

Optogenetics



Traditional methods for the functional analysis of neurons have relied on direct stimulation by tiny electrodes, although the effectiveness is undermined by the limited spatial and temporal precision with which individual cells can be selectively targeted. As such, the recent emergence of optogenetic tools — genetically encoded switches that allow neurons to be turned on or off with bursts of light — promises to revolutionize the study of how neurons operate singly and as members of larger networks, and could ultimately offer new hope for patients suffering from vision impairment or neurological disorders such as epilepsy or Parkinson's disease.

TURN-ONS AND TURN-OFFS

At a basic level, the nervous system can be thought of as a highly complex electrical circuit. Every neuron contains a variety of pump and channel proteins that control the flow of ions across its membrane, maintaining a negative membrane potential in the resting neuron. Activation signals, for example from neurotransmitters, cause positively-charged ions to flow into the cell from the external environment via these channel proteins, resulting in membrane depolarization. At a certain threshold, this triggers an action potential — a rapid influx of sodium ions that effectively reverses the voltage inside the cell, initiating a chain reaction of sodium-ion influx that propagates down the length of the axon, eventually causing the release of neurotransmitters that stimulate or inhibit the production of electrical impulses in neighbouring neurons.

Microelectrodes have historically proven useful for the direct stimulation (less so

for the inhibition) of neurons in neurophysiological studies, although the poor resolution limit imposed by this experimental regime has left neuroscientists hungry for alternatives. The development of 'caged' neurotransmitters — chemically modified to remain inactive unless triggered ('uncaged') by laser illumination — and chemically modified photo-switchable ion channels have allowed notable improvements in precision for functional studies, although with limited possibilities for application.

However, the real revolution came with the discovery of the algae protein channelrhodopsin, which allows influx of positive ions in response to illumination with blue light to act as an 'on' switch¹ (Fig. 1). A few years later, scientists recognized the potential of the archaeal protein halorhodopsin, which triggers influx of negatively-charged chloride ions in response to yellow light and thereby hyperpolarizes the cell, to act as an 'off' switch². Both of these proteins can readily be introduced into target cells by various techniques, allowing scientists to rapidly and accurately turn individual neurons on and off without the need for additional drugs or chemicals.

CONTROLLING NEURAL CIRCUITS WITH LIGHT

Although this field of optogenetics is relatively new, scientists have already made remarkable progress in mapping functional brain circuitry over long distances — for example, charting neuronal processes that link the two hemispheres of the cerebral cortex in mice. Other preliminary studies have applied this approach to the study of brain disorders, using halorhodopsin and channelrhodopsin to characterize the neural circuits targeted by deep brain stimulation, which is an effective but poorly

understood therapeutic strategy for late-stage Parkinson's disease.

A lot of effort is currently being devoted to refining optogenetic techniques. Although viruses offer an effective and clinically applicable means for delivering the genes that encode these rhodopsins, it is still a laborious process to develop constructs that maximize the efficiency of gene delivery and expression. In addition, although naturally-occurring channel rhodopsin and halorhodopsin work well, there is a suggestion that modified versions of these proteins might offer improved light sensitivity and therefore more rapid switching. Better modes of light delivery will be required to improve the accuracy and efficiency of optogenetic strategies³; alternatives under investigation include arrays of separately addressable light-emitting diodes (LEDs) that effectively cover multiple brain sectors of interest, and infrared illumination systems that can penetrate deep within the dense tissues of the brain.

LASER-GUIDED NEUROSCIENCE

The core optogenetic methodologies are well established; having shown that these light-activated rhodopsins are tolerated and functional in the mammalian brain, scientists are now focused on using these tools for basic and clinical research.

Breakthroughs in materials science now allow the cultivation of neurons in complex predetermined patterns. The capacity to stimulate or silence individual cells selectively within these engineered cultures, in conjunction with reagents that allow the direct visualization of neuronal activity, promises to yield insights that could inform the design of artificial neural networks based on the natural principles underlying brain structure and function.

Although the light-gated cation channel channelrhodopsin2 (ChR2) is used as the main optogenetic tool in hundreds of neurobiologically oriented laboratories worldwide, little is known about the basic molecular mechanism. Scientists at the Max

Planck Institute of Biophysics in Frankfurt have recently demonstrated that ChR2 is a leaky light-driven proton pump, whereby the leak represents the channel properties (Feldbauer, K. *et al. Proc. Natl Acad. Sci. USA* 106, 12317–12322, 2009).



