Optogenetics: Using Light to Investigate and Potentially Treat Neurological and Psychological Disorders

Michael Kub

ABSTRACT

Optogenetics is an innovative neuromodulation technique involving the use of light and light-sensitive proteins to control molecular events within a genetically modified cell. The fundamental mechanism behind optogenetics is the deliberate shining of light at light-sensitive cellular membrane proteins which causes some sort of change within a cell. These proteins, called opsins, come in many forms including ion channels, pumps, and G protein-coupled receptors and they are found in a wide range of organisms from vertebrates to prokaryotes [1]. When utilizing optogenetics, researchers must make several considerations including the light source to be used to control the cellular event, the type of cell to be activated by the light, and the tools to be utilized for measuring such cellular activity.

We reviewed in detail the mechanism behind optogenetics and the considerations researchers make in employing this technique. We also reviewed outcomes from several studies centered around it and its current limitations. In conducting this review, we utilized web-based archives such as PubMed, Nature, and ScienceDirect.

The studies that we specifically reviewed include the application of optogenetics for analyzing the effect that grafted cells have on relieving Parkinson's Disease symptoms in animal models [2], the capability of optogenetics in instantly controlling depression-like states in mice [3], and the capability of optogenetics in regulating epilepsy in cultured animal brain models [4]. In each of these studies, the type of cell that was sought to be controlled was the neuron, which all studies had substantial success in doing so. One area which was not addressed in these studies and which should be in future studies, is the plausibility that optogenetics could someday be used on humans.

Based on the outcomes of these studies and the overall indication that optogenetics is an effective and precise technique in evoking cellular events, we conclude that optogenetics will likely have an enormous impact on research for years to come. Furthermore, given concerns over safety and use on humans, which we get into later in this paper, we also conclude that optogenetics has an uncertain future for clinical application.

INTRODUCTION

A main focus in the field of neural engineering has been to establish accurate and reliable techniques for modulating the activity of the nervous system. At the forefront of neuromodulation field are electrical, chemical, and biological techniques. These techniques include for example deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), pharmaceutical delivery, and peripheral nerve stimulation [11]. A technique that over the last decade has been gaining the interest of researchers and is proving to be both a temporally precise and spatially accurate method in modulating neural activity is a technique referred to as optogenetics. In optogenetics, cells are introduced with a gene that encodes for a light sensitive protein, and certain colored lights are delivered to these modified cells to either activate or suppress their activity [18]. In this paper, we explore this technique and its impact on neuroscience as well as elaborate on its potential for scientific and clinical application in the future.

HISTORICAL BACKGROUND

The beginnings and transformation of this technique into what is today is the result of research that has accumulated over the past fifty years. In 1971, Walther Stoeckenius, of University of California, San Francisco, in collaboration with Dieter Oesterhelt discovered that *Halobacterium halobium* contains a transmembrane protein that functions as a light-driven proton pump [6]. In 1977, Matsuno-Yagi and Mukhata who were studying a protein they referred to as halorhodopsin, discovered that it also functions as a light-driven pump, pumping chloride ions into the cell when exposed to light [7]. In 1999, Francis Crick of University of San Diego suggested at a conference using light to control a neuron by means of light-sensitive

proteins [7]. In 2002, Boris Zemelman and Gero Miesenbock employed Francis Crick's idea and successfully activated mice neurons with light using opsins from fruit flies. Though successful, these proteins were not always instantaneously responsive to light. In 2005, Karl Deisseroth and two students Edward Boyden and Feng Zheng expanded on Zemelman et al.'s, work and used opsins from bacteria called channelrhodopsin-2 (ChR2) instead of opsins from fruit flies for activating mice neurons [7]. Their modification resulted in cells that would within milliseconds reliably respond to light. Their work helped earn optogenetics Nature Magazine's Method of the Year in 2010 and catapulted the technique to an unprecedented level as fields across neuroscience including neural network research, visions research, brain mapping behavior research, neurodegenerative research, and neuroprosthetic research began experimenting with it [18].

