

# Optogenetics: Light of the Future

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Neuroscientists have long known that the mammalian brain is just as complex as it is difficult to study. It consists of billions of intertwined neurons with many more connections exchanging precisely timed, millisecond-scale, electrical and chemical signals and a wealth of biochemical messengers. Prevailing techniques cannot activate or inhibit specific groups of neurons with the precision and timing needed to comprehensively decipher neurological phenomena [1]. Declared Method of the Year across all fields of science and engineering in 2010 by *Nature Methods* [2-4], the emerging field of optogenetics provides neuroscientists with opportunities beyond the reach of traditional methods. It has also been recognised by *Science* as one of the breakthroughs of the past decade because it is already yielding potentially powerful insights into the mysteries of neuroscience [5].

According to Stanford University psychiatrist and neuroengineer Karl Deisseroth, one of its foremost proponents, optogenetics is the combination of optics and genetics to control well-defined events within any specific cells of living tissue, even within moving animals [1]. It involves the discovery and insertion of genes conferring light responsiveness into cells, developing technologies for delivering light to cells of interest, and assessing the effects of this optical control via readouts. By using opsins, a group of light-sensitive proteins that convert light into electrochemical signals, researchers can conduct extremely precise, cell-targeted experiments in living and freely-moving animals.

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To study neurons of the brain in particular, researchers must first identify a gene that expresses a suitable opsin. They must then combine this opsin gene with a genetic element called a promoter that will cause the gene to be active only in a specific cell type. The modified opsin gene is then inserted into an engineered virus, which can be injected into the brain of an experimental animal. The virus infects many neurons but, because of the promoter, only one type of neuron will eventually produce the opsin protein. Fibre optic probes then inserted into the animal's brain can now use light flashes to trigger firing by specific neurons



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on command and control specific patterns of neural activity. Researchers can also then observe the effects of these light flashes on the animal's behaviour [1,6]. Mice are often used for these experiments, but there are already examples of optogenetics being applied to non-human primates and other animals, with the possibility of human testing [7-9].

While optogenetics itself is a recent development, the underlying ideas and methodology were available in principle long before. In a 1979 *Scientific American* article [10], Francis Crick, Nobel Laureate for his discovery of the structure of DNA with James Watson, suggested that the major challenge facing neuroscientists was the need to control one cell type in the brain while leaving others untouched. Electrophysiology and its electrode-based methods, which had delivered significant insights concerning the mechanisms underlying the action potential — the voltage impulse that neurons often use to convey information to each other — could not meet this challenge.

The problem with electrode-based methods is that, although they can measure the voltage or current on a scale as small as a single ion channel protein on the membrane of a neuron, they stimulate all cells at their insertion site without distinguishing between different cell types and their signals, nor can they turn neurons off with precision [11]. This lack of precision is particularly problematic because researchers often try to shed light on the functions of specific brain regions by turning them off. A more precise alternative is to remove the brain region using ablative surgical procedures, but these cause irreversible lesions. Due to the shortcomings of these two techniques, any differences observed in animals subject to behavioural tests may not necessarily be wholly attributable to the region in question.

Crick later speculated in 1999 that light could be used as a control tool to meet his challenge since it could be delivered in precisely timed pulses in various colours and locations [12]. At this time, it was not known how specific cells could be made to respond to light even though for the past forty years biologists had known that some micro-

organisms produce opsin proteins that directly regulate the flow of electric charge across their membranes in response to visible light. Among them was bacteriorhodopsin, a single-component protein ion pump that could be briefly activated by green light photons. Its function had been discovered by Walther Stoeckenius and Dieter Oesterhelt at the University of California San Francisco in 1971 [13], long before Crick first raised his challenge. Other single-component microbial opsins such as halorhodopsin and channelrhodopsin have since been identified and found to respond to different photons [14,15].

