

● INVITED REVIEW

Optogenetics and its application in neural degeneration and regeneration

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Abstract

Neural degeneration and regeneration are important topics in neurological diseases. There are limited options for therapeutic interventions in neurological diseases that provide simultaneous spatial and temporal control of neurons. This drawback increases side effects due to non-specific targeting. Optogenetics is a technology that allows precise spatial and temporal control of cells. Therefore, this technique has high potential as a therapeutic strategy for neurological diseases. Even though the application of optogenetics in understanding brain functional organization and complex behaviour states have been elaborated, reviews of its therapeutic potential especially in neurodegeneration and regeneration are still limited. This short review presents representative work in optogenetics in disease models such as spinal cord injury, multiple sclerosis, epilepsy, Alzheimer's disease and Parkinson's disease. It is aimed to provide a broader perspective on optogenetic therapeutic potential in neurodegeneration and neural regeneration.

Key Words: light-activated proteins; neural plasticity; spinal cord injury; epilepsy; Parkinson's disease; Alzheimer's disease; multiple sclerosis; neural engineering; memory retrieval; neuron inhibition; neuron activation; neural regeneration

Introduction

Optogenetics is a technology that combines optics with genetics to induce a precise gain or loss-of-function in cells or tissue by applying light (Yizhar et al., 2011a). This biological technique involves: 1) engineering a gene that must be delivered in a cell specific manner and expressed at adequate levels, 2) developing a mode to deliver light for *in vitro* and *in vivo* studies, and 3) detecting the effect of optogenetics (*i.e.*, dendritic density, immunofluorescence, electrophysiology, behaviour studies) (Yizhar et al., 2011a). This light-sensitive technology has revolutionized the study of neuroscience with single-cell and millisecond precision control of neurons (Deisseroth et al., 2006; Deisseroth, 2011). Accurate spatial and temporal control is especially important to a system as complex as the nervous system, containing a network of billions of cells.

Current methods for neurological treatment involve targeting the nervous system with deep brain stimulation (DBS) and pharmacologic therapy (Oluigbo et al., 2012; Connolly and Lang, 2014). While DBS provides precise temporal control of neurons, it lacks spatial specificity. On the other hand, pharmacologic therapy can give spatial specificity, but it lacks temporal control of cellular processes (Li et al., 2012). Consequently, it is challenging for the neuroscience field to create a technique with these dual features. In the early 1970s, Francis Crick postulated that the field of neuroscience needed a tool that could be used to control neurons with

cellular and temporal precision (Crick, 1979). Crick suggested light activation of neurons may be the solution to these drawbacks. It took nearly three decades to achieve what he had envisioned with the introduction of optogenetics to the study of the nervous system (Boyden et al., 2005). This review aims to briefly, yet concisely, introduce optogenetics and summarize its use as a tool to understand pathological circuitry and a possible treatment for neurological diseases.

Major Components of Optogenetics

Optogenetics works by transducing light-stimulated electrical currents directly into specific cells (Terakita, 2005). To achieve this purpose, this technique is comprised of three major components: 1) light-activated proteins, 2) light, and 3) mode of delivery.

Light-activated proteins

Light-activated proteins were first discovered approximately 40 years ago. There was the discovery of bacteriorhodopsin (proton pump) in 1971, halorhodopsin (chloride pump) in 1979 (Oesterhelt and Stoeckenius, 1971; Matsuno-Yagi and Mukohata, 1977), and channelrhodopsin-1 (proton channel) and -2 (cation channel) in 2002 and 2003 (Nagel et al., 2002, 2003), respectively. These channels and pumps called opsins are microbial 7-transmembrane domain proteins containing trans-retinal cofactors that are activated upon light exposure.

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In general, these microbial opsins have the common characteristic of directly inducing electrical currents into cells upon light activation. This feature is different from rhodopsin (opsin found in the retina of vertebrates), which indirectly transduces electrical current *via* intracellular G-proteins (Oesterhelt and Stoerkenius, 1971; Stryer, 1986).

Despite their common role as cellular electrical transducers, each of these prokaryotic opsins brings about different effects on membrane potential upon activation with light. Upon light-induced electrical transduction, some opsins such as halorhodopsin (HR) hyperpolarize the membrane potential whereas other opsins such as channelrhodopsin (ChR) depolarize the membrane potential (**Figure 1**) (Zhang et al., 2006). Halorhodopsin hyperpolarizes the membrane potential by pumping chloride ions into cells, resulting in spiking and neurotransmission inhibition, and ChR depolarizes cell membranes by allowing cations to diffuse into the cells by an electrochemical gradient, which could induce an action potential (Nagel et al., 2003; Kikukawa et al., 2015). This is especially applicable in neurons because of their electrophysiological properties of generating action potentials.

Initially, although the optogenetics method seemed revolutionary, there was a lot of scepticism about its application in neuroscience. There were concerns about whether photocurrents, which are currents induced by photons, would be too weak and slow to activate and inactivate neurons with millisecond precision. Moreover, it was thought that opsins may be toxic or not expressed at high enough levels in neurons to mediate a desired effect. Since opsins require trans-retinal cofactors for activation, it was postulated that optogenetics would require a multicomponent delivery similar to the novel molecular strategies, which were engineered at the time (Zemelman et al., 2002; Banghart et al., 2004; Deisseroth, 2011).