EXPERIMENTAL CONSIDERATIONS

One of the strengths of optogenetics that makes it such a practical option for so many fields is its flexibility [5]; this technique can be used to influence cell activity in various cell types, and it can be applied to numerous species. As long as a number of basic steps are performed, a researcher can control cells of their interest and measure the desired activity of those cells [5]. First, a researcher must design a gene construct that includes a tissue-specific promoter, a gene that encodes for the light-sensitive protein, a marker, and a stop. Second, the gene construct must be delivered into the cells of interest. Third, a light source must be chosen to control the genetically modified cells. Finally, the researcher must be able to record the cellular activity that was manipulated with light.

As previously mentioned, the types of proteins optogenetics relies on are called opsins. Opsins for optogenetics come in many forms: ion channels, pumps, or G protein-coupled receptors, and examples of opsins preferably utilized include channelrhodopsin, bacteriorhodopsin, and halorhodopsin which are naturally found in various microbial organisms (Fig. 1) [9]. These types of opsins, considered more generally to be rhodopsins, are chromoproteins made up of an opsin and 11-*cis*-retinal, a pigmented molecule derived from vitamin A [8]. Activating these proteins requires that they are illuminated with a particular wavelength of light. If exposed to that wavelength of light, the retinal it contains photo-isomerizes to the *trans* state, inducing a conformational change of the protein and an alteration in cellular activity [8].

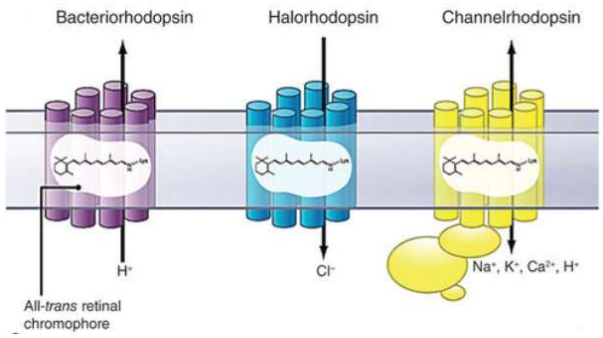

Fig. 1. Common light-activated proteins used in optogenetics. Activation occurs upon alteration of the retinal chromophore found within these proteins from the 11-*cis* state to the all-*trans* state

The type of opsin to choose for a study depends heavily on several factors. Some factors include how much time is desired between when the protein is exposed to light and when the cell should respond to that light exposure, how many cells are being simultaneously illuminated, whether the same activity is desired of those cells, their location (*in vitro* or *in vivo)*, etc. For activation of neurons, the seemingly default opsin of choice is Channelrhodopsin-2 (ChR2) [5]. This is because ChR2 is a cation channel that reliably excites neurons within milliseconds of exposure to blue light. Another type of channelrhodopsin that is advantageous for neural activation is the ChR1/VChR1 channelrhodopsin, which is activated by red-shifted light. This type of rhodopsin is particularly beneficial for control of cells *in vivo*, mainly because red light tends to scatter less than other visible forms of light and thus would have an increased chance of reaching the ChR1/VChr1 than blue light would have reaching ChR2 [5]. If a researcher desires to inhibit neural firing, an opsin he or she could choose is halorhodopsin or bacteriorhodopsin, as these proteins open channels to ions (chloride ions and protons, respectively) which hyperpolarizes the cell when stimulated with light [5]. An interesting wild card that could be used by a researcher is a type of opsin referred to as stabilized step-function opsins (SSFP). This type of opsin remains activated beyond its initial exposure to light and can sustain activity for long durations of time up until it is deactivated with another wavelength of light. A researcher would find the strongest benefit from such an opsin when there is need for an excitation or inhibition for an extended period of time such as research on depression and anxiety. [12].

Once the proteins have been chosen for study, the researcher must decide how to transfer them into the cells of interest. In almost all situations, the proteins must be created in the cell and thus the genes which encode for those proteins must be transferred into it. For *in vitro* experiments, a construct is typically delivered into a cell via transfection [5]. This involves inserting the construct with the protein-specific gene into a bacterial plasmid,

increasing the permeability of the cell with calcium phosphate or short pulses of electricity, and injecting the plasmid nearby the cells so that it can be taken up by them. For *in vivo* experiments, a construct is typically introduced into a cell via viral transduction [5]. In transduction, the construct is incorporated into the genome of a virus and the virus is stereotaxically injected into the target tissue. Assuming that the virus infects the cells in that tissue, the viral DNA which contains the construct will be taken up by those cells and it will combine with those cells' genomes. Adeno-associated viruses (AVV) and lentiviruses are most frequently used for transduction [5].

