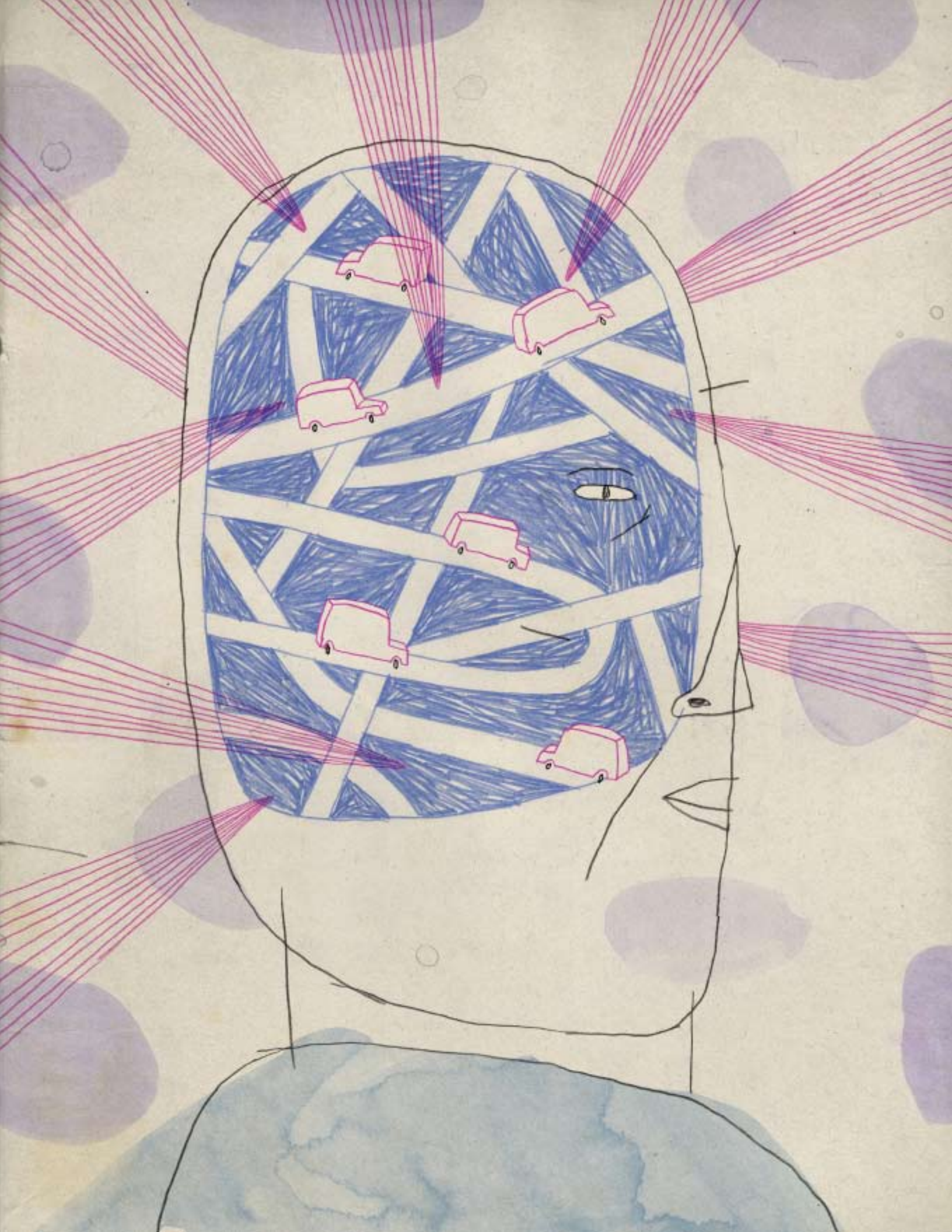
head lights

MIXING FIBER OPTICS WITH GENETICS HAS CREATED A REVOLUTIONARY TOOL FOR STUDYING THE BRAIN

BY BRUCE GOLDMAN
ILLUSTRATION BY BRIAN REA

The brain wears many hats. It thinks and dreams, it loves and hates, it recollects and predicts, it directs our moods and our movements. But it's a tough nut to crack. • "The human brain is probably the most complicated object in the universe," says Karl Deisseroth, MD, PhD, who has hit on such an effective way to finally discover how it works, colleagues say it could win him a Nobel Prize some day. • A most marvelous instrument, the brain comprises on the order of 200 billion nerve cells, or neurons, each of which may connect to 10,000 other neurons. Pulses of information in the form of electrical signals race along nerve fibers like sports cars on a speedway. • Yet what do you see when you look at a brain? Inscrutability. A shimmering, gelatinous mass of fatty fibers, snaking and threading and heading who knows where. • What if you could install traffic signals along the neurons threading through a living brain, so that you could start or stop traffic on them and observe the effect? Maybe you could learn something. • Deisseroth, an associate professor of bioengineering and of psychiatry and behavioral sciences, and his colleagues have created just such a system. The new technology, called optogenetics, mixes optics, genetic engineering and several other disciplines. It literally uses lights to control the messages zinging along our nerves: The go signal is blue, and the stop signal is yellow. Both are photosensitive proteins called opsins, originally discovered in microbes.



