Optogenetics — The Might of Light

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Using light to control neuronal activity has been the dream of neuroscientists for decades. Because light does not interfere with normal brain function and can be targeted with exquisite spatial and temporal precision, it has long held the promise of providing a more powerful and selective way to influence brain activity than conventional electrophysiological or pharmacologic approaches. This promise has been realized thanks to a revolutionary approach termed "optogenetics," which involves the use of light-sensitive proteins expressed in specific neurons under genetic control. The 2021 Albert Lasker Basic Medical Research Award recognizes the contributions of Drs. Karl Deisseroth, Peter Hegemann, and Dieter Oesterhelt to the discovery of light-sensitive microbial proteins and their development of this initial discovery into an indispensable tool for neuroscience. The path to this achievement has taken place over decades and has involved the collaborative efforts of many scientists from diverse disciplines. It is a remarkable story that illustrates how important medical advances can arise from the most exotic corners of basic research.
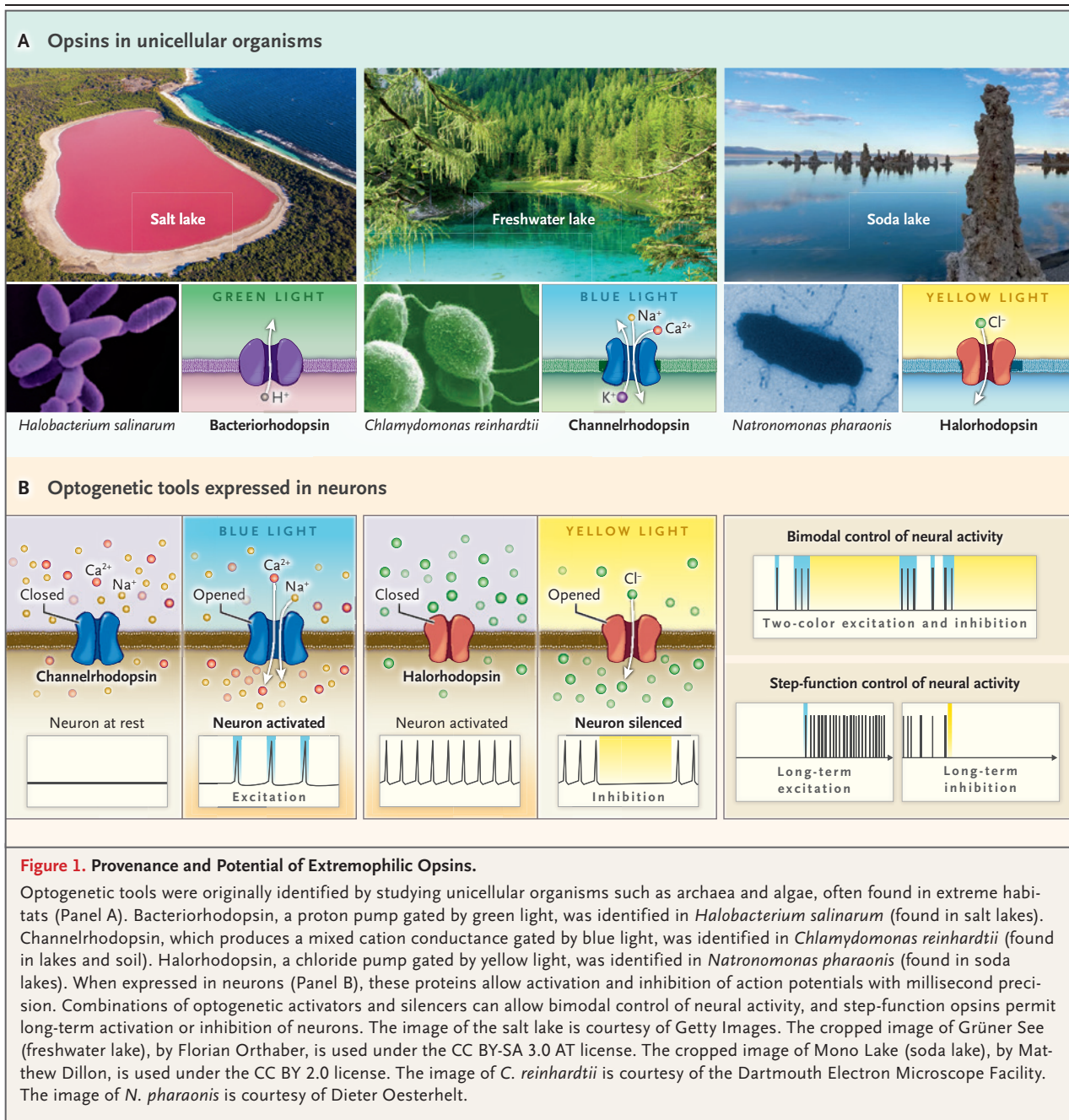
The story begins with a unicellular prokaryote, *Halobacterium salinarum*, which lives in extreme ecologic niches such as pink salt lakes (which owe their color to this remarkable archaeon). The halobacteria contain a "purple membrane" that serves as the powerhouse of this simple organism: it turns light into a proton gradient that can in turn be used to synthesize ATP. Working together with Walter Stoeckenius at the University of California, San Francisco, in the early 1970s, Dieter Oesterhelt discovered that the purple membrane achieves this feat with a single protein, bacteriorhodopsin (a molecule with close structural homology to vertebrate rhodopsins), which acts as a light-gated proton pump[1]: a beautiful molecular machine for converting light into electrochemical signals. The later cloning of the gene encoding bacteriorhodopsin and its heterologous expression in *Xenopus laevis* oocytes, showing that it can produce a light-activated current, established the principle that underpins the current field of optogenetics: the use of a single gene to drive expression of a single protein that activates a transmembrane current in response to light.