The challenges of optogenetics application in neuroscience were overcome by Boyden et al. in 2005 (Boyden et al., 2005). Boyden's group delivered the first opsin into neurons by transfecting cultured hippocampal cells with lentivirus containing channelrhodopsin-2 (ChR2). In their study, they showed activation of neurons within milliseconds of light stimulation, synaptic neurotransmission, and neuronal spike trains resembling normal neuron electrophysiology. A significant observation was that cell health and electrophysiology properties were not affected by ChR2 expression. Furthermore, since retinoids were found present in sufficient amounts in mature mammalian brains, this conferred optogenetics as a single-component strategy to control neuronal activity (Deisseroth et al., 2006; Zhang et al., 2006). Complementary tools that could inhibit neuronal activity were then introduced following the discovery of natronobacterium pharaonic halorhodopsin (NpHR) (Han and Boyden, 2007) and a new class of inhibitory opsins called archaerhodopsin, which are outward proton pumps (Chow et al., 2010).

Since the serendipitous application of optogenetics to neural systems, the field has vastly expanded (Boyden, 2011). However, the introduction of optogenetics to neuroscience has not been without its challenges. For example, there were challenges in increasing the cell membrane transport of NpHR since it was observed to accumulate intracellularly at high expression levels (Gradinaru et al., 2007). To increase its cell surface expression, the C-terminal endoplasmic reticulum (ER) export peptide sequence from Kir2.1 channel was added, which resulted in the synthesis of the opsin enhanced natronobacterium pharaonic halorhodopsin (eNpHR) (Gradinaru et al., 2008). Moreover, NpHR was inefficient in inhibiting neurons since it pumped one chloride ion per photon. The finding of high-resolution crystal structure of

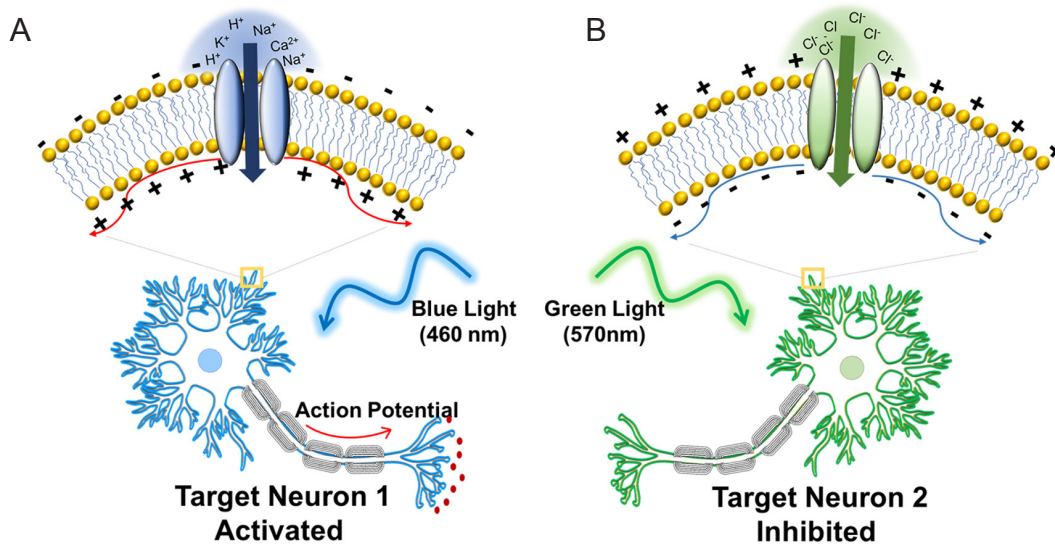


Figure 1 Optogenetic control of neural activities.

Opsins can be delivered to neurons to either activate or inactivate their activity. (A) Channelrhodopsin-2 is a cation channel, which is activated by 460 nm wavelength light. Upon activation, influx of sodium (Na⁺), potassium (K⁺), hydrogen (H⁺), and calcium (Ca²⁺) occurs into the cell and depolarizes the membrane potential. If the membrane potential reaches the threshold, this can induce an action potential to activate the neuron. (B) Halorhodopsin is a chloride pump, which is activated by 570 nm wavelength light. Upon activation, halorhodopsin pumps chloride into the cell to hyperpolarize the membrane potential, essentially inactivating the neuron.

ChR2 facilitated the reconstruction of its ion pore to conduct chloride ions instead of cations into the cell, which changed its properties to a neuron inhibitory channel and created a more efficient inhibitory opsin (Kato et al., 2012; Berndt et al., 2014; Wietek et al., 2014). NpHR also had a slow recovery rate from the inactivated state upon continuous stimulation, which was unfavourable to its application in neurons (Hegemann et al., 1985; Bamberg et al., 1993). High-throughput screening enabled the discovery of a new inhibitory opsin called archaerhodopsin-3 (outward proton pump) that mediates strong currents and spontaneously recovers from light-induced inactivation (Chow et al., 2010). With advanced understanding of opsin kinetics, structure, molecular trafficking, and optical properties, it has been possible to optimize their properties (e.g., increase membrane expression, light sensitivity, and expression safety) or modify them for diverse applications (e.g., slower or faster closing and/or opening kinetics, shifting wavelength of activation) (Zhang et al., 2008; Lin et al., 2009; Gradinaru et al., 2010; Gunaydin et al., 2010; Boyden, 2011).

Light

A major component in optogenetics is the light wavelength to activate a specific opsin. For example, ChR2 is activated by ~460 nm light and NpHR is activated by ~570 nm light. This allows the coexpression of these opsins into a neuron or more complex system to simultaneously activate and inactivate neurons (Matsuno-Yagi and Mukohata, 1977; Nagel et al., 2003). Since these two opsins work antagonistically (i.e., ChR2 activates neurons; NpHR inactivates neurons), this strategy would not be suitable if one is interested in synergistically activating two different groups of neurons. Zhang et al. addressed this issue by identifying and characterizing a cation-conducting channelrhodopsin (VChR1) with red-shifted absorption spectrum and maximum absorption wavelength ~70 nm higher than ChR2 (531 nm) (Zhang et al., 2008). Since ChR2 and VChR1 both depolarize membrane potentials and are activated by different wavelengths, it is possible to simultaneously activate different groups of neurons within a neural circuit.