Perhaps the most necessary part of a gene construct enabling its expression in the first place is the promotor. The promoter is the region of DNA that initiates gene transcription. Adding to the complexity that is inducing cells to express a certain gene-specific opsin, a researcher must decide which cells he or she desires to express the proteins. It is well found that different types of cells contain unique promoters and transcription factors [5]. They also contain general promoters and transcription factors which are found ubiquitously in many cell types [5]. In a study in which a researcher desires an opsin to be expressed ubiquitously in many cell types, the best approach would be to add a ubiquitous promoter to a gene construct. Examples of ubiquitous promoters a researcher could use are elongation 1α, synapsin, and cytomegalovirus (CMV) [5]. In the case that a researcher would like for the opsin to be expressed in only a specific cell-type, cell-specific promoters should be incorporated into the gene construct. For example, a researcher interested in having the opsin expressed in only dopaminergic neurons should add to the gene construct a tyrosine hydroxylase promoter since this is a type promoter found only in such neurons [10].

Another major consideration that a researcher must make following successful introduction of an opsin into a cell is the type of light source that is desired to control that cell. Most importantly, the light source should be able to emit light of the wavelength that would stimulate the particular opsin. Aside from that, there are many options. The primary technologies used for stimulating cells include light emitting diodes (LED) and lasers, most popular in *in vitro* studies, and one-photon lasers, optical fibers, and LED based systems, commonly utilized in *in vivo* studies [7]. Finally, as for the recording of cell activity manipulated through optogenetics, the measuring tool would depend on the type of cell being influenced. Considering exclusively neural activity, such activity could be measured using for example electrodes, functional magnetic resonance imaging (fMRI), calcium imaging, and behavioral assays [7].

RESEARCH APPLICATIONS

As previously mentioned, the applications of optogenetics are enormous. We first consider the use of optogenetics for studying depression. Depression is a serious psychotic disorder that affects more than sixteen million U.S. adults in a given year, about 6.9 percent of the U.S. population [16]. The standard treatment option for depression are antidepressants, which it was recently estimated that only 28 percent of patients who take them achieve full remission. Additionally, for patients who are considered to have an extreme version of depression referred to as major depressive disorder, it was estimated that as high as thirty to forty percent do not respond whatsoever to antidepressants [17]. The strong need for a better understanding of depression and improved treatment has encouraged researchers to turn to optogenetics for answers. For instance, one group in 2013, led by Dr. Dipesh Chaudhury and Dr. Jessica Walsh, successfully employed optogenetics to instantly induce depression-like states in mice [3]. They did so by adding ChR2 proteins into neurons in a region of the mouse brain called the ventral tegmental area (VTA), implanting an optical fiber into this region, and thereafter illuminating neurons in this region so that they would fire at either rapid rate (i.e. phasic rate) or a slow rate (i.e. tonic rate) [3]. They learned through modulating neurons of the VTA in this way that the more rapid the rate particular neurons of this region are fired (that is, VTA neurons projecting into the nucleus accumbens) the more susceptible the mouse was to depression [3]. This was mainly evidenced to the team by higher social avoidance during a social interaction test with another mouse ('target mouse') and lower preference for sucrose compared to controls and mice whose VTA neurons were modulated to fire at a tonic rate. This is illustrated in Fig. 2 below (included with the study) [3].

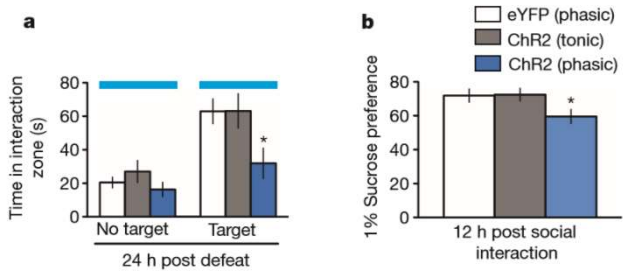

Fig. 2. Phasic stimulation of mice VTA neurons while simultaneously assessing behavior indicates an increased susceptibility to depression. (a), Social interaction data in control, tonic and phasic groups. (b), Sucrose preference measured over a 12 hour period following social interaction test.

