For the first time, science has cracked the code to controlling parts of the brain and even behaviour. **Dan Cossins** explores a revolution in the making.

Light switch

Artist's depiction of a neuron which has been genetically modified so that it 'switches on' when exposed to blue light. As the blue light hits the neuron, light-sensitive proteins known as opsins (shown here as small green spots) open.

IMAGE COURTESY OF THE MCGOVERN INSTITUTE FOR BRAIN RESEARCH AT MIT

A BROWN MOUSE stands on its hind legs, twitches its whiskers and looks around curiously for the best way out of a white square container. It doesn't seem to notice the fibre-optic cable protruding from the top of its head. Suddenly, the cable lights up bright blue and the mouse starts racing around in counter-clockwise circles. After 10 seconds, the light goes off and the mouse stops.

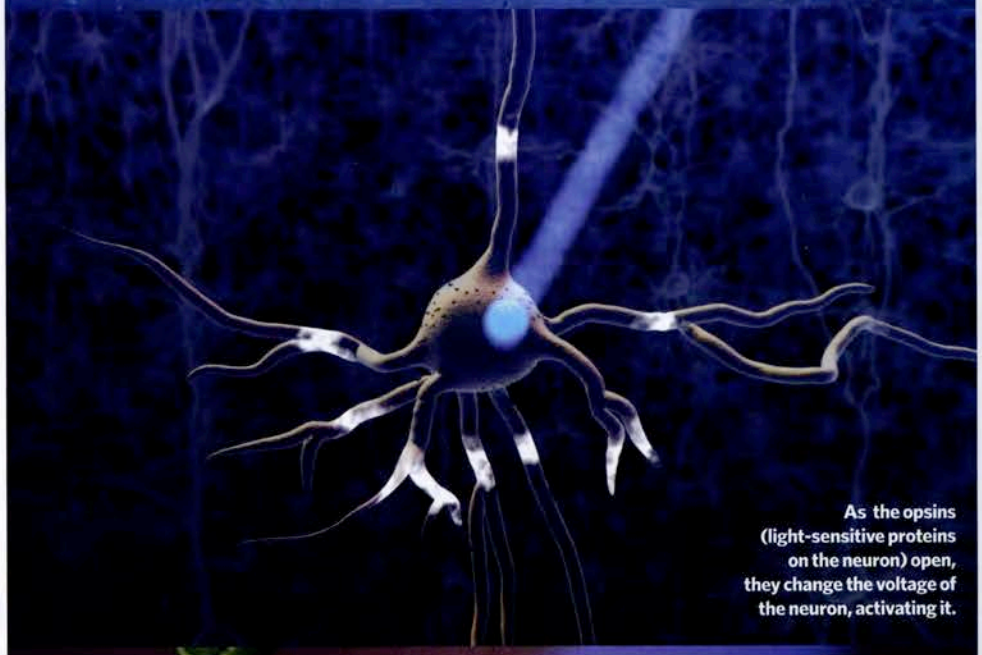
It's the summer of 2007 in a lab in the Department of Bioengineering at Stanford University, Palo Alto, California. A group of post-doctoral researchers led by neurobiologist Karl Deisseroth have carefully threaded the cable through the animal's skull and into its brain tissue. Their target is a specific class of brain cells in the right secondary motor cortex that has been genetically modified to activate when exposed to blue light.

When the researchers switch on the light via a computer, those cells fire, sending electrical signals racing round the animal's brain that make it run in manic circles, constantly turning left. The animal could be forgiven for being slightly confused; but the scientists are delighted, for they have created a remote-controlled mouse.