Microbial opsin genes were not introduced into animal cells for such a long time because it was initially thought that the animal cells could not manufacture the opsins efficiently or safely and that the proteins would likely be too slow and weak to be effective. It was also thought that an additional factor, a vitamin A-related compound called retinal, would be needed to absorb the photons because an opsin-retinal interaction in photoreceptors of the vertebrate eye gives rise to vision. Luckily, the opsins were eventually found to work in mouse cells because all mammalian tissues happen to contain significant quantities of retinal, meaning that nothing beyond a single opsin gene needed to be added to target neurons [1].

These concepts and discoveries eventually came together when a team led by Oxford University physiologist Gero Miesenböck founded optogenetics in 2002 by developing a multi-component strategy which used a protein regulating

neurons as well as another protein that spurred the opsin into action when it was triggered by ultraviolet light [16,17]. However, it was only when Karl Deisseroth's team at Stanford University began using a single-component system based on channelrhodopsin in 2005 that optogenetics became more widely used, as it now required only one gene to be expressed in order to work [18]. The term "optogenetics" itself was coined by Deisseroth the following year [19] and numerous laboratories have since used microbial opsins in their research.

One of the significant applications of optogenetics is to validate many scientific findings generated by functional magnetic resonance imaging (fMRI), a technique which is usually credited as providing detailed maps of neural activity in response to various stimuli. However, fMRI only shows changes in blood-oxygen levels in different brain areas, suggesting that it may not reflect actual neural activity. Experiments involving optogenetics have eliminated this uncertainty by demonstrating a positive correlation between neural activity and blood-oxygen levels detected by fMRI scanners [20]. A combination of these two techniques has also been used to map functional neural circuits with a precision surpassing that possible using electrodes or pharmaceutical drugs. By validating the findings of previous fMRI-based studies, optogenetics is helping to confirm and advance much scientific literature [20].

Clinically, optogenetics has already led to insights into various neurological and psychiatric disorders. Patients with



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Parkinson's disease often undergo deep-brain stimulation, in which an implanted device similar to a pacemaker applies oscillating electrical stimuli to specific areas deep in the brain. The promise of this technique is partially limited because it is dependent on electrodes, which stimulate nearby brain cells without selectivity. Moreover, medical understanding of what stimuli should be applied is far from complete. Studies using optogenetics have suggested that deep-brain stimulation may be more effective when it targets not neurons but rather synapses, the connections between them [21]. Similarly, optogenetics has been used on cells which have been found to enhance oscillations that are abnormal in both schizophrenia and autism as well as on neurons implicated in chronic sleep disorder to find that specific types of electrical activity set off awakening [22,23]. The importance of such studies is that they demonstrate how specific kinds of activity in specific cells can produce complex behaviours.

“ **Optogenetics also provides an important reminder of the importance of ecological conservation and pure basic science** ”

Apart from the benefits that it promises to bring to science and medicine, optogenetics also provides an important reminder of the importance of ecological conservation and pure basic science. Many opsins were first isolated from microbial organisms occupying rare and specialised environmental niches. For example, halorhodopsin was first isolated from the archaeobacterium *Natronomonas pharaonis* in highly saline soda lakes in Egypt and Kenya [1,24]. Furthermore, opsins were studied for their own sake by microbiologists and biophysicists long before their applications to neuroscience began to be considered. This is of particular concern as pure basic science is increasingly under threat from funding systems that reward immediate results or applications of research rather than research as an end in itself.

Like many other techniques used in research concerning the brain, optogenetics has strong ethical implications that need to be addressed. It is perhaps safer than some prevailing approaches, such as using pharmaceutical drugs or surgical lesions, in that the consequences of optogenetic methods are neither systemic as is sometimes the case for the former, nor irreversible as is the case for the latter. Nevertheless, there is potential for abuse in that optogenetics could be used on patients who are unsuspecting or reluctant, and its power to control specific neural circuits quickly and on demand may very well pave the way towards methods of manipulating people and denying them self-control.

As is the case with all approaches towards deciphering the brain's function, optogenetics operates on the underlying assumption that all aspects of our mental state fundamentally have a biochemical and electrophysiological basis. The latent potential of optogenetics concerning the control of such key components of the mind raises questions not only as to whether it is appropriate or justifiable to make significant modifications, but also concerning the very nature of the self and the will and whether it is acceptable to manipulate these in the name of science [1].

