

Optogenetic Technology and Its In Vivo Applications

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Optogenetics is a novel technology with the widely acknowledged potential to revolutionize cell biology and neuroscience. Essentially, optogenetic methods integrate optical and genetic tools to control the activity of whole cells or subcellular events. In recent years, optogenetics has been used to activate and to inhibit genetically defined neuronal populations within neural circuits. As such, it has been used to show the sufficiency or the necessity of specific neuronal cell types

in generating behaviors across a number of animal species. When employed in rodent models of human neurological and psychiatric disorders, optogenetics has provided clinically relevant insights into the function of pathologic neural circuits. Recent progress in the in vivo applications of this methodology is reviewed in this article, with particular focus on behavioral applications in nematodes, fish, rodents, and nonhuman primates.

INTRODUCTION

The field of neuroscience has witnessed the emergence of a novel methodological approach that allows for the optical control of neuronal activity. This technology, recently dubbed “optogenetics,” combines optical and genetic tools in order to establish temporally and spatially precise control of activation and inhibition of specific neuronal cell types within the brain. So far, most of these studies have been carried out in genetically tractable organisms such as flies, worms, and mice, but the technology is applicable to other organisms such as rats and even nonhuman primates (Deisseroth, 2011). Recently, articles on optogenetics have appeared in the popular-science magazine *Scientific American* (Deisseroth, 2010) and the science section of the *New York Times* (Schoonover and Rabinowitz, 2011). This is due not only to the novelty of the techniques involved, but also to the intriguing and almost science fiction–like power ascribed to optogenetics as empowering scientists to “control the brain with light.” Despite the hype, however, scientists indeed consider optogenetics a powerful and promising technique; *Nature Methods* declared it the 2010 “Method of the Year” (2011). This brief review describes some of the insights afforded to neuroscience by this novel technology. Particularly, it focuses on its in vivo applications, after providing a concise introduction to the technology.

IN VIVO APPLICATIONS OF OPTOGENETIC TECHNOLOGY IN NEUROSCIENCE

Optogenetic technology encompasses several methodologies, each making an important contribution to a growing and promising experimental toolbox. An integral part of optogenetics is a photosensitive element that, upon absorption of light, produces some change in the activity of the cells under study. While multiple-component systems, which require a conducting ion channel and an additional photosensitive element, have existed for some time (for example, photolabile caged ligands [purinergic ion channels P2X₂ coupled with caged ATP used in *Drosophila*) [Lima and Miesenbock, 2005], and

chemically modified photosensitive ion channels [light-gated ionotropic glutamate receptor] [Szobota et al., 2007]), the novel realization that a family of ion channel proteins encoded by opsin genes could serve as single-component photosensitive elements has recently greatly advanced and revolutionized the technique (Fiala et al., 2010; Deisseroth, 2011).

The most prominent and widely used photosensitive element in nervous-system applications is the light-sensitive channelrhodopsin-2 (ChR2) protein isolated from *Chlamydomonas reinhardtii*. ChR2 is a nonspecific cation channel that is activated by a covalently bound photosensitive chromophore, retinal (a form of vitamin A ubiquitous in vertebrate cells) (Nagel et al., 2003). When blue light (~470 nm) is absorbed by retinal, the change in its conformation from *trans* to *cis* opens the ChR2 cation channel, permitting the conductance of positive ions Na⁺, Ca²⁺, and K⁺. As the driving force for Na⁺ and Ca²⁺ ions is greater than that of K⁺, the inward current of positive charge depolarizes the cell. In the case of neurons, if this depolarization meets the threshold, an action potential is produced. Whereas ChR2 is used to excite neurons, another light-sensitive protein, halorhodopsin (NpHR), a chloride pump isolated from a halobacterium, *Natronomonas pharaonis* (Matsuno-Yagi and Mukohata, 1977), is used to inhibit neuronal activity. NpHR's inhibitory function stems from the activation of chloride conductance upon illumination with yellow light (~590 nm). Negative chloride ions flowing into the neuron hyperpolarize the cell, lowering cellular excitability by making it more difficult to generate an action potential. These two proteins, ChR2 and NpHR, are the most widely used light-sensitive activity switches in neuronal applications of optogenetics.