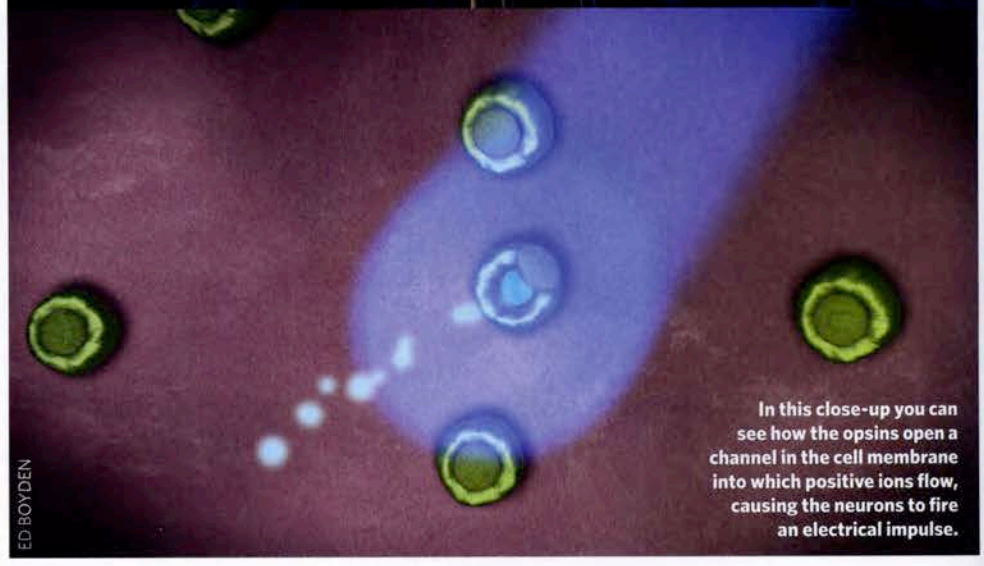
That may seem like a sinister thing to do. By hijacking the animal's mind, or at least the parts that govern locomotion, Deisseroth and his team proved that a beam of light could control brain activity in a living mammal with genuine precision. In the process, they demonstrated the power of a technique that is now revolutionising our understanding of how that lump of pinkish-grey flesh inside your skull works.

YOUR BRAIN IS a vast, staggeringly complicated tangle of 100 billion brain cells, called neurons, and trillions of fibre-like connections. Within that network of networks, each neuron is itself an astonishing electrical device. It receives, computes and sends impulses several times a second, every second, for as long as you live. Thanks to that immense activity, your brain controls everything you do – every thought, feeling, movement, memory, decision and sensation. It defines who you are and mediates every experience you'll ever have. >>

When blue light hits a neural network in which one kind of neuron is light-sensitive, only that kind of neuron is activated.



As the opsins (light-sensitive proteins on the neuron) open, they change the voltage of the neuron, activating it.



In this close-up you can see how the opsins open a channel in the cell membrane into which positive ions flow, causing the neurons to fire an electrical impulse.

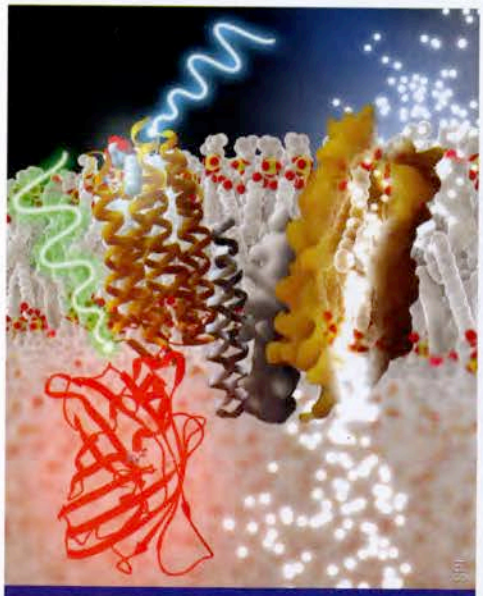
ED BOYDEN

>> And yet, despite having this incredible instrument at our disposal, we know desperately little about how it works or what happens when it goes awry. Despite decades of study, the brain remains our most enigmatic organ: maddeningly difficult to understand, even harder to manipulate or control.