One of the challenges of optogenetics is targeting deeper brain structures while minimizing invasiveness of implanting optical fibers to deliver light. A strategy to overcome this is to develop opsins with red-shifted activation wavelengths. This will reduce light scattering and allow deeper light penetration (**Figure 2B**) (Pansare et al., 2012). Recent developments in opsin engineering to address this issue include the cation channels (activation wavelengths): red-activatable ChR (ReaChR) (~590–630) (Lin et al., 2013); Channelrhodopsin-1/Volvox Channelrhodopsin (C1V1; E122T mutation; ChR1/VChR1 chimera) (~600 nm) (Yizhar et al., 2011b); Chrimson (~660 nm) (Klapoetke et al., 2014); fast red-activatable Channelrhodopsin (bReaChES) (~590 nm) (Kim et al., 2016); Volvox Channelrhodopsin (VChR1; similar to channelrhodopsin-1) (~531 nm) (Zhang et al., 2008) and the chloride pump Jaws (~635 nm) (Chuong et al., 2014). Moreover, the ChR2 gene has been modified to pro-

duce a “bistable” channel also known as step-function opsin (SFO). This channel is opened by one wavelength and closed by a different wavelength. This allows control of how long a channel can stay in the opened state (Berndt et al., 2009). Since these channels can be kept open for longer periods of time, their conduction efficiency is higher, which reduces the light intensity necessary to activate a neuron or other cells.

Mode of delivery

Opsin delivery

Similar to the genetically encoded GFP, opsins can be specifically delivered to a subset of cells. One of the delivery methods used is transfection of lentivirus or adeno-associated virus (AAV) packaged with the opsin gene, a marker such as enhanced green fluorescent protein (EGFP) or enhanced yellow fluorescent protein (EYFP) and a cell-specific promoter (e.g., calcium/calmodulin-dependent protein kinase II alpha (CaMKII α) promoter for excitatory neurons, glial fibrillary acidic protein (GFAP) promoter for astrocytes, and preprohypocretin/preproorexin (ppHcr) promoter for hypocretin neurons). Another method includes using transgenic mice to create a uniform expression of the opsin in a specific group of cells (Zhang et al., 2010; Adamantidis et al., 2014). However, some cells (e.g., parvalbumin interneurons, dopaminergic neurons) contain promoters with weak transcriptional activity, which makes it difficult to express opsins in a cell-specific manner at necessary levels on the cell membrane. To overcome this, conditional expression systems using Cre recombinase-locus of crossover in P1 (Cre-loxP) are used to increase the expression of opsins in these cells (Atasoy et al., 2008; Kuhlman and Huang, 2008; Yizhar et al., 2011a). Briefly, transgenic mice are engineered to express Cre recombinase in a cell-specific manner (e.g., in PV⁺ GABAergic neurons). A vector containing an upstream ubiquitous promoter, a stop cassette flanked by unidirectional lox-P and a downstream opsin gene is then delivered to the region of interest. In this manner, cells that uptake the vector but do not express Cre recombinase will not be able to express the opsin because of the stop cassette. However, cells containing Cre recombinase will excise the stop cassette and allow high expression of opsin under the control of a ubiquitous transcription promoter (Zeng and Madisen, 2012).

Light delivery

For *in vitro* studies, a laser or light emitting diode (LED) can be directly coupled to the microscope light path (Zhang et al., 2010). For *in vivo* optogenetic stimulation of superficial layers of the cortex (e.g., layer 5 of primary motor cortex), small LED bulbs can be implanted on thinned skull directly above the target region (Gradinaru et al., 2007; Huber et al., 2008). However, since light power density drops to as low as 1% upon 1 mm penetration of tissue, a fiber-optic-based optic neural interface has been developed in which LED or laser diode systems can be coupled to lightweight flexible optic fibers to deliver light to deeper brain tissue (**Figure 2C**) (Adamantidis et al., 2007; Aravanis et al., 2007).

One of the challenges in neuroscience research is to ob-

serve how neural activity results in behaviour changes. This requires the *in vivo* recording of neural activity, which was previously done with conventional 1 or 2-photon imaging. However, previous methods required the stereotaxic stabilization of the head, which prevents studying freely moving animals and cannot image deep structures such as the hippocampus and amygdala (Doronina-Amitonova et al., 2013; Gunaydin et al., 2014). To overcome this issue, fiber-optic imaging/recording systems, which involve fluorescence modalities such as: 2-photon, scanning confocal fluorescence, and epifluorescence have been developed (Helmchen et al., 2001; Flusberg et al., 2005; Sawinski et al., 2009). Coupled with optogenetics technology, it is possible to simultaneously stimulate and record intracellular Ca^{2+} , electrical activity, and/or fluorescent proteins in specific groups of neurons in any brain structure and observe the resulting behaviour in freely moving animals (Gradinaru et al., 2007; Miyamoto and Murayama, 2016). Szabo et al. made this possible by coupling a microscope to a fiber bundle containing a micro-objective to activate neurons and simultaneously image and record their activity (Szabo et al., 2014). This technology, which is called a fiberscope, is capable of fluorescence imaging with epifluorescence, structured illumination, or scanless multipoint confocal microscopy. Other developments have included the use of an optrode, which combines an optic fiber with an electrode to simultaneously stimulate neurons and record their activity with single-neuron resolution (Arenkiel et al., 2007; Tamura et al., 2012). Recent advances in light delivery have implemented closed-loop circuits, which allow real-time control of neurons based on error of desired and measured output, and wireless light stimulation, which does not require restraining animals during placement of optical fibers (Grosenick et al., 2015; Montgomery et al., 2015; Park et al., 2015). Similarly, a wireless optrode has been developed (Gagnon-Turcotte et al., 2017). These technologies provide a more natural environment for optogenetic stimulation and neuron activity recording (Figure 2).