The precise temporal and spatial delivery of photoelectric opsin proteins and activating light of specific intensity and wavelength to a relatively large volume of tissue are challenging problems that affect specificity of the technique (Kravitz and Kreitzer, 2011). For delivery of protein, the three main approaches are transfection, viral transduction, and generation of transgenic mouse lines (Pastrana, 2011). For in vivo applications, creation

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of stable transgenic mouse lines is preferable. Cell-type specificity can be achieved by using tissue-specific promoters. The activating light is applied using experimentally appropriate methods. For example, in behavioral experiments, illumination is delivered with the use of optic fibers implanted stereotactically into the brain.

One of the seminal challenges of neuroscience is to understand how particular neuronal populations within well-defined brain regions control behavior. Part of the appeal of optogenetics lies in its potential to help establish definitive mechanistic links between the activity of defined neuronal ensembles and behavior, potentially providing insight into the mechanisms that generate disease conditions. However, without a means of showing a causal link between neuronal activity triggered by illumination and its outcome, optogenetics would probably lose its main appeal. Therefore, complementary methods have been devised or modified from existing techniques to monitor the effects of illumination on cellular, tissue, and organismal levels. In brain slices or anesthetized animals, cellular activity is measured with conventional patch/sharp microelectrodes or extracellular single/multi-unit recording electrodes, respectively. Additionally, various fluorescent activity indicators, for example calcium-sensitive dyes, are used. However, in awake, unrestrained animals, where such methods are unsuitable, specific behavioral assays are used to establish a causal link between the illumination and the outcome. More recently, optoelectrodes, also referred to as optrodes, have been devised, combining optic fiber for illumination and an array of metal electrodes for recording neuronal activity (Zhang et al., 2009).

The first in vivo experiments used invertebrate organisms such as the model nematode *Caenorhabditis elegans*. In order to examine specific behavioral changes triggered by optogenetic tools, researchers expressed ChR2 in muscle cells throughout the body and, in a different experiment, in the mechanosensory cells, which trigger an escape response in the worm (Nagel et al., 2005). Because *C. elegans* lacks an endogenous chromophore retinal, groups of transgenic worms were grown in media in the absence or presence of retinal. Wild-type worms, not expressing ChR2, also served as a control. Nematodes expressing ChR2 in muscle cells responded to illumination by strong contraction of all muscles in the body. Muscular contractions subsided after the termination of illumination. Lower-intensity and shorter-duration illumination produced contractions of a lesser degree. Based on these and additional experiments, researchers concluded that muscle contraction was a result of ChR2-induced depolarization, which recruits voltage-sensitive Ca^{2+} channels and ryanodine-sensitive Ca^{2+} channels. When transgenic nematodes expressed ChR2 in several types of mechanosensory neurons, illumination with blue light produced a robust withdrawal reflex. The effect was even greater in animals that lacked mechanosensitive ion channels, indicating that

depolarization solely due to ChR2 activation triggered the escape response. Although the behaviors triggered in these experiments were simple reflex responses, the results nevertheless constitute the first proof of principle that optogenetics can be used to control the behavior of a whole organism using single-component light-sensitive ion channels.

The extensively used and genetically tractable zebrafish, *Danio rerio*, provides another example of the successful use of the optogenetic toolbox to trigger a more complex escape response (Douglass et al., 2008). In these experiments, researchers transiently expressed ChR2 coupled to enhanced yellow fluorescent protein (EYFP) (to visualize the cells) in Rohon-Beard and trigeminal neurons of zebrafish, located in the spinal cord. Illumination with 488 nm light (blue), but not with 680 (red) nm light, triggered a robust escape response. Characteristics of the response, such as onset latency and kinematics, were similar to naturally occurring touch-evoked escapes in the fish, while no response to the light stimulus was observed in control fish expressing only EYFP. Additionally, destroying Rohon-Beard and trigeminal neurons by introducing neurogenin-1 antisense morpholino oligonucleotide also eliminated light-induced escape responses. These results demonstrate that optogenetics can be used to trigger more-complex behaviors in vertebrate animals. Moreover, experimenters found that stimulation of only one sensory cell was frequently sufficient to produce a response, suggesting efficient coding of sensory stimuli in primary sensory neurons involved in a vital behavior.