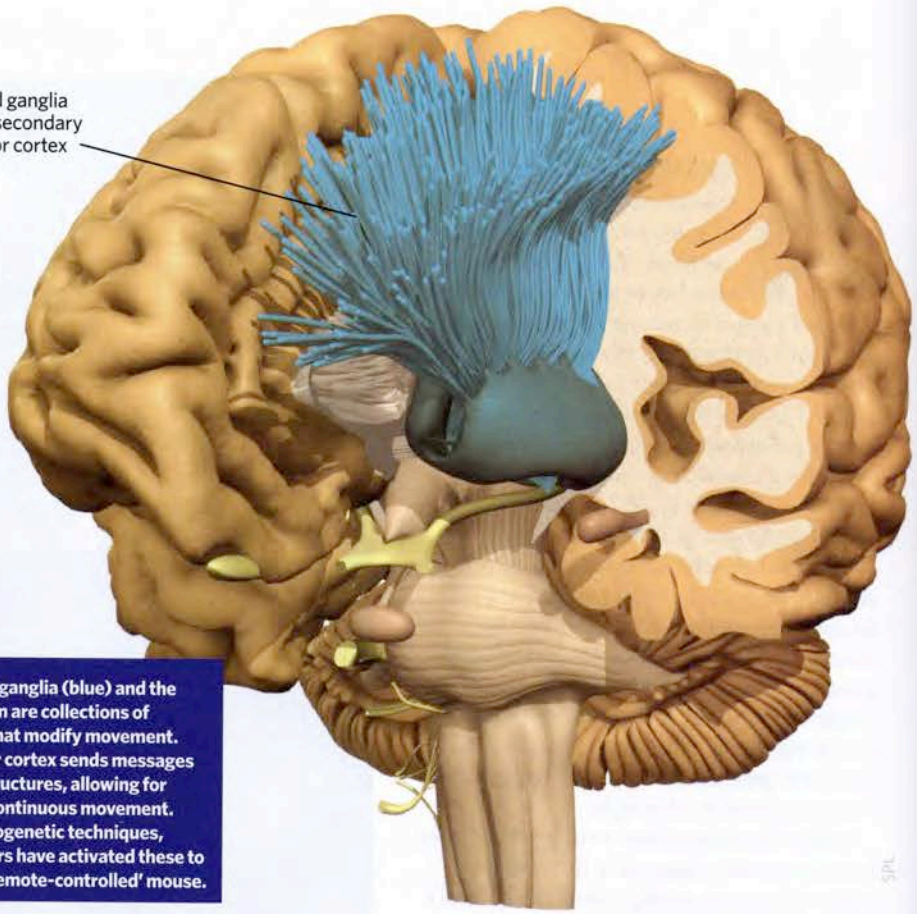
That's the challenge for synthetic neurobiologists, scientists seeking to invent new tools to understand the brain at a level that will allow them to engineer its function. By far the most promising innovation so far is optogenetics, a technique by which light-sensitive proteins are expressed in brain cells so scientists can instantaneously activate or silence them with light. In other words, it's a light switch for brain cells. And as well as creating remote-controlled mice, it promises to illuminate the inner workings of the brain and its malfunctions as never before.

By simply turning specific sets of brain cells on or off, scientists can now test exactly what these cells do in living animals. In the process, they're revealing fresh insights into intractable brain disorders and pointing the way to more effective treatments for everything from Parkinson's disease to anxiety, addiction and depression.

"It's like a renaissance for neuroscience," says Garret Stuber, assistant professor of molecular physiology and psychiatry at the University of North Carolina's Neuroscience Centre in Chapel Hill. "The level of precision it provides means that we can really get at the fine details of how specific populations of neurons govern behaviour. We now have a set of tools that we can use to answer questions that were previously out of reach."



A channelrhodopsin protein molecule embedded in a cell membrane. In unicellular green algae, these proteins control the flow of ions across the membrane in response to light. Scientists have found a new use for them in optogenetics.



The basal ganglia (blue) and the cerebellum are collections of neurons that modify movement. The motor cortex sends messages to both structures, allowing for smooth, continuous movement. Using optogenetic techniques, researchers have activated these to create a 'remote-controlled' mouse.

Boyden and Deisseroth considered several ideas, but were drawn to rhodopsins, a type of protein found in algae and other micro-organisms that regulate the flow of ions across cell membranes in response to light. While their natural use is to help organisms 'navigate' light, Boyden and Deisseroth saw that the special properties of rhodopsins meant they could also be used to mediate electrical spiking in brain cells, the language with which the brain communicates.

Thanks to this immense activity, your brain controls everything you do – every thought, feeling, movement, memory, decision and sensation.