The discovery of bacteriorhodopsin inspired the search for other light-activated transmembrane proteins in a wide range of microbes (Fig. 1). A light-gated pump selective for chloride was characterized in *H. salinarum* by Oesterhelt and colleagues and dubbed halorhodopsin. *Natronomonas pharaonis*, an archaeon found in soda lakes, yielded another halorhodopsin, and subsequent structural analysis indicated that the archaeal rhodopsins form a family with similar structural motifs for transport of protons and other ions.

The next chapter of the story involves a completely different unicellular organism, *Chlamydomonas reinhardtii*, a type of green algae commonly found in freshwater lakes that exhibits phototaxis: it can move in the direction of the light, allowing it to maximize photosynthesis (its source of energy). Peter Hegemann and colleagues showed that chlamydomonas exhibits extremely fast light-gated currents[2] mediated by a rhodopsin-like protein. In a highly fruitful collaboration among Peter Hegemann, Georg Nagel, and Ernst Bamberg,[3] sequence mining of the chlamydomonas genome identified genes encoding two light-activated proteins, channelrhodopsin 1 (ChR1) and channelrhodopsin 2 (ChR2), which were shown to produce a directly light-gated proton conductance and mixed cation conductance, respectively.

This range of microbial proteins represented a molecular toolkit: the stage was set for its deployment in neurons. Pioneering work by Gero Miesenböck and colleagues in 2002 showed that

Figure 1. Provenance and Potential of Extremophilic Opsins.

Optogenetic tools were originally identified by studying unicellular organisms such as archaea and algae, often found in extreme habitats (Panel A). Bacteriorhodopsin, a proton pump gated by green light, was identified in *Halobacterium salinarum* (found in salt lakes). Channelrhodopsin, which produces a mixed cation conductance gated by blue light, was identified in *Chlamydomonas reinhardtii* (found in lakes and soil). Halorhodopsin, a chloride pump gated by yellow light, was identified in *Natronomonas pharaonis* (found in soda lakes). When expressed in neurons (Panel B), these proteins allow activation and inhibition of action potentials with millisecond precision. Combinations of optogenetic activators and silencers can allow bimodal control of neural activity, and step-function opsins permit long-term activation or inhibition of neurons. The image of the salt lake is courtesy of Getty Images. The cropped image of Grüner See (freshwater lake), by Florian Orthaber, is used under the CC BY-SA 3.0 AT license. The cropped image of Mono Lake (soda lake), by Matthew Dillon, is used under the CC BY 2.0 license. The image of *C. reinhardtii* is courtesy of the Dartmouth Electron Microscope Facility. The image of *N. pharaonis* is courtesy of Dieter Oesterhelt.