Application of Optogenetics in Neuroscience and Neurological Disorders

Optogenetics could be an invaluable tool in the field of neuroscience. It induces gain or loss of function in neurons and behavior with precise control for quick and specific outcomes. Since its introduction for neuroscience application in 2005 (Boyden et al., 2005), this light-sensitive technology has vastly improved our understanding of treatment mechanism, pathology of neurological disease, neural circuitry, and physiological processes. It has been used to advance our understanding of complex processes such as: learning (Schroll et al., 2006), sleep (Adamantidis et al., 2007), vision (Farah et al., 2007), addiction (Witten et al., 2010), and movement (Chen et al., 2014; Proville et al., 2014). Moreover, it has been used to find new targets for therapy. Thus, optogenetics is a powerful tool in basic science research.

One of the challenges of optogenetics is its translatability to the clinic. Opsin gene delivery is invasive, since it would require injecting viruses into nervous tissue. Delivering light to deep brain regions would require optic fiber implantation, which may cause direct trauma. Moreover, applying light to the brain may cause heat damage. However, once these problems are overcome, optogenetics can be another tool to treat neurological diseases. Applying optogenetics to several neurological disease models such as spinal cord injury, epilepsy, Alzheimer's disease (AD) and Parkinson's disease (PD) may provide an alternative treatment with less side effects than current therapies. The nervous system is made up of complex neural networks that work with spatial and temporal precision. Therefore, the ideal treatment would restore normal physiology, and optogenetics is a capable tool. In the following session, we will focus on the application of optogenetics in these diseases and discuss the challenges of translating this technique to the clinic.

Spinal cord injury

Spinal cord injury (SCI) is a devastating traumatic event disconnecting the brain to the peripheral nervous system (PNS). Patients may suffer from urinary and bowel incontinence,

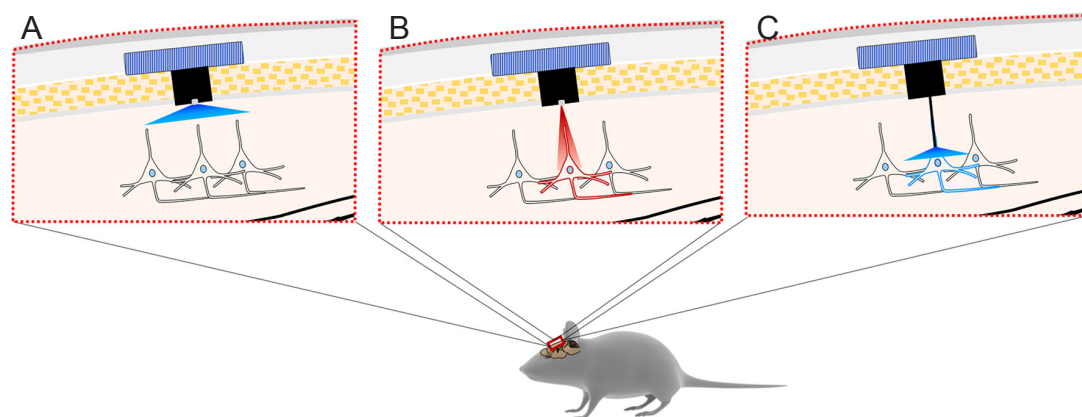


Figure 2 Improvements in optogenetics.

Wireless delivery of light allows for more natural mobility of the rodent. (A) Optogenetic stimulation using blue light. The blue light scattering may prevent control of neurons in deep locations in the brain. (B) The application of red-shifted opsins. Using light with longer wavelength to activate opsins will reduce light scattering and allow targeting of deeper brain locations. (C) An alternative strategy to target deeper brain structures. Although more invasive, optic fibers allow the targeting of deeper brain structures by implanting the fiber near the region of interest.

sexual dysfunction, and sensorimotor paralysis (Furlan et al., 2011; Simpson et al., 2012). It is a global health problem with an estimated prevalence in the range of 236–1,009 per million and costing a patient up to \$1 million in the first year and \$185 thousand every year thereafter (Cripps et al., 2011; NSCISC, 2016). Although many research efforts have advanced our understanding of pathophysiological mechanisms, new treatments have been inadequate. Unlike the PNS, the adult mammalian central nervous system (CNS) is unable to regenerate. Two mechanisms are recognized that prevent axonal regeneration in the CNS: 1) the environment following injury is inhibitory for axonal regeneration (Yiu and He, 2006; Fitch and Silver, 2008; Hackett and Lee, 2016), and 2) neurons do not have sufficient intrinsic capacity to regenerate (He and Jin, 2016).