THE OPTOGENETICS REVOLUTION began back in 2000, when Karl Deisseroth and Ed Boyden, then doctoral students at Stanford University, were looking for ways to control the electrical activity of neurons. "One of the core problems with understanding the brain is that it's made up of so many different kinds of cell," says Boyden, now a professor of bioengineering at the Massachusetts Institute of Technology (MIT) in Boston. "If you want to understand what they all do, then ideally you have to be able to turn them on and off."

The idea wasn't completely novel. Francis Crick, of double helix fame, had speculated that light might be used to control specific sets of brain cells in a *Scientific American* article in 1979. In the following decades, several research groups worked toward light-activated neurons, some experimenting with genes derived from algae. But no one had actually got rhodopsin proteins to work as light-activated channels in mammalian neurons.

Building on work that identified the Channelrhodopsin-2 (ChR2) protein as being suitable for the purpose, Deisseroth and Boyden decided to give it a try. In February 2004 they took the ChR2 gene, inserted it into a virus and infected a dish full of lab-cultured neurons. The cells took up the genetic material and synthesised the proteins, inserting them into the membrane. Then, to Deisseroth and Boyden's delight, the neurons fired in response to pulses of blue light.

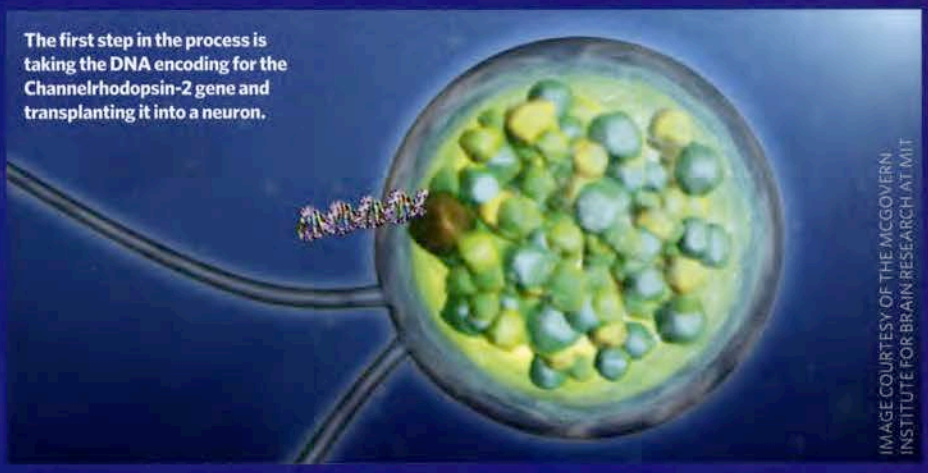
"It was kind of surprising that it worked on the first attempt," says Boyden. "In algae, these molecules are not found on the cell membrane, they're found on a little eyespot. So the fact that [the molecules] didn't get stuck in the neuron and instead ended up on the surface, where they could convert light to electricity, was a surprise."

In a 2005 paper in *Nature Neuroscience*, Deisseroth and Boyden announced the potential of their new technique. Simply put, they had shown that by modifying brain cells to express ChR2 proteins, you could drive their activity with light (see "The optogenetics pathway", right).

Compared to previous methods used to probe brain functions, their approach has several advantages. With electrophysiology, a technique in which electrodes are implanted into the brain to record neuronal activity, researchers can see how that activity changed during behaviour. But that only provides evidence of correlation. With optogenetics, neuroscientists have the chance to go beyond that to prove that certain neurons actually cause that behaviour.

"It provides a level of precision that we never had before," says Boyden, citing the ability to target specific brain cells and manipulate their activity on the millisecond timescale at which brain cells operate.

Robert Desimone, director of the McGovern Institute for Brain Research at MIT, described it as "God's gift to neurophysiologists" – and others agree. "This hugely improves our ability to explain how the brain's innumerable microcircuits can work together to produce behaviour," says Ethan Scott, a research fellow at the University of Queensland. "It allows for brain activity to be observed and manipulated over wide regions and tight time-windows, while still being specific to the cells and circuits of interest. That means we're able to tackle questions that have long been inaccessible." >>



The first step in the process is taking the DNA encoding for the Channelrhodopsin-2 gene and transplanting it into a neuron.

