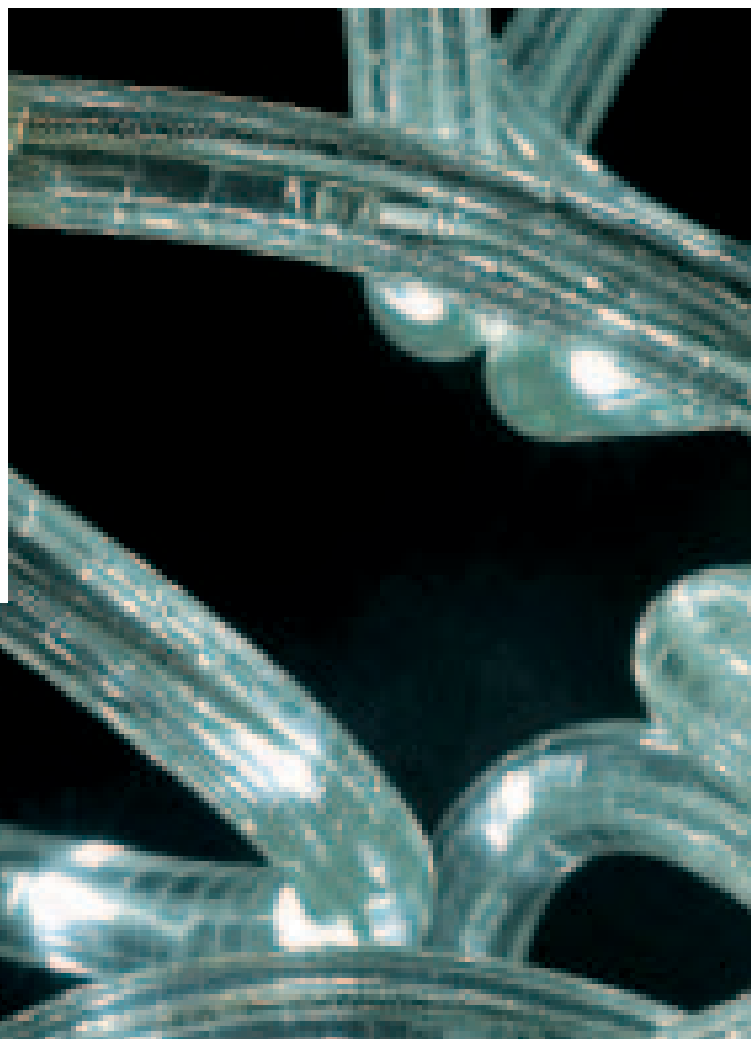
Yet for all that optogenetics has revealed in experiments with rodents, it’s not clear how or even whether similar techniques might work in the human brain. To answer that question, researchers need to demonstrate that opsins can control neurons in primates and affect their behavior.

An important step came in 2009, when Ed Boyden, working with MIT neuroscientist Robert Desimone and then postdoctoral scholar Xue Han, showed that he could optogenetically control the firing of ChR2 neurons in the rhesus monkey brain, safely and over the long term. Then Boyden worked with MGH’s Vanduffel to manipulate a subtle behavior in primates. In a study published in the Sept. 25, 2012 issue of *Current Biology*, the scientists targeted a well-defined neural circuit involved in a task often used to study visual perception in monkeys and humans.

For this task, two monkeys gaze at a focal point on a computer screen and move their gaze only when there’s a certain visual cue in the periphery of their view. If successful, they receive a sip of apple juice. The researchers inserted ChR2 (the light-activated protein that excites neurons) into a brain region in which signals from the retina mingle with those from the front of the brain containing information about what to pay attention to and what rewards to expect. By illuminating those neurons, both monkeys performed the task measurably faster, though nothing else about their behavior was altered. That meant the intervention had discretely targeted a key neural pathway involved in this task, and it augmented the neural circuit and enhanced performance.

Simultaneous fMRI scans of the monkeys’ brains showed that the focused stimulation activated a distributed network of brain regions previously seen in brain imaging studies of the task. “That confirmed that the stimulated region was functionally connected and was doing what it was supposed to do,” says Rosen, who helped with this part of the study.

For this technology to become useful in people, it will have to overcome two significant obstacles—optogenetics’ physical invasiveness and the need to use gene therapy to infect human neurons with light-activated opsins.



On the first count, though optogenetics would require implants into the brain, optic fibers are smaller, narrower and more pliable than Deep Brain Stimulation (DBS) electrodes, and scientists are developing materials that will be more compatible with brain tissue. Boyden is working on less invasive techniques, including a wireless method to control light probes. He's also developing opsins that respond to red light, because red light waves travel farther through the body's tissue than other kinds of light, and so could illuminate a larger area and require fewer implants. On the horizon, artificial cellular receptors that are activated by drugs rather than by light could eliminate the need to have an optic device in the brain or body.

Moreover, while past gene therapy trials have had problems, Rosen believes the research is progressing, noting examples of genetic manipulation using viruses to target immune systems in end-stage cancer patients. Most likely, the first optogenetic applications will be for severely ill patients who would be candidates for DBS—people with Parkinson's, epilepsy or severe depression—and also for certain kinds of blindness and spinal cord injury, which would not require tampering with the brain itself. In the case of blindness, opsins introduced into the retinal cells could fire upon exposure to natural light, perhaps filtered and preprocessed by specialized eyeglasses.

Farther down the road, Boyden envisions being able to use optogenetics to overcome cognitive and other deficits in

patients with disabilities or neurodegenerative disorders.

Yet if and when optogenetics has direct therapeutic applications, the value of those are likely to be dwarfed by continuing discoveries in basic science, suggests Stanford's Deisseroth. Already, he says, "just having a better understanding that these behavioral symptoms result from an explicit problem in the brain's circuit can help my patients seeking to understand their troubling symptoms."

And while optogenetics may never answer all of the questions about the brain's daunting complexity, it could continue to help us understand what's going on in the brains of millions of people with mental illnesses, depression or uncontrolled aggression, or those suffering from trauma, developmental brain disorders and degenerative brain diseases. And that knowledge, says Boyden, an engineer at heart, could lead to new approaches to therapy. Once you know how a complex system goes wrong, he says, you can figure out how to fix it. ■

→ DOSSIER

1. **"A History of Optogenetics: The Development of Tools for Controlling Brain Circuits with Light,"** by Edward S. Boyden, *F1000 Biology Reports*, May 3, 2011. This account of the development of optogenetics, by one of its inventors, includes middle-of-the-night "aha!" moments, with credit given to other researchers and to serendipity.
2. **"Dopamine Neurons Modulate Neural Encoding and Expression of Depression-Related Behaviour,"** by Kay M. Tye et al., *Nature*, January 2013. This study provided new insights into the role of dopamine in symptoms of depression by probing for the underlying neural circuits. The researchers integrated optogenetics with electrophysiology and pharmacology and used specially designed devices to precisely track behavior.
3. **"Optogenetics, Sex, and Violence in the Brain: Implications for Psychiatry,"** by David J. Anderson, *Biological Psychiatry*, June 15, 2012. This study found a surprising neural link between aggression and sex, raising provocative questions about whether faulty wiring could account for some sexual pathologies.