As last year's Method of the Year continues to be used more widely in laboratories around the world and gains acceptance among the scientific community as an important and valid technique for solving some of the great mysteries of neuroscience and biology, it is important to remember its humble origins in microbial opsins whose applications were not realised for decades. This marriage of optics and genetics provides us with not only significant implications for science and medicine, but also questions about what measures we are willing to take to learn more about ourselves. Most importantly, this emerging field demonstrates just how little we know about where science is taking us and what tools and ideas will be needed to understand more about our world. ■

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#### References

1. Deisseroth K. Controlling the Brain with Light. *Sci Am.* 2010;303(5):48-55.
2. Method of the Year 2010. *Nat Meth.* 2011;8(1):1.
3. Pastrana E. Optogenetics: controlling cell function with light. *Nat Meth.* 2011;8(1):24-5.
4. Deisseroth K. Optogenetics. *Nat Meth.* 2011;8(1):26-9.
5. The News Staff. Stepping Away From the Trees For a Look at the Forest. *Science.* 2010 December 17, 2010;330(6011):1612-3.
6. Zhang F et al. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nat Protocols.* 2010;5(3):439-56.
7. Arenkiel BR et al. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron.* 2007;54(2):205-18.
8. Diester I et al. An optogenetic toolbox designed for primates. *Nat Neuro.* 2011;14(3):387-97.
9. Zhu, P, Narita Y, et al. Optogenetic dissection of neuronal circuits in zebrafish using viral gene transfer and the Tet system. *Front Neural Circuits.* 2009;3(21):1-12.
10. Crick F. Thinking about the Brain. *SciAm.* 1979;24(3):181-8.
11. Schneider MB, Gradinaru V, Zhang F, Deisseroth K. Controlling neuronal activity. *Am J Psychiatry.* 2008;165(5):562.
12. Crick F. The Impact of Molecular Biology on Neuroscience. *Philos Trans R Soc Lond B Biol Sci.* 1999;354(1392):2021-5.
13. Oesterhelt D, Stoekenius W. Rhodopsin-like protein from the purple membrane of Halobacterium halobium. *Nature.* 1979;233(39):149-152.
14. Hartmann R and Oesterhelt D. Bacteriorhodopsin-mediated photophosphorylation in Halobacterium halobium. *Eur. J. Biochem.* 1977;77:325-35.
15. Nagel G. et al. Channelrhodopsin-1: a light-gated proton channel in green algae. *Science* 2002;296 (5577):2395-8.
16. Zemelman BV et al. Selective Photostimulation of Genetically ChARGed Neurons. *Neuron.* 2002;33(1):15-22.
17. Zemelman BV et al. Photochemical gating of heterologous ion channels: Remote control over genetically designated populations of neurons. *Proceedings of the National Academy of Sciences.* 2003;100(3):1352-7.
18. Boyden ES et al. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci.* 2005;8(9):1263-8.
19. Deisseroth K et al. Next-Generation Optical Technologies for Illuminating Genetically Targeted Brain Circuits. *The Journal of Neuroscience.* 2006;26(41):10380-6.
20. Lee JH et al. Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. *Nature.* 2010;465(7299):788-92.
21. Gradinaru V et al. Optical Deconstruction of Parkinsonian Neural Circuitry. *Science.* 2009 April 17, 2009;324(5925):354-9.
22. Cardin JA et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature.* 2009;459(7247):663-7.
23. Sohal VS et al. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature.* 2009;459(7247):698-702.
24. Gradinaru V, Thompson K, Deisseroth K. eNpHR: a *Natronomonas* halorhodopsin enhanced for optogenetic applications. *Brain Cell Biology.* 2008;36(1):129-39.
25. Available from: [http://www.niehs.nih.gov/news/newsroom/releases/assets/images/larger\\_image\\_for\\_mouse.jpg](http://www.niehs.nih.gov/news/newsroom/releases/assets/images/larger_image_for_mouse.jpg)
26. Available from: <http://www.flickr.com/photos/jepoirrier/954701212/>.