IMAGE COURTESY OF THE MCGOVERN INSTITUTE FOR BRAIN RESEARCH AT MIT

The optogenetics pathway

1 MAKE THE GENETIC CONSTRUCT

Researchers create a genetic construct containing the Channelrhodopsin-2 (ChR2) gene – a gene taken from green algae that encodes for blue-light-sensitive proteins – along with a specific 'promoter', a snippet of DNA that will drive the expression of the proteins encoded in the ChR2 gene.

2 INFECT BRAIN CELLS

Next, the DNA is packaged into a harmless virus and injected into the animal's brain, where it infects the neurons. The targeted brain cells then use their own protein-making machinery to produce the light-sensitive proteins encoded in the opsin gene and install them all over the membrane of the cell, like solar cells on a roof.

The proteins are only expressed in the targeted brain cells – those with the necessary machinery to activate the specific promoter. That way, researchers can have the proteins installed in specific sets of cells or connections and leave others unaffected.

3 PREPARE THE LIGHT SOURCE

A fibre-optic cable known as an 'optrode' is carefully (and painlessly) threaded through the animal's skull and into the brain tissue, allowing the researchers to send pulses of blue light into the brain. The pulses are sent in a rapid-fire fashion, on the millisecond-by-millisecond timescale at which the brain's signalling system naturally operates.

4 ACTIVATE THE BRAIN CELLS

When the pulses of light hit the targeted neurons, the light-sensitive proteins open a channel in the cell membrane into which positive ions flow, causing the neurons to fire an electrical pulse. Simply put, it turns the selected cells on but leaves surrounding cells completely unaffected.

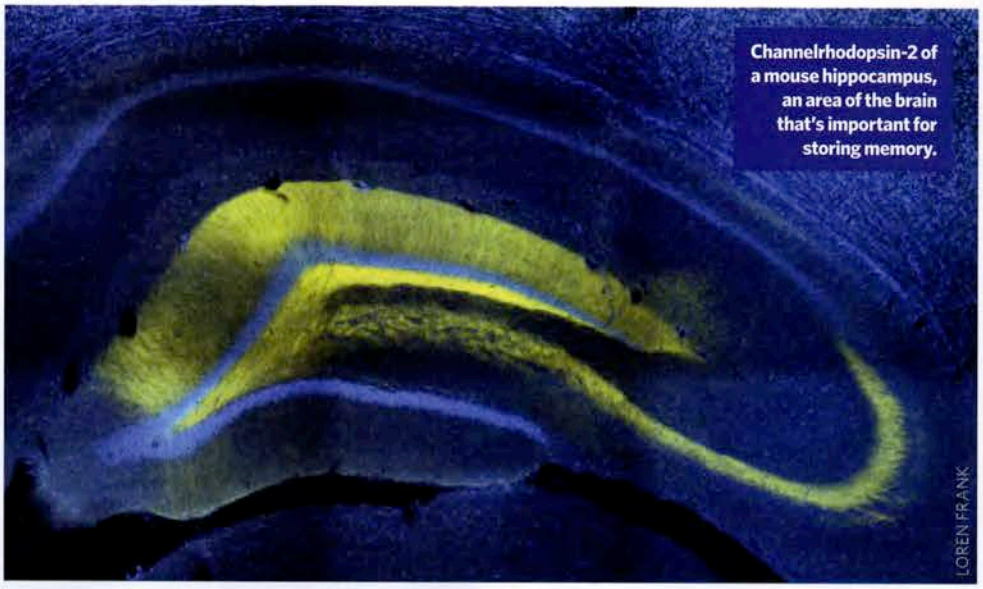
The researchers can now switch the brain cells on with pulses of light sent through the fibre-optic implant. That means precise control over the activity of the brain cells, so they can see exactly what behavioural changes occur as a result of their activation.

ANATOL KREITZER

By activating specific neurons using the optrode, neuroscientists can distinguish which neurons provoke different behaviours.

>> **AS THE TECHNOLOGY** spread to labs around the world, scientists began to generate a plethora of new discoveries. Like enthusiastic children playing with the latest cool toys, in early experiments they showed they could make nematode worms stop wiggling and drive mice round in circles.

Then a group at the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland, used the techniques to restore vision in mice where retinal degeneration had caused complete blindness – and detailed it in *Nature Neuroscience* in 2008. In the same year and the same journal, another group at Case Western Reserve University's School of Medicine in Cleveland, Ohio, managed to use optogenetics to rescue nerve function in mice paralysed by spinal cord injuries.