Optogenetics is an intriguing treatment for SCI, and applying optogenetics in SCI regeneration studies has just been emerging. Upon axonal injury, there is an increase in intracellular calcium (Ziv and Spira, 1995, 1997). Calcium serves a multitude of purposes following injury including: regulating gene expression through mitogen-activated protein kinase (MAPK) and Ca^{2+} /calmodulin-dependent protein kinase (CaMK) phosphorylation of cAMP response element binding protein (CREB); activating proteases to cleave spectrin to reduce membrane tension; promoting vesicle fusion to the plasmalemma *via* Ca^{2+} dependent SNARE protein association; activating delta-like 1 homolog (DLK1) to promote retrograde signalling to the soma; and stimulating growth cone formation to promote neural regeneration (Figure 3) (Ziv and Spira, 1997; Deisseroth et al., 1998; Wu et al., 2001; Kamber et al., 2009; Ghosh-Roy et al., 2010; Watkins

et al., 2013; Hendricks and Shi, 2014). Further increase in intracellular calcium occurs through action potentials, which are induced by retrograde membrane depolarization following axon transection *via* voltage-gated sodium channels (Mandolesi et al., 2004). This depolarization is crucial for axon regeneration since studies have shown that blockade with tetrodotoxin (*i.e.*, voltage-gated sodium channel blocker) prevents neurite regeneration (Mandolesi et al., 2004). Furthermore, other studies have shown that electrical stimulation of peripheral neurons enhances neurite elongation (Brushart et al., 2002; Udina et al., 2008). However, electrical stimulation of axotomized rubrospinal neurons (*i.e.*, CNS neurons) has failed to show enhancement of neurite outgrowth (Harvey et al., 2005). This may be due to the counteracting molecules in the CNS such as myelin-associated inhibitors and chondroitin sulfate proteoglycans (CSPGs), which inhibit regeneration (Yiu and He, 2006). Interestingly, expression of constitutively active CREB in DRG neurons was sufficient to overcome myelin-associated inhibitors of axon regeneration in spinal cord axons (Gao et al., 2004). However, calcium levels are likely inadequate to alter gene expression into a regenerative state through CREB following CNS injury, since intracellular calcium elevation is transient and not significantly different than those observed during physiological burst activities (Friel and Chiel, 2008; Rishal and Fainzilber, 2010). Therefore, optogenetics using ChR2 for induction of membrane depolarization and action potentials to enhance the elevation of intracellular calcium following injury is a possible treatment option for SCI. Moreover, ChR2 is permeable to calcium ions, so it would also directly increase intracellular calcium (Nagel et al., 2003).

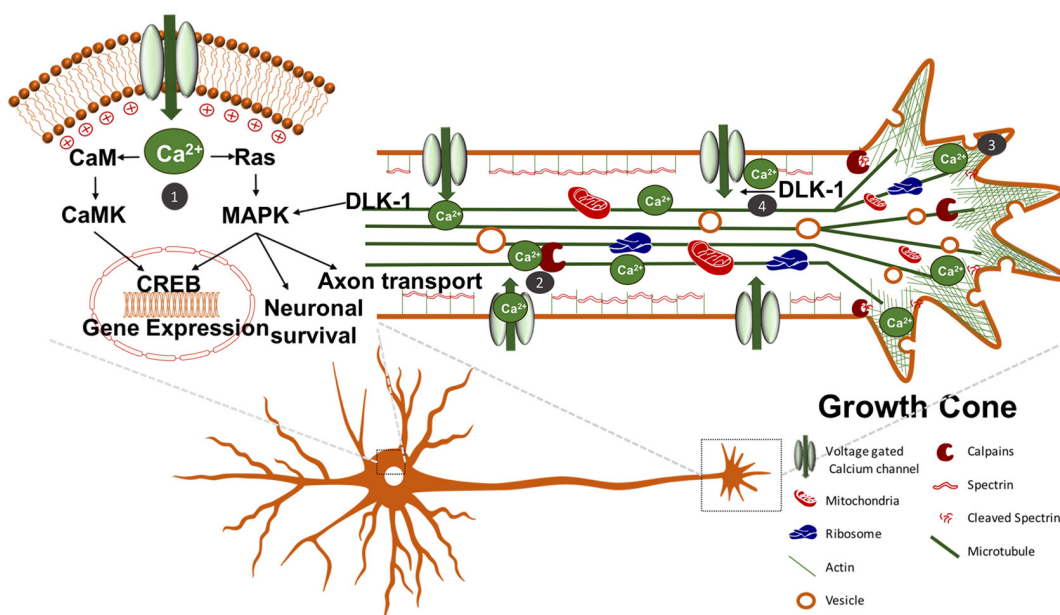


Figure 3 Augmenting intracellular calcium signalling to induce axon regeneration.

After axon transection, calcium signalling has been shown to be very important in generation of the growth cone and promoting axon regeneration through a cascade of events. Optogenetics can be used to augment calcium signalling by inducing membrane depolarization and opening voltage-gated calcium channels. Calcium 1) alters gene expression through Ca^{2+} /calmodulin-dependent protein kinase (CaMK) and mitogen-activated protein kinase (MAPK) phosphorylation of cAMP response element binding (CREB) protein, 2) activates proteases such as calpains, which then cleave spectrin and actin to reduce membrane tension to allow the membrane to seal, 3) promotes vesicle fusion to the spectrin free plasma membrane, and 4) activates delta-like homolog 1 (DLK-1), which promotes retrograde signalling to the soma to alter gene expression *via* mitogen-activated protein kinase (MAPK).