OPTOGENETICS HAS taken neuroscience by storm. Since Deisseroth published the first paper describing how it works in 2005, thousands of researchers around the world have started using it to define the deficits behind schizophrenia, autism, addiction, Parkinson's disease and more. In December 2010, the peer-reviewed *Nature Methods* named optogenetics the journal's "method of the year." That same month, *Science* magazine kicked off a roundup of 10 "insights of the decade" with a nod to Deisseroth.

"Optogenetics is the solution to our long-standing problem of lack of precision," says Anatol Kreitzer, PhD, a UC-San Francisco neuroscientist who recently collaborated with Deisseroth on a study of Parkinson's disease. "It lets us selectively inhibit or activate exactly the cells we're interested in. Karl's work is really revolutionary."

Deisseroth is a practicing psychiatrist as well as a researcher. The patients he sees suffer from severe, debilitating mental disorders such as autism, schizophrenia and depression. He hopes to find a way to give them their lives back — and he's painfully aware of psychiatry's limited ability to help him do so.

"Psychiatry has a long way to go," he says. "That's not because psychiatrists are anything but thoughtful, well-trained and observant. It's because we've lacked the tools to tease apart the component circuits that make up a working brain and examine their functions, one by one."

In the absence of such tools, it's even tougher to learn what's wrong with a brain that isn't working so well. Until now, most brain studies have relied on electrodes or drugs. Electrodes work fast. But they stimulate in a non-predictable way, igniting many different nerve-cell types in many different circuits. Plus, even though the stimulation is local, nerve fibers innocently passing through can get stimulated and trigger consequences far away. And while electrodes can activate neurons, they can't inhibit them, which is just as critical to studying brain function.

Drugs can selectively activate or inhibit neurons, but not always just the ones you want (that's one reason they produce side effects). Plus, they ooze everywhere and can't be mopped up quickly, making them lousy on/off switches.

Without precise techniques, how are you ever going to make sense out of 100 billion sentient spaghetti strands winding to and fro like midday traffic in some 3-D Manhattan?

Nothing to lose

IN 2004, DEISSEROTH was a new assistant professor at Stanford. He was eager to improve the lives of patients with psychiatric disorders, and dissatisfied with brain scientists' inability to map the malfunctioning nervous circuitry behind those disorders.

Here's what he was thinking: Neurons transmit electrically coded information down long, skinny fibers that project to other neurons near and far. What if you could coat their surfaces with photosensitive molecules so that when light hit those fibers, it would make them propagate — or resist propagating — electrical waves on demand? Suppose further that you could control which set of neurons would carry those molecules on their surfaces, and that you could direct the light to just the place you wanted. Then, at the flick of a switch, you'd be able to turn on or turn off the flow of impulses in the neurons of interest, and learn a huge amount about what they're doing.

Deisseroth knew that photosensitive molecules called opsins had been isolated from microbes such as *Chlamydomonas reinhardtii*, aka pond scum. Opsins are porelike proteins that open in response to particular wavelengths of light, allowing currents consisting of electrically charged particles to flow either in or out (depending on the particular type of opsin) across cell surfaces.

In theory, opsins were made to order for Deisseroth's approach. In practice, few had tried it and nobody had pulled it off, for plenty of reasons.

In living cells, proteins are created using recipes carried on genes. These days, plucking a gene (say, for an opsin) from one organism and plunking it into another organism's genome is a standard technique. But getting that gene into a living organism's brain without deleterious consequences is hardly a no-brainer. And it doesn't guarantee the protein the gene specifies will actually get made. (All your cells have virtually the same DNA inside them, yet skin cells, for instance, make entirely different batches of proteins than liver or blood cells do.)

Plus, the opsin molecules have to show up not just anywhere inside of neurons, but on their surfaces where all the electronic impulse-passing action is. Proteins aren't pets. Once made, they don't simply go where you want them to because they love to make you happy. They go where myriad biochemical imperatives direct them. Whether microbial opsins would really wind up on the surfaces of mammals' neurons — the only place where they could do any good — would be a bit of a crapshoot.

On top of all that, proteins are complex and finicky, working well only under the right conditions (heat, acidity and the companionship of chemicals called cofactors). Mammalian cells' biochemistry differs in numerous ways from that of microbes. Would an opsin molecule work as well in a mammalian neuron as it does in a pond-scum cell?