Channelrhodopsin-2 of a mouse hippocampus, an area of the brain that's important for storing memory.

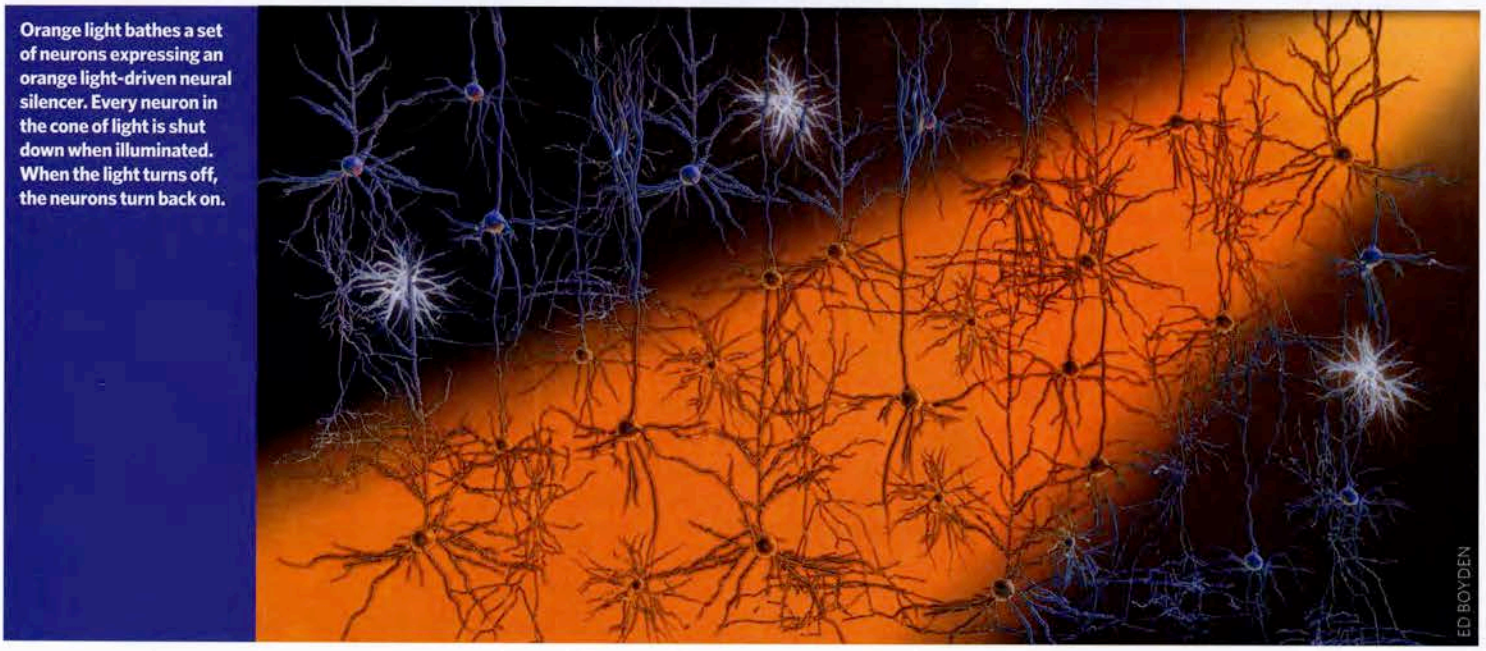
LOREN FRANK

Despite decades of study, the brain remains our most enigmatic organ: maddeningly difficult to understand, even harder to manipulate or control. Until now.

More recently, researchers have begun to control neurons in an attempt to study the brain circuits implicated in a range of human brain disorders. It's early days, but their initial insights hold promise for clinical application further on. A good example is a series of experiments exploring Parkinson's disease, a neurodegenerative disorder that affects motor control, causing patients to suffer from shaking, impairment of movement and loss of balance.

Since the 1990s, the symptoms of some Parkinson's sufferers have been relieved by the use of an implant technology known as deep brain stimulation (or DBS), which applies an oscillating electrical stimuli to the brain. But DBS has its drawbacks. The current floods an entire brain region, often resulting in unwanted side effects such as tingling, muscle contractions and problems with speech and balance. It is also unclear which sets of cells DBS affects, so the technique adds nothing to our understanding of the neural circuitry behind the condition. In short, it's a blunt instrument. Optogenetics, by contrast, is a scalpel.