Optogenetics has been used to treat respiratory dysfunction in Sprague-Dawley rats following cervical SCI by transfecting spared motor neurons that have lost their presynaptic input. In this study, intermittent optogenetic stimulation of the phrenic motor neuron pool following cervical 2 (C2) spinal hemisection resulted in return to normal hemidiaphragm electromyography (EMG) activity in synchrony with the non-lesioned side (Alilain et al., 2008). Interestingly, long periods of intermittent stimulation caused long-lasting return of rhythmic diaphragm activity even after stimulation ceased. This was not observed in control animals, indicating optogenetic stimulation induced neuroplasticity. To support this, optogenetics has been shown to induce synaptic plasticity by long-term potentiation (LTP) of neurons in the hippocampus (Zhang and Oertner, 2007). Neuroplasticity is of paramount importance for recovery following SCI as spared pathways can be recruited to replace damaged axons (Sandrow-Feinberg and Houlié, 2015). Furthermore, neuron electrical activity, which can be augmented with optogenetics, has been shown to increase proliferation and differentiation of oligodendrocyte and neuron precursor cells, increase myelin thickness, and enhance motor skill in mice (Gibson et al., 2014).

Optogenetics has also been studied in another type of CNS injury, stroke, which is similar to SCI in which there is neuronal death and scar tissue formation (Choudhury and Ding, 2016). In a study by Cheng et al. (2014), stimulation of layer 5 primary cortex with ChR2 in the region immediately adjacent to the stroke lesion resulted in significant increase in neuroplasticity markers, cerebral blood flow, rotating beam test performance and weight gain (Figure 4). Similarly, a dif-

ferent study showed optogenetic stimulation of transplanted neural stem cells containing ChR2 promoted motor function recovery, increased expression of neural plasticity markers, and downregulated transcription of pro-inflammatory genes in a stroke model (Daadi et al., 2016). More recently, optogenetic stimulation of the lateral cerebellar nucleus (LCN) in a middle cerebral artery (MCA) stroke model improved mouse performance in rotator beam. This study also showed an increase in the neuroplasticity marker growth-associated protein 43 (GAP43) in the ipsilesional cortex (Shah et al., 2017). The successful application of optogenetics for stroke recovery suggests its therapeutic potential for SCI. Moreover, optogenetics has been combined with a flexible electrophysiology recording probe to interrogate the normal neural circuitry in spinal cord for movement of hindlimbs (Lu et al., 2017). This technology demonstrated feasible fiber probe integration into the spinal cord with minimal trauma, which shows its potential application for circuitry restoration in SCI. In summary, optogenetics has a potential to improve SCI through: 1) increasing intracellular calcium leading to activation of intrinsic regeneration mechanisms, 2) enhancing neuroplasticity of spared axons, 3) promoting axon myelination, and 4) restoration of normal circuitry. Whether optogenetics can promote regeneration of severed axons and subsequently functional recovery in SCI animal models remains to be determined.

Multiple sclerosis

Multiple sclerosis (MS) is a progressive inflammatory demyelination disease affecting several tract fibers of the CNS (Hauser and Oksenberg, 2006). Patients may present with

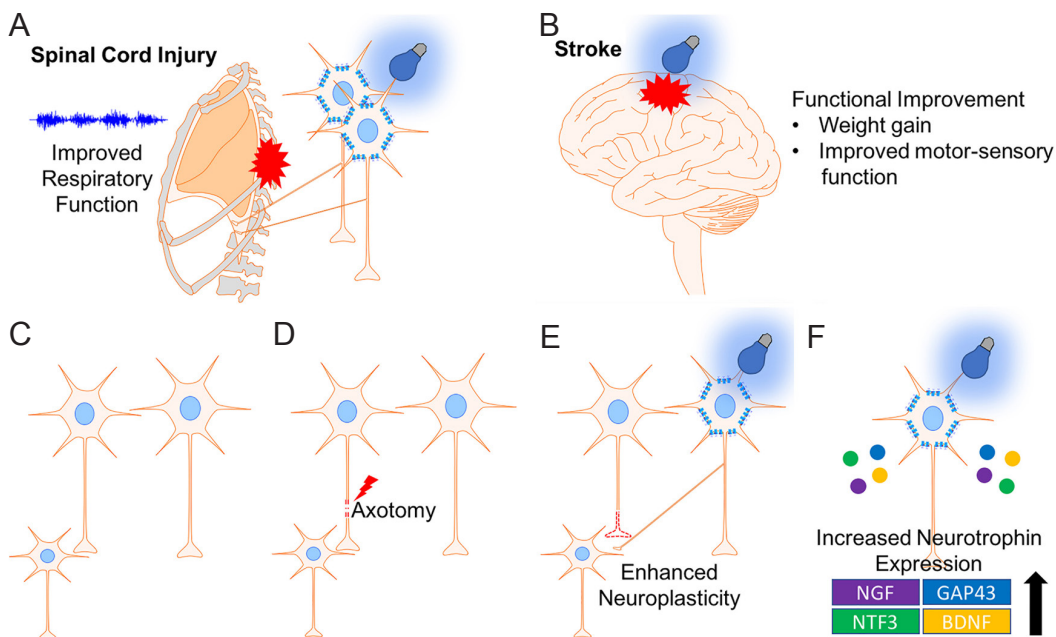


Figure 4 Optogenetics promotes recovery following central nervous system injury.