Another nail-biter: Microbial proteins on mammalian cell surfaces are sitting ducks. If the immune system, which abhors foreign substances, sees them, it just might chew the neurons they're sitting on into shreds, or at least

produce profound inflammation.

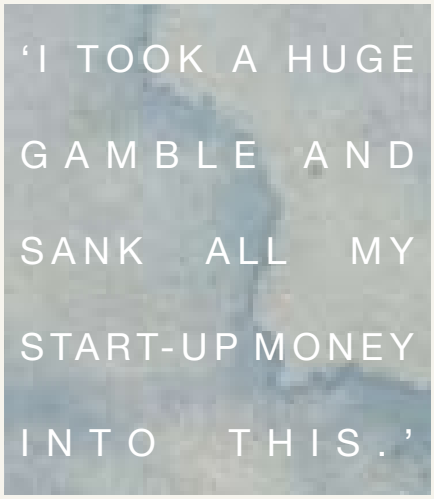
It added up to one risky proposal. "I was turned down for funding by a lot of people who thought this couldn't possibly work," Deisseroth says. "They figured if it worked it would have been done already. People had known about opsins for decades."

But he wanted to take a shot at it. He was young, with nothing to lose — no competing projects in urgent need of completion or renewal, no inventory of expensive equipment whose costs had to be amortized via other studies. He had a brand-new federal grant, and the support of the chairs of both departments he worked in. "I took a huge gamble and sank all my start-up money into this."

Deisseroth recruited two grad students, Feng Zhang and Ed Boyden. Zhang knew chemistry, molecular biology and virology. Boyden was adept at electrophysiology. They plunged in.

"Karl's lab was completely empty," says Zhang, now an assistant professor at MIT. He recalls having to go door to door in the building housing Deisseroth's lab, asking to borrow equipment from neighbors and hustling to get it back in time for the owners to use it in their own scheduled experiments.

A researcher at the Max Planck Institute had recently found an algae-derived gene coding for an opsin that, when stimulated by blue light, passed electrical current in a way that, in principle, could cause neurons to fire. Deisseroth got hold of the gene and suggested that Zhang try to fit it into some kind of system that could shuttle it into living mammalian neurons.



"I guess I didn't know any better," Zhang says. "It seemed worth a try."

Zhang's closest colleagues were trial and error. Eventually he settled on a defanged virus. If there's one thing a virus is good at, it's breaking into cells and commandeering their genetic machinery. To use a virus as a genetic-engineering tool, you take away its disease-causing weapons and replace them with a gene or genes you've taken from somewhere else. Then you inject your customized gene shuttle into an experimental animal. When the virus gets inside a cell's nucleus, it delivers the alien gene into that cell's own genome.

To ensure that just the right cells would produce the protein, Zhang affixed a kind of bar code to the opsin gene. Typically, genes have short "come hither" sequences of DNA right in front of them that tell cells' gene-reading machines which genes to perch on and when to make the proteins they specify. These little DNA tags are called promoters, and gene-readers in different cell types are attracted to different promoters. A gene — say for hemoglobin — with a particular promoter sequence may get hit on all the time by the gene-reading machines in a red blood cell, but never in a skin cell.

Deisseroth's team surmounted every hurdle. They succeeded in virally delivering opsin-encoding genes into rodents' nervous tissue. They were able to restrict opsins' production to neurons, or even just a selected type of neuron. The protein popped up on nerve-cell surfaces as hoped, and they bioengineered it further so it would do so more readily. Blue light made selected neurons fire.

To test this in live, freely moving, opsin-injected rodents, Deisseroth's group inserted a customized tube, or cannula, into the rodents' brains. During experiments, they threaded an ultra-thin optical fiber (outer diameter one-tenth of a millimeter) through the cannula. This way they could, at will, send pulses of laser light through the fiber to exactly the desired brain area. It worked like a charm, eventually.

As for the immune-reaction heebie-jeebies, a tight seal called the blood-brain barrier appeared to exempt experimental animals' brains from patrol by bulky antibodies and cellular cops. The suspicious molecules, like the proverbial falling tree thumping to the ground in an empty forest, apparently went undetected.

The Deisseroth group published their results with the excitatory blue-light opsin in 2005 in *Nature Neuroscience*. Not long afterward, they got an inhibitory, yellow-light-sensitive opsin, isolated from yet another one-celled organism, to work. Labs around the world are now routinely using both of them.

Optogenetics, applied

WHILE THE NEW methodology has terrific potential in psychiatric research, it has obvious limitations. Experiments that introduce foreign genes for light-responsive, nerve-impulse-triggering proteins into human beings aren't safe just yet. That's where experimental mice come in.