In collaboration with Deisseroth, neuroscientist Anatol Kreitzer, of the Gladstone Institute of Neurological Disease at University of California, San Francisco, has used that scalpel to tease apart a particular set of cells that cause Parkinsonian symptoms in mice. Located in the basal ganglia, a part of the brain associated with movement, the cells had already been implicated in the disease, but Kreitzer wanted to test the hypothesis. "A lot of what we knew was indirect," he



Orange light bathes a set of neurons expressing an orange light-driven neural silencer. Every neuron in the cone of light is shut down when illuminated. When the light turns off, the neurons turn back on.

ED BOYDEN

says. "We had ideas, but we hadn't been able to test them. Optogenetics allows us to do that in a very precise way."

In a study published in *Nature* in 2010, he showed that the activation of indirect-pathway medium spiny projections (or MSNs) was enough to produce the symptoms in the mice. In contrast, activation of indirect-pathway MSNs reduced those symptoms. "That tells us that this particular set of cells might be a key target to think about [manipulating] in order to treat Parkinson's," says Kreitzer.

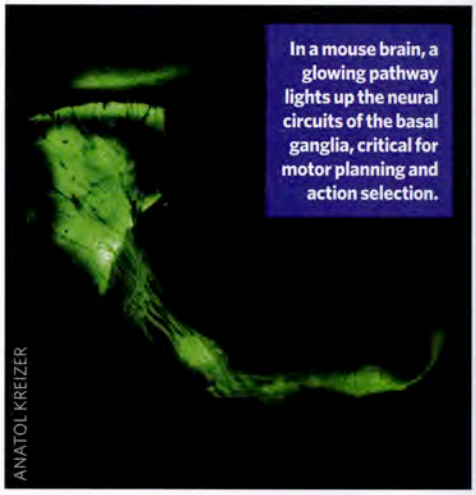
"We know that if we increase the activity of this particular type of cell, we can reduce the symptoms. Now we can focus on finding a strategy to affect that in a precise way that doesn't affect extraneous circuits and cause unwanted side effects."

ELSEWHERE, SCIENTISTS ARE making use of the optogenetic toolbox to illuminate the brain circuits involved in neuropsychiatric disorders. Herbert Covington III, assistant professor of psychology and neuroscience at Duke University in Durham, North Carolina, has adopted the technique to examine the role of brain cells in the prefrontal cortex in clinical depression.

In a paper published in the *Journal of Neuroscience* in December 2010, he showed that optogenetic excitation of a particular set of neurons in mice that were susceptible to depression exerted a potent anti-depressant effect.

"At this stage, it's all about using this to understand the roles of specific types of cells," he says. "But that will inevitably lead to better treatments in the form of pharmacology and psychotherapy. If we know the circuits that are important, we can begin to target them."

Over at the University of North Carolina, Garret Stuber has isolated the neural networks responsible for controlling reward-seeking behaviour like drug addiction. Again working on mice, he used light to activate a specific set of cells that connect the amygdala (an almond-shaped clutch of neurons responsible for the processing and memory of emotional reactions) and the nucleus accumbens (a group of neurons thought to play an important role in reward, pleasure, laughter, addiction, aggression, fear, and possibly the placebo effect).


ANATOL KREIZER

In a mouse brain, a glowing pathway lights up the neural circuits of the basal ganglia, critical for motor planning and action selection.

"We wanted to see whether the animals would find that stimulation rewarding, whether it would make them learn to respond in order to receive optical stimulation," he says. And that's exactly what happened.

Stuber then performed the opposite experiment, using halorhodopsin – another opsin that inhibits neuron activity when exposed to yellow light – to see whether shutting down particular neurons would suppress the animal's urge to consume a sugary drink. (Opsins are a group of light-sensitive, membrane-bound protein-coupled receptors found in photoreceptor cells of the retina.)

The control animals learned to recognise a cue for the sucrose delivery, licking in anticipation. But those with optogenetically-inhibited pathways did not lick in anticipation, and consumed less sucrose when it arrived.