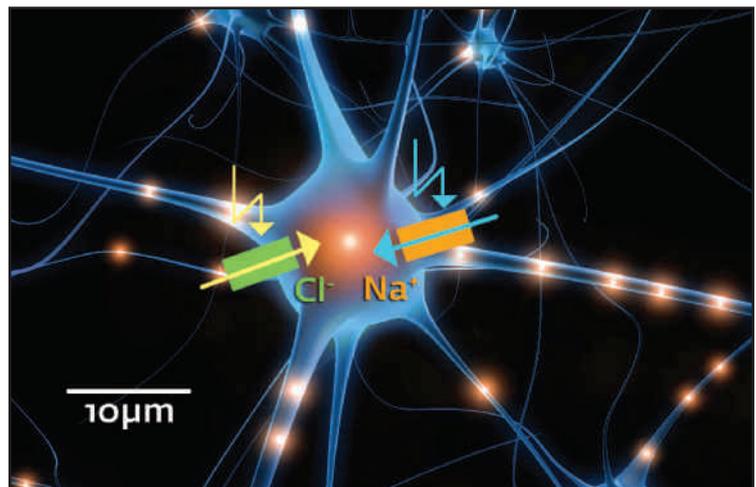
- Light-responsive proteins are allowing scientists to turn neurons on or off selectively with unprecedented precision.
- Introducing these proteins into cultured cells or the brains of live animals allows investigation of the structure and function of neural networks.
- These 'optogenetic' tools also hold clinical promise, with the potential for modulating activity of brain circuits involved in neurological disorders or restoring vision loss.

Even the complex environment of the living brain is within reach of these techniques⁴. For example, it is now possible to use 'light pipes' to deliver illumination to certain areas of the mouse brain, activating olfactory circuits, inducing whisker movement or switching on motor centres to trigger physical activity (Fig. 2)^{5,6}. These successes in animal models are merely a prelude to a longer-term goal: optogenetic gene therapy in humans. Studies have already shown that light sensitivity can be restored to photoreceptor-deficient mice by delivering the channelrhodopsin gene into retinal cells^{7,8}. Eventually, viral-delivery systems could allow similar therapeutic strategies in humans to treat blindness resulting from macular degeneration and other disorders — a promising alternative to more invasive implant-based therapeutic approaches.

Early work is also underway using optogenetics to improve the treatment of Parkinson's disease⁹. Deep brain stimulation uses small electrodes for targeted excitation of certain brain sectors, but suffers from a lack of spatial resolution and a tendency to cause unintended inactivation of non-target neurons. As such, the use of optogenetic factors could deliver a much needed improvement in precision, with micrometre-scale rather than millimetre-scale resolution. In principle, a similar approach could be applied to treat epilepsy, using halorhodopsin to enable selective inhibition of brain regions involved in the onset of seizures. Treatment of these and other neurological disorders could, for example, theoretically entail the pairing of a carefully crafted gene-therapy strategy and an implantable device for optogenetic stimulation.

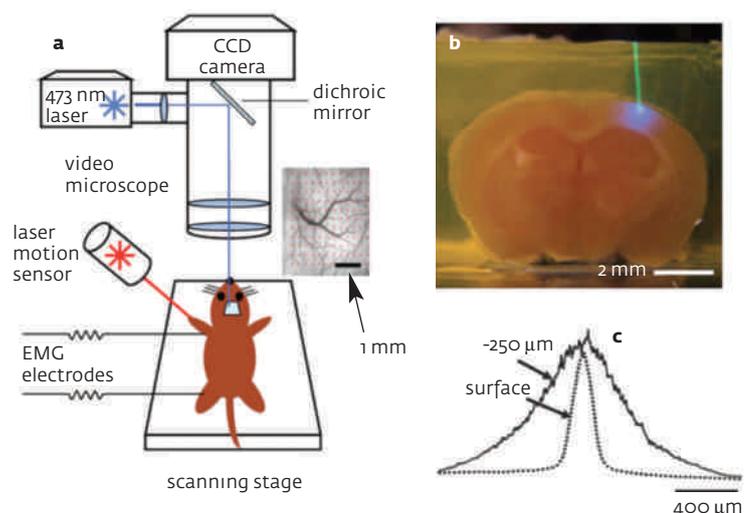
Optogenetics already offers great opportunities for basic neuroscience research, as has already been demonstrated by many laboratories worldwide; although biomedical applications still face unpredictable challenges and risks, these areas of research offer great promise for redefining neurological therapeutic strategies in the future.

Fig. 1 | Schematic representation of the action of channelrhodopsin and halorhodopsin on neural cells.



Activation with blue light opens the channel (channelrhodopsin), allowing in sodium ions and turning the neuron 'on'; yellow light (onto halorhodopsin) introduces chloride ions, turning the neuron 'off'.

Fig. 2 | Automated light-based mapping of the mouse motor cortex.



- a** Experimental setup. Anaesthetized transgenic mice are stimulated by laser beam. Motor response is detected by electromyography (EMG) electrodes.
- b** Slice of brain tissue embedded in agarose targeted by a stimulation laser.
- c** Intensity profile of the area illuminated as the beam passes through the agarose and through the cortical surface.

Reproduced from ref. 6.