But when you're watching a mouse, it's a whole lot easier to observe its movements than its mental state. So a nice way to check out optogenetics' potential for brain research is to examine the animal equivalent of Parkinson's disease, a disorder in which patients gradually lose their ability to control movement. About 1 million people in the United States, mostly over age 65, are affected, making Parkinson's the second-most common neurodegenerative disease after Alzheimer's.

While Parkinson's ultimate causes are unknown, the disease clearly involves the loss of a set of neurons located in a structure deep within the brain whose signals feed directly into two separate circuits crucial to controlling voluntary movement.

Recently, Deisseroth, UCSF's Kreitzer and their colleagues optogenetically unravelled the workings of those two nerve-cell circuits and proved that one of the two facilitates normal movement, while the other inhibits it. Using both the blue-light-responsive, nerve-revving opsin and the yellow-light-responsive, nerve-blocking one, the researchers showed that imbalances in these two circuits' function can produce Parkinson's-like symptoms in mice — and that optogenetic interventions can exacerbate or alleviate those symptoms. By stimulating one of the two opposing circuits, they could restore normal movement in mice even after destroying the upstream nervous circuit that normally drives this activity and whose loss is the hallmark of Parkinson's disease.

Those results were published last year in *Nature*. "That we could completely rescue motor behavior by stimulating this pathway using optogenetics was surprising," Kreitzer says. "This is the first time anyone's ever reversed Parkinsonian symptoms using activation of a specific neural circuit. These mice became indistinguishable from their pre-lesioned, healthy state."

The finding implies that Parkinson's patients' conditions could someday benefit from new drugs that might be able, unlike current treatments, to stimulate the circuit that facilitates movement but not the circuit that inhibits it.

With ingenuity, it's possible to explore, optogenetically, not only the control of movement but more subtle workings of the brain, such as those involved in addiction and depression. Deisseroth and his Stanford colleagues have mapped the circuitry of the brain's reward system, illuminating the biological basis of addiction and depression. In a study published last year in *Science*, they showed the importance of a class of neurons whose role in reward couldn't have been nailed down by less-specific approaches. They used a fairly standard experimental design employing two "rooms." A mouse entering one room gets cocaine; entering the other, it gets nothing. It soon starts to strongly prefer the former. But the scientists could induce the same degree of preference by using blue light to optogenetically stimulate a solitary circuit comprising only 1 percent of the neurons in a particular brain structure. Furthermore, the mice's cocaine-induced preference for one room over another was obliterated when, during cocaine administration, the researchers shut down that same circuit by shining yellow light on it, thus inhibiting its firing. Now these mice couldn't care less which room they wandered into.

Similar efforts are identifying key circuits' roles in sleep disorders, schizophrenia, epilepsy and autism. In schizophrenia, the neurons of interest are sparsely sprinkled throughout the brain, Deisseroth says. "They form a network that seems to fire in synchrony." Disrupting the network's natural firing rates by delivering optical pulses at different frequencies impairs information flow in the brain. Fine-tuning neuronal networks' firing frequencies at will is yet another example of optogenetics' superiority over earlier neuroscience methodologies.

Spreading it around

DEISSEROTH HAS SHIPPED his viral opsin-gene shuttles to 800 labs around the world. And thanks to Stanford's multidisciplinary research complex, Bio-X, directed by neurobiology and biology professor Carla Shatz, PhD, he's been able to set up a training lab inside Bio-X's main piece of turf, the Clark Center. Guest researchers jet in for three-day training sessions conducted by this lab's director, Maisie Lo, PhD.

"What is really neat is that this technique can be used for any cells in the body, not just nerve cells," says Shatz.

Optogenetics will probably reach far beyond neuroscience. It can be adapted to trigger cascades of biochemical events inside all kinds of cells, including those of the heart and pancreas. Deisseroth is busy expanding opsins' range of colors and effects. Combining two kinds of excitatory opsins that respond to different colors will let you see what happens when you switch from exciting one circuit to another, or stimulate both simultaneously. Bioengineered opsins that, after a single pulse of light, turn nervous impulses on or off for long periods will let animals "go wireless" — researchers deliver a pulse that sustains activity or inhibition, then set the animal free. Alternatively, fast-acting opsins capable of delivering hundreds of pulses per second will allow scientists to explore frequency-dependent brain-circuit effects in more depth than ever dreamed of before.

While direct therapeutic applications of optogenetics — such as restoring neuromuscular function in paraplegics, proof of principle for which was established in mice in a collaboration this year with bioengineering professor Scott Delp, PhD — can be imagined, they will have to await the successful introduction of gene therapy in humans, which is still a ways off. "There will always be a risk/benefit trade-off," Deisseroth says. "By far the biggest impact of optogenetics will be the new understanding it makes possible." **SM**

Bruce Goldman is at goldmanb@stanford.edu

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