Another area of research optogenetics has been utilized is in epilepsy research. Epilepsy affects more than 50 million people worldwide [13]. Like depression, one of the better treatment options for epilepsy are drugs, which helps reduce excitation of neural networks that trigger an epileptic event. However, drugs appear to be only effective for two thirds of patients who take them, leaving the other

third with having to choose alternatives, which can be scarce [4]. The alternative treatment options these patients may be eligible for are surgical resection of the tissue causing the epileptogenic activity or implantation of a device which halts an epileptic event immediately after it begins through electrical stimulation. The latter option, while having shown to be effective, is limited because electrical stimulation lacks specificity, potentially causing negative side effects [14]. Optogenetics, on the other hand, as previously mentioned, is highly specific and researchers, aware of that fact, have in recent studies used optogenetics to control neural networks associated with epilepsy. In one study, for example, led by Jan Tonnessen, his team incorporated halorhodosin proteins from *Natronomonas Pharaonis* (NpHR) *in vitro* into neural networks of the hippocampus of a mouse, regions which are associated with epilepsy [4]. As halorhodopsin normally pumps chloride ions into a cell and the transfer of chloride into a neural cell has been indicated to cause hyperpolarization, the team hypothesized that by incorporating these proteins into these neural networks, and thereafter illuminating them, they could inhibit neural activity associated with epilepsy. To test this hypothesis, they first induced an epileptic event in the brain tissue of the mice by electrically stimulating hippocampal regions known as CA1 and CA3, and immediately afterwards stimulating halorhodopsins incorporated within cells of these regions with orange light. Upon illumination, inhibition of neural activity did occur, which is illustrated in Fig. 3 (included with the study) [4].

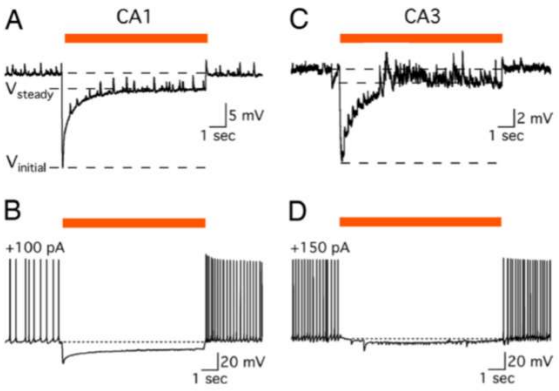

Fig. 3. Illumination of CA1 and CA3 pyramidal neurons with orange light hyperpolarizes these neurons and inhibits the production of action potentials. (A and C), Initial and steady voltage hyperpolarizations that occurs with NpHR activation in the CA1 and CA3 neurons. (B and D), Inhibition of current injection-induced actions potentials upon illumination of CA1 and CA3 neurons with orange light. The orange bars represent time of light illumination.

We finally explored the use of optogenetics in Parkinson's Disease (PD) research. Parkinson's disease is caused by the deterioration of midbrain dopamine neurons, which typically results in reduced dopamine levels and noticeable symptoms such as tremors and difficulty moving. One role that optogenetics has had thus far in PD research is enlightening researchers on the influence that transplanted neurons have in alleviating PD symptoms [2]. For some time now, stem cell therapy has been shown to be greatly effective in reducing PD symptoms in animal models [2]. But it is not exactly clear why grafting of these stem cells into the damaged midbrain has led to a reduction of PD symptoms: do the new cells induce the remaining intact cells in the brain to produce dopamine to make up for the dopamine shortages? This is possible since other studies have shown that transplanted cells can release signaling factors that can influence host cell activity. Do they help repair degenerated cells? Again, this is not out of the question since transplanted cells have been shown to induce remyelination and perform other repair functions [2]. Or lastly, do the newly introduced cells actively replace the deteriorated cells and produce dopamine themselves? In a recent study, led by Julius Steinbeck, him and colleagues sought to answer this question with the aid of optogenetics. They did so through a number of steps. First, they produced a unilateral lesion in the brains of a group of mice resulting in their display of PD symptoms (for this experiment, the main PD symptom they observed was a biased preference for sugar pellets ipsilateral to the lesion). Next, they modified stem cells by incorporating inhibitory chloride pumps proteins, eNpHR3.0, into them, ultimately transforming them into light-deactivating cells [2]. Then, they implanted these light-deactivating cells into the midbrain of a lesioned mice and examined for reduction of PD symptoms, which for the experiment was an unbiased preference for sugar pellets on both sides of the mouse. Once reduction of the mices' symptoms had been observed as expected, Steinbeck and his team positioned a laser near the grafted cells and illuminated them with green light. They were able to show that illuminating these cells had reintroduced the motor deficit symptoms originally observed in the lesioned mice. Furthermore, they were able to show that those motor deficit symptoms could again be removed by turning off the light, which had the effect of reactivating the grafted cells. To determine if it was the inhibition of dopamine release from the grafted cells that was the cause for why mice reverted to their original diseased stated when exposed to green light, the team injected a dopamine-receptor-agonist, apomorphine, into the graft site before additional optogenetic stimulation and observed the effect it had on the mice during an extra round of testing. Even upon inhibition of the grafted cells with green light the mice did not relapse to the PD state, suggesting to team that the link between graft function and behavior is in fact dopamine released from these newly introduced cells. The results from these tests are displayed in Fig. 4. below (included with the study) [2].