"It seems that by shutting down these cells, we could reduce their reward-seeking behaviour," says Stuber. "That implies that, if you can find some sort of pharmacological method to shut down activity only in that pathway, you would, in my prediction at least, be able to reduce reward-seeking behaviour in humans with, say, a drug addiction."

FOR THE MOMENT, making use of these new therapeutic targets means optimising standard ways to manipulate the brain, namely by using drugs and DBS. In future, though, optogenetics itself could become

Flipping fields

IF OPTOGENETICS offers game-changing opportunities for neuroscientists, it also presents them with considerable personal challenges. The molecular biology that underpins the technique lies well outside their traditional areas of expertise, so even the most experienced brain researchers have to come to grips with an unfamiliar set of skills.

When Loren Frank first came across it, he was both energised and slightly intimidated. "It was immediately clear that this was the kind of tool that many of us had been hoping for, so it was incredibly exciting," says Frank, a principal investigator at the Keck Centre for Integrative Neuroscience at the University of California in San Francisco. "But to be able to use these techniques you have to have some sense of how they work. The viruses have to be engineered to deliver a particular protein to particular cells."

Frank, who studies how cells in the hippocampus govern the storage and retrieval of memories, was not alone. Neuroscientists everywhere suddenly found that if they wanted to make the most of optogenetics, they had to revisit the textbooks and learn a whole new suite of skills that lay way beyond their experimental comfort zone.

"One of the biggest issues is finding which combination of virus, rhodopsin and promoter is the right one for your particular experiment," says Frank. "There is no manual for that." Switching to trial and error, he tried a range of combinations to see which gave him the sort of protein expression he was after, where he wanted it. "We also had to overcome the technical challenges in inserting a fibre-optic device into the brains of living rats and making sure it would shine light where we want," he adds.

While it may be tricky, the pay-off is worth the effort. "It's opened up a whole new world where we can control these processes as they happen," he says. "There are never any silver bullets, but this is the thing that has come closest, because it helps us make the leap between getting at correlation and causation in the brain. It's changing the way we do our science."

>> the treatment. Indeed, some are optimistic that in the coming decades we'll see people with implants in their skulls that use light to control the neural circuits responsible for particular brain diseases.

While these 'neural prosthetics' may appear a remote prospect now, some of the technical obstacles are already being overcome. Boyden himself has developed a wirelessly powered LED light device, weighing around 1.5g, to deliver light to neurons without the need for a tether. Meanwhile, Michael Lin at the University of California, San Diego is working on 'red-shifting' the proteins: engineering them to be sensitive to red light, which penetrates deeper into brain tissue than blue light, which is interrupted on its journey because it is more easily-absorbed by surrounding tissue.

Perhaps the biggest challenge is to prove that rhodopsins can be safely and effectively delivered into the human brain without any harmful side effects. Optogenetics is, after all, a form of gene therapy, and although promising, there have been mishaps and, as yet, no gene therapy has been approved for clinical use. Clearly, that's likely to be a long and expensive process, but early experiments in macaques have already shown that the rhodopsin proteins can be expressed in – and used to control – neurons in the primate brain without eliciting an immune response.

Some are optimistic that in the coming decades we'll see people with implants in their skulls that use light to control the neural circuits responsible for particular brain diseases.

"We have to go through the proper scientific and pre-clinical processes, really look at practical problems and potential side effects of any new instruments, but those are things people are already trying to resolve," says Boyden. "It's a very difficult area and nobody wants to rush it, but I would predict that if the science is borne out, then there certainly could be scope for applications in humans."

Talk of implants and genetic modification can sound pretty scary: will it really take off as a treatment for brain diseases? Garret Stuber believes the answer is yes. "You would need to confirm that the expression of the proteins doesn't damage the brain over long periods," he says. "But if people are prepared to use deep brain stimulation, you would think they would be happy to use an optogenetic device. From a purely physical perspective, there is really very little difference."

With the suite of techniques being refined and updated all the time, Ed Boyden is optimistic that optogenetics will one day make a real difference for patients. "Around a billion people worldwide suffer from some sort of brain disorder," he says. "Neural prosthetics could be part of the solution."

Dan Cossins is a British freelance science journalist and former staff writer at BBC Magazines.