expression of a drosophila phototransduction cascade requiring three genes yielded light-evoked responses in mammalian neurons,[4] demonstrating that an optogenetic strategy could work in principle. In 2005, Karl Deisseroth and colleagues reported a landmark study showing that a simple, single-molecule microbial opsin could act as an optogenetic tool[5]: ChR2 expression in cultured h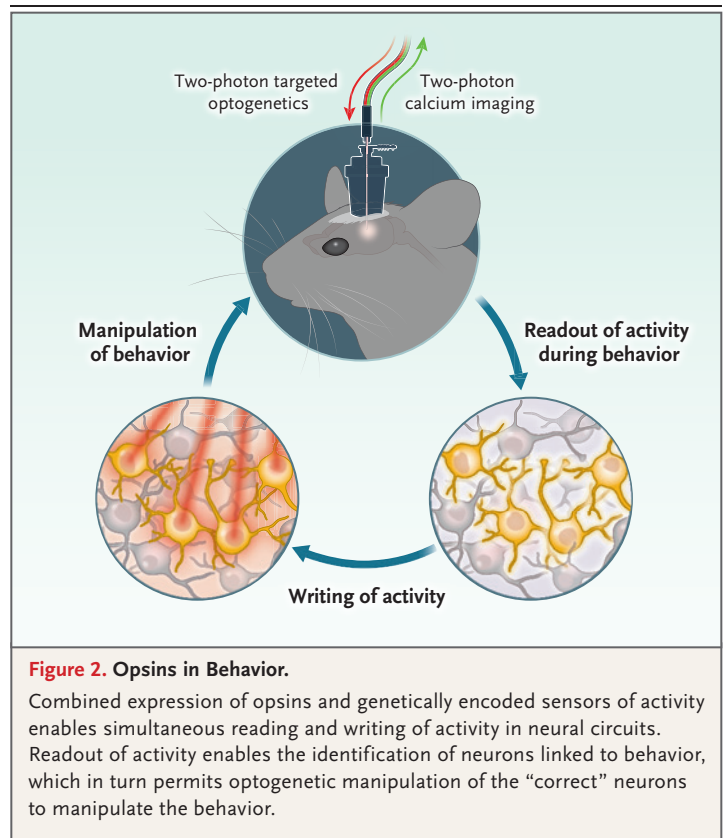ippocampal neurons allowed action potentials to be triggered by light with millisecond precision. Crucially, they showed that it was not necessary to externally supply the additional cofactor retinal (the aldehyde of vitamin A) to enable ChR2 to operate normally. This study was rapidly followed by the work of many groups showing that ChR2 could be used for optogenetic activation in a range of systems, including in vivo models.

The next key development was the use of mi-

crobial opsins to silence neurons. The laboratories of Karl Deisseroth[6] and Edward Boyden[7] showed that expression of halorhodopsin from *N. pharaonis* could enable millisecond-precise silencing of neuronal activity. Importantly, both studies showed that coexpression of channelrhodopsin and halorhodopsin in the same cells could allow bidirectional, two-wavelength control of activity, thus enabling crucial gain-of-function and loss-of-function experiments in neural circuits.
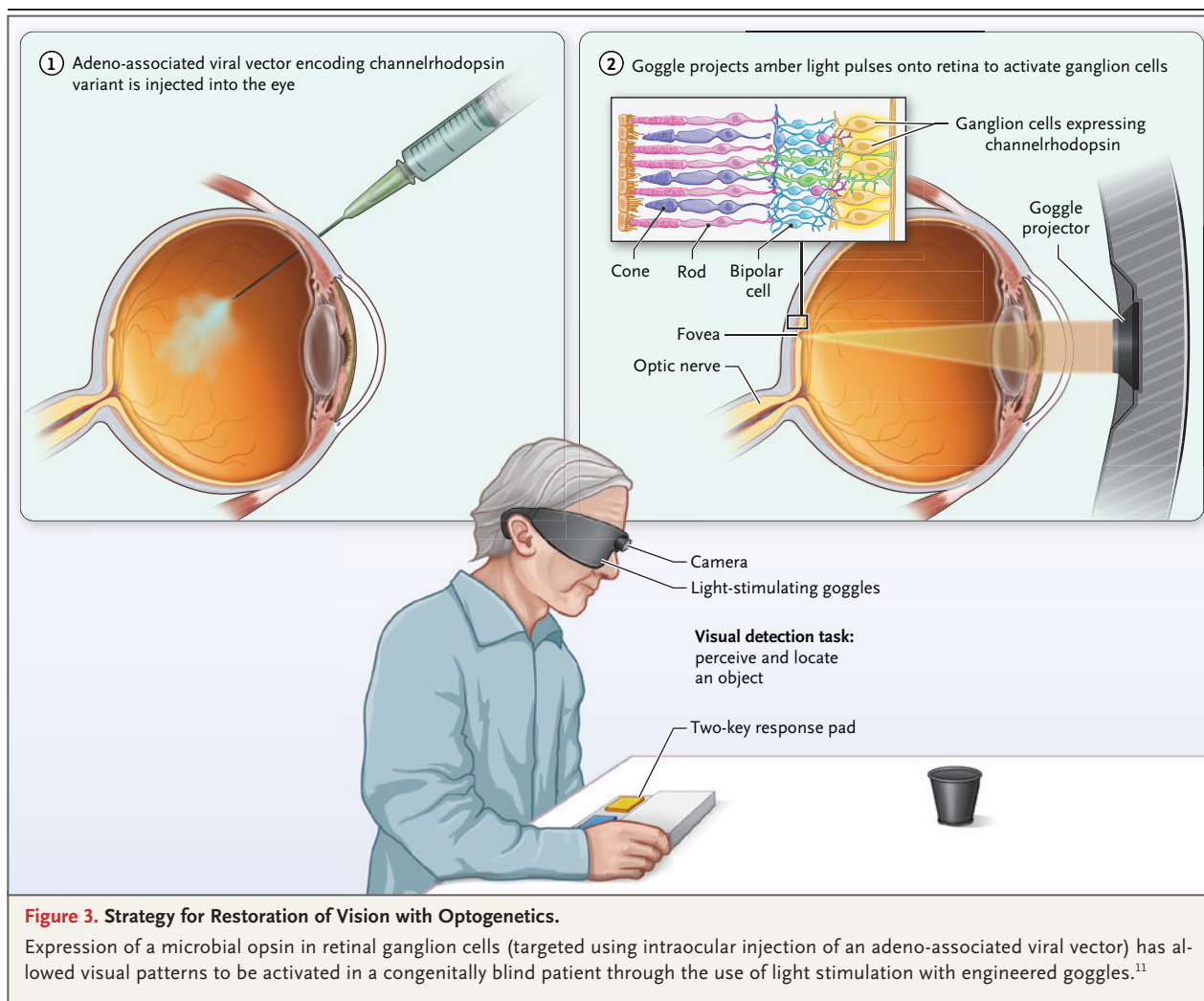
In the years since the annus mirabilis of 2005, there have been remarkable developments in the field of optogenetics. We now have a palette of microbial opsins, both derived from natural sources and optimized by means of site-directed mutagenesis. In addition to allowing rapid activation and silencing of neurons with the use of a range of wavelengths and kinetics, opsins have also been engineered to enable more potent optogenetic inhibition through redesigning of the channel pores to favor chloride flux, allow step-function activation, and trigger activation of biochemical cascades.[8] Strategies involving viral expression (principally with the use of adeno-associated virus vectors) and transgenic expression have been developed to enable in vivo expression in specific cell types. The use of fiberoptic-coupled diode technology, pioneered by the Deisseroth group, permits activation of specific neurons deep in the brains of freely moving animals. The development of opsins activatable by two-photon illumination allows for specific neurons to be targeted in physiological spatial and temporal patterns, and the simultaneous expression of genetically encoded calcium indicators and opsins provides an "all-optical" strategy for readout and control of neural circuits (Fig. 2). This offers the prospect of closed-loop control of activity,[9] which could provide the basis of novel brain–machine interfaces.