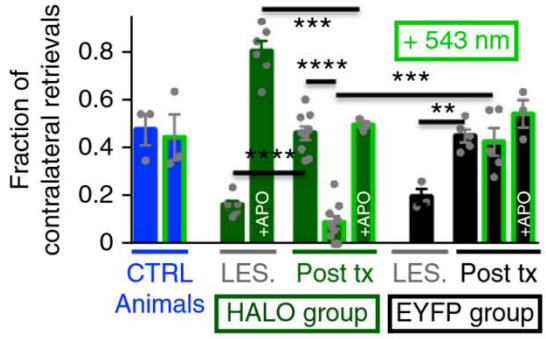

Fig. 4. Results from behavioral corridor test in the presence and absence of green light optical stimulation of grafted cells: Illumination of HALO-expressing grafted cells (POST TX) with green light (neon green bars) resulted in the display of motor deficits symptoms in mice (a strong preference for food ipsilateral to lesion). Absence of green light to these cells showed a more balanced retrieval of food on both sides of the mouse. Injection of apomorphine (APO) into tissue of the grafted cells prevented a relapse to the PD state, even in the presence of green light stimulation.

LIMITATIONS AND HURDLES

While there are many benefits to using optogenetics as discussed above, there are limitations to using this technique as well as barriers that this technique has encountered and will continue to encounter in the future. For instance, with respect to making optogenetics a reality for use someday on humans, optogenetics has been confronted by a major hurdle in regulatory organizations such as the FDA. This is mainly because foreign opsin expression in humans, except for a few cases, has yet to be approved, and for it to be granted use someday on humans, it would have to be undoubtedly proven (particularly for the FDA) that it is safe and effective, which can be a difficult and lengthy task. The difficulty in proving this is particularly true because previous studies have demonstrated that overexpression of opsin proteins can have cytotoxic effects, and it is not clear what safe levels of expression are. Furthermore, the incorporation of foreign genes into host cells could trigger an unwanted immune response [12]. This is compounded by the fact that there may be unintended consequences to optical stimulation such as tissue damage from excess heating when illuminating the cells or general physical damage from the implantation of the optical device.

Besides this, limitations exist that put into question the robustness of optogenetics. For example, it has been previously proven difficult to deliver light to regions found deep within the brain, as light tends to scatter as it penetrates through tissue. Similarly, it has been proven problematic in some studies to ensuring that light is uniformly spread over a region of interest without causing tissue damage from overheating [12].

CONCLUSION

Since its conception in 2005 following Deisseroth's ground-breaking research on control of neural cells with blue light, optogenetics has firmly established itself as one of the primary neuromodulation techniques currently used by researchers. This is because not only has it shown to have extraordinary feasibility and flexibility, it also has the advantage over other techniques of being both temporally precise and spatially accurate. Optogenetics will likely continue to have a significant impact on research for many years to come. Furthermore, it will likely continue to have an uncertain future for clinical application given hurdles like the FDA and concerns over safety with respect to its use on humans.

ACKNOWLEDGMENTS

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