Functional significance of populations of specific cell types within widely distributed neural circuits is of great interest to neuroscientists. Studies in transgenic mouse models that integrate optogenetics with conventional electrophysiology and behavioral assays have attempted to tackle this problem. One recent study (Witten et al., 2010) investigated the functional significance of a sparse population of cholinergic interneurons in the intricate circuitry of the nucleus accumbens (NAc), an area involved in reward behavior such as cocaine-conditioning. The researchers used Cre-recombinase technology with the cholinergic neuron-specific choline acetyltransferase promoter and stereotactically injected Cre-inducible adeno-associated virus vector carrying either the gene encoding ChR2 or halorodopsin containing a sequence for enhanced yellow fluorescent protein to visualize the cells. Using this method they were able to express opsin channels specifically in cells that also express choline acetyltransferase, which presumably are only cholinergic neurons. Studies by the Deisseroth group (2010, 2011) delineated a better-defined role for cholinergic interneurons in the nucleus accumbens circuitry and provided a causative link between the activity of these neurons and reward-related behavior. The group first employed conventional slice electrophysiology to demonstrate that optogenetically activating cholinergic interneurons

leads to an increase in the frequency of the postsynaptic inhibitory currents recorded in medium-spiny neurons (MSNs) of NAc, the main output neurons of this brain region. Mechanistically, this suggests that acetylcholine released from light-activated cholinergic interneurons activates muscarinic receptors on nearby inhibitory interneurons, which in turn synaptically inhibit MSNs. In *in vivo* experiments where optrodes were implanted in the basal ganglia of anesthetized animals, the increased frequency of postsynaptic currents translated into a significant decrease in the firing rate of MSNs. Finally, to test how this inhibition is involved in reward-related behavior, the researchers used optogenetics in a cocaine-conditioned place preference (CPP) paradigm to explore the role of cholinergic interneurons in the modulation of this type of conditioning. In this paradigm, mice are conditioned to associate one part of the cage with cocaine, which makes the animals prefer that part of the cage even in the absence of cocaine. Results indicated that the cocaine-induced place preference was almost completely abolished in the mice expressing halorhodopsin and in which cholinergic neurons were silenced during cocaine conditioning. Their littermate controls (mice not expressing halorhodopsin), however, exhibited normal CPP. This is strong evidence for the necessity of these neurons in cocaine-induced CPP, and, more broadly, a causative link between the activity of cholinergic interneurons and the induction of conditioning behavior. Thus, the authors postulate that silencing cholinergic interneurons can reduce cocaine-induced behavioral effects without interruption of other non-drug-induced behaviors, which may be of interest in the clinical treatment of addiction.

In principle, optogenetic technology is applicable even in higher vertebrates such as primates. As proof of principle, Han and colleagues (Han et al., 2009) stereotactically delivered a lentiviral construct containing the gene for ChR2-GFP (green fluorescent protein) expressed under a CaMKII promoter to the frontal cortices of two macaque monkeys. The single injection of lentivirus (~1 µl) resulted in ChR2-GFP expression in excitatory pyramidal neurons of the frontal cortex in a spherical volume approximately 1.5 mm in diameter. Laser illumination and recording of electrical activity in this area over a period of a few months revealed that this methodology neither produced light-induced physical damage to the nervous tissue nor triggered an immune response to expressed channelrhodopsins. The findings indicated that the blue light activated excitatory pyramidal neurons with remarkable temporal precision. Moreover, neuronal activity decreased to below-baseline levels after the discontinuation of laser stimulation, suggesting that pyramidal cell firing also activated downstream inhibitory interneurons. Overall, these results show that optogenetic activation of specific cell types in the primate cortex can be used to study functional interactions between cell types in neocortical neural networks in conscious animals.

CONCLUSION

This mini-review illustrates, using examples from the literature, how the rapidly evolving optogenetic toolbox can be applied to *in vivo* investigations in neuroscience. This toolbox provides an invaluable approach in investigating the functional roles of genetically defined ensembles of neurons in widely interconnected neural circuits. The power of optogenetics, however, is not limited to basic science. Its impact is already felt in translational research. Advances generated by optogenetic tools in the studies of Parkinsonian rodents not only help us to better understand the mechanisms of deep-brain stimulation, an established therapeutic method to treat Parkinson's disease, depression, and other neurological and psychiatric disorders (Krack et al., 2010), but also provide a significantly novel approach to investigate pathological neural circuits (Gradinaru et al., 2009). Finally, there is a proposal that optogenetic intervention aimed at modulating activity in the prefrontal cortex and globus-pallidus subthalamic nuclei may be therapeutically beneficial in treating schizophrenia (Peled, 2011). Thus, optogenetics has the potential to become a method of choice both in the clinic and in the research laboratory.

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