This powerful "optogenetic toolkit" has become part of the standard repertoire of thousands of neuroscience groups and has largely replaced the use of conventional electrical stimulation electrodes for activating neural pathways. The benefit of this approach is clear: it enables genetically defined subsets of neurons to be manipulated with millisecond resolution without directly perturbing neighboring neurons. This allows functional connectivity to be probed with unprecedented precision in vivo and causal relationships to be established between the activity

Figure 2. Opsins in Behavior.

Combined expression of opsins and genetically encoded sensors of activity enables simultaneous reading and writing of activity in neural circuits. Readout of activity enables the identification of neurons linked to behavior, which in turn permits optogenetic manipulation of the "correct" neurons to manipulate the behavior.

of specific neurons and behavior. Similarly, applying optogenetic tools to probe mechanisms of circuit dysfunction in disease models is revealing how specific pathways may contribute to Parkinson's disease, epilepsy, anxiety, depression, and other neurologic and psychiatric disorders.

What is the prospect of using optogenetic tools in humans? This requires that a range of challenges be addressed, such as achieving safe and efficient delivery of exogenous genes to the neurons of interest, ensuring the long-term absence of immunogenicity or genotoxicity, and engineering adequate light delivery to the relevant brain regions. Intensive testing and validation in nonhuman primates has provided encouraging results, indicating that these challenges may soon be solved.[10] Vision restoration has long been viewed as the "low-hanging fruit" among the many medical applications of optogenetics, given the accessibility of the human retina. This potential has very recently been dramatically validated with the demonstration of partial recovery of visual function in a blind patient after optogenetic therapy with a microbial opsin (Fig. 3).[11]

Figure 3. Strategy for Restoration of Vision with Optogenetics.

Expression of a microbial opsin in retinal ganglion cells (targeted using intraocular injection of an adeno-associated viral vector) has allowed visual patterns to be activated in a congenitally blind patient through the use of light stimulation with engineered goggles.[11]

The work of Deisseroth, Hegemann, Oesterhelt, and others has yielded optogenetic tools and a strategy for their deployment to control neural activity. Originally identified in humble unicellular organisms, these microbial proteins may provide the keys to understanding and treating the most complex diseases of the brain. The most exciting chapters in the story of optogenetics are yet to be written.

Disclosure forms provided by the author are available at NEJM.org.

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