Previous studies have shown that optogenetics can be used to control denervated motor neurons controlling respiratory function following spinal cord injury (A) and aid in functional recovery after stroke (B). (C) Schematic drawing shows neural connections in the central nervous system before injury. (D) Upstream axotomy leads to denervation of distal axons. Optogenetic stimulation around the lesion site has been shown to enhance neuroplasticity (E) and increase neurotrophic factors (F) in a stroke model. NGF: Nerve growth factor; GAP43: growth associated protein 43; NTF3: neurotrophin 3; BDNF: brain-derived neurotrophic factor.

a wide range of symptoms including, but not limited to, optic neuritis, paraesthesia, motor weakness, and ocular movement dysfunction (e.g., internuclear ophthalmoplegia). Treatments have focussed on anti-inflammatory agents to reduce symptomatic episode duration, frequency, and severity (Goldenberg, 2012). However, current therapy is only partially effective and contains several side effects. An intriguing therapy for MS is to increase neuronal activity, which has been shown to increase myelination and oligodendrocyte proliferation with electrical stimulation (Li et al., 2010). However, electrical stimulation is non-specific and can cause several side effects. To overcome this, a previous study demonstrated the potential of optogenetics for MS treatment. Using ChR2 optogenetic stimulation of the premotor cortex in mouse models, Gibson et al. reported that enhanced neuronal activity increased axon myelin thickness and oligodendrocyte proliferation (Figure 5A) (Gibson et al., 2014). Optogenetics was mainly used in this study as proof-of-concept (i.e., enhancing neural activity increases myelination), establishing a target for MS treatment. Since MS is a disease of demyelination, electrical stimulation and optogenetics are potential techniques to use in this disease.

Epilepsy

Epilepsy is a disease characterized by neuron hyperexcitability causing recurrent seizures and affecting about 50 million people worldwide (Banerjee et al., 2009). Current treatments include pharmacologic targeting, vagus nerve stimulation, and DBS (Perucca and Tomson, 2011; Fisher, 2012; Orosz et al., 2014; Schmidt and Schachter, 2014; Ben-Menachem et al., 2015). However, these treatments contain several side effects including neurocognitive side effects and hypersensitivity reaction from pharmacotherapy and hallucinations and memory impairment from DBS (Walia et al., 2004; Andrade et al., 2006; Girardeau et al., 2009; Hessen et al., 2009; Yang et al., 2011). Moreover, resistance to pharmacotherapy is a major concern (Picot et al., 2008). Another challenge is that traditional DBS requires continuous stimulation of internal brain structures. Since seizures occur erratically with potentially long interictal periods, there is unnecessary DBS that may contribute to the side effects observed (Hartikainen et al., 2014). To overcome the continuous DBS, the closed-loop device NeuroPace RNS[®] System has been developed and approved for use in medication-resistant partial onset seizures (Morrell, 2011; Morrell and Halpern, 2016). This system detects seizure activity and ablates the seizure focus on-demand and has been shown to improve quality of life and to have no effect on mood and cognition (Morrell and Halpern, 2016). Although this device has temporal specificity, it lacks spatial specificity, which may alter normal brain regions (Li et al., 2012). Optogenetics may be beneficial to circumvent this problem with its high temporal and spatial resolution.

Optogenetics has been shown to be effective *in vitro* and *in vivo*. In a study by Tønnesen et al., *in vitro* optogenetic inhibition of hippocampal principal cells of regions CA1 and CA3 in a pharmacoresistant model was sufficient to reduce seizure activity (Tønnesen et al., 2009). Furthermore, in an

in vivo model of neocortical epilepsy, which is commonly pharmacoresistant (Schuele and Luders, 2008), transfection and inhibition of hyperexcitable cortical pyramidal neurons with halorhodopsin reduced seizure activity (Figure 5B) (Wykes et al., 2012). Optogenetics has also shown efficacy in thalamocortical seizures following stroke using halorhodopsin inhibition of neurons (Paz et al., 2013), temporal lobe epilepsy by activation of parvalbumin positive gamma-aminobutyric acid (GABAergic) interneurons with channelrhodopsin-2 (Krook-Magnuson et al., 2013), and for penicillin induced absence seizures by inhibition of thalamic neurons with enhanced *Natronomonas pharaonis* halorhodopsin (eNpHR) (Figure 5B) (Han et al., 2015). Moreover, optogenetics has opened insight into the role of the cerebellum in modulating temporal lobe epilepsy. In a study by Krook-Magnuson et al., a closed-loop seizure device was used to detect temporal lobe seizure activity and deliver light to lateral or midline cerebellar regions containing ChR2 or NpHR expressing Purkinje cells (Krook-Magnuson et al., 2014). This study showed that cerebellar-directed intervention can inhibit seizure activity and increase interictal period. Although optogenetics has been shown effective for treating epilepsy in animal models, translating this into the clinic is challenging, considering not all epilepsies are the same. To overcome this challenge, a study showed optogenetic activation of the deep/intermediate layers of the superior colliculus suppressed seizures originating from diverse networks including thalamocortical (absence), brainstem, forebrain (complex partial), and forebrain plus brainstem (Soper et al., 2016). Therefore, this is an intriguing target for clinical application.

Previous methods to decipher pathological neural circuitry in epilepsy include pharmacological stimulation and glutamate photo-uncaging. However, these methods are limited by long latency in the former and long-lasting effect and requirement of glutamate replenishing in the latter (Zemelman et al., 2003; Shepherd and Svoboda, 2005; Jin et al., 2006; Zhang et al., 2012). Optogenetics overcomes these limitations by its short evoked-potential latency period and fast “on-off” kinetics allowing for effective neuron stimulation with millisecond interpulse intervals (Boyden et al., 2005). In studies by Meeren et al. and Sorokin et al., optogenetics was used to understand the pathway responsible for generalized absence seizures, which was previously questioned (Meeren et al., 2005; Sorokin et al., 2017). They found that rhythmic synchronized spiking or phasic firing of thalamocortical neurons was necessary and sufficient to induce synchronous spike wave discharge (SWD) and behaviour activity indicative of absence seizures. Moreover, optogenetics was used to identify the mechanism of low-frequency DBS for the treatment of temporal lobe epilepsy. The efficacy treatment was found to be through activation of glutamatergic CaMKII α -positive neurons in the entorhinal cortex, which then activate GABAergic principal cells in the hippocampus to ablate seizure activity (Xu et al., 2016). In summary, optogenetics can be applied in epilepsy research, especially as a tool to improve our understanding of pathophysiology of different types of epilepsy.

Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive cognitive decline (Qiu et al., 2009) and the pathological markers: extracellular senile plaques made up of beta-amyloid protein and intracellular neurofibrillary tangles (Kumar et al., 2015). Most of the research has focussed on these pathological markers and memory dysfunction in later stages of AD (Selkoe, 2001, 2002; West and Bhugra, 2015). However, studies have shown aberrant synaptic phenotypes (Terry et al., 1991; Jacobsen et al., 2006), decreased dendritic spine density in the dentate gyrus, LTP impairment, and dysfunctional perforant pathway input from the entorhinal cortex to the dentate gyrus in mouse models of memory impairment prior to formation of senile plaques (Jacobsen et al., 2006). These findings have increased interest in earlier stages of AD.

A recent study by Roy et al. showed that early memory deficits in AD are a result of aberrant memory retrieval and not memory consolidation (Roy et al., 2016). In their study, early AD mouse models were used by overexpressing the delta-9 variant of presenilin-1 in combination with the Swedish mutation of amyloid precursor protein. To deduce the mechanism of memory impairment, contextual fear conditioning (CFC) was used. In their model, the unconditioned stimulus electric shock was paired with the chamber "Context A". This caused the conditioned response "freezing". Placing control mice in "Context A" elicited freezing response, but early AD mice did not freeze after placement in "Context A" because of memory impairment. However, following optogenetic activation of engram cells (*i.e.*, neurons holding traces of specific memory) in the dentate gyrus with ChR2, mice responded with freezing even in a different environment "Context B". This showed that memory impairment in early AD is not the result of impaired memory storage but dysfunctional memory retrieval. Moreover, this study also showed the potential of neuron stimulation in the treatment of AD. As mentioned above, reduced dendritic spine density in the dentate gyrus is observed in early AD. Remarkably, optogenetic induction of LTP between the perforant path synapse and dentate gyrus engram cells recovered spine density to control levels, and this correlated with improved long-term memory (**Figure 5C**) (Roy et al., 2016).

Optogenetics has been used to clarify the causal relationship between synaptic plasticity and memory encoding, which was previously challenging to elucidate (Stevens, 1998). In a study by Nabavi et al. (2014), memory from cued-fear conditioning was inactivated and reactivated by long-term depression (LTD) and LTP, respectively. This was observed by conditioning mice to reduce lever pressing (conditioned response; CR) by pairing the unconditioned stimulus (US) "foot shock" with blue light stimulation (conditioned stimulus; CS) of oChIEF-(mammalian-codon optimized variant of ChIEF; ChIEF is a variant of ChEF with isoleucine 170 mutated to valine); ChEF is a chimera of channelrhodopsin 1 and 2) expressing neurons in the medial geniculate nucleus projecting to the lateral amygdala, which is an essential pathway for fear conditioning (Repa et al., 2001). After

the training phase, mice showed reduced lever pressing upon blue light stimulation, which indicated successful conditioning. Interestingly, pairing light stimulus with the foot shock resulted in LTP, which was detected by measuring the ratio of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to N-methyl-D-aspartate (NMDA) receptor influence in optogenetically driven synaptic responses in amygdala slices. Furthermore, optical induction of LTD caused the loss of conditioned response to blue light stimulation, and this response was reactivated by inducing optical LTP. Therefore, this study showed that memory can be inactivated and reactivated using LTD and LTP, respectively, and clarified the causal relationship between these synaptic processes and memory. Collectively, optogenetics was mainly used as a tool to elucidate memory encoding in animal models. In addition, it was used as a proof-of-concept to show that stimulation of dentate gyrus engram cells in AD mice restores spine density, which correlated with memory retrieval.

Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease affecting 1–2% of the population above 60 years. It is characterized pathologically by loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and clinically by bradykinesia, cogwheel rigidity, resting tremor, and shuffling gait (Lang and Lozano, 1998; Tanner and Aston, 2000; Dauer and Przedborski, 2003). Current therapies have been focussing on improving symptoms and quality of life but have not been successful in reversing the disease progression (Connolly and Lang, 2014). The two main therapies used for PD are pharmacotherapy and DBS. However, these therapies have many potential side effects such as: nausea, constipation, orthostasis, psychosis, impulse control disorders, depression, and mania (Weintraub et al., 2006; Temel, 2010; Connolly and Lang, 2014). Moreover, medication-associated resistance and dyskinesia may develop following long-term therapy with levodopa, which is one of the most effective treatments for PD (Fahn, 2000; Aquino and Fox, 2015). DBS is an effective treatment option for patients with insufficient response to pharmacotherapy and/or develop dyskinesia. However, this treatment is invasive since it requires a surgical procedure to implant electrodes into deep brain nuclei (Okun, 2012). Moreover, since DBS requires electrical stimulation, it is a non-specific targeting treatment (Li et al., 2012).

Optogenetics provides more specific neuron targeting with high temporal resolution. This light-guided technology has been used in the PD field to study movement, dopaminergic neuron transplantation and pathological circuitry. To understand the role of dopamine in movement, Howe and Dombeck used 2-photon intracellular calcium (by expressing green fluorescent protein (GFP)-Calmodulin fusion protein 6f (GCaMP6f) in dopaminergic neurons) recording of dopaminergic neurons (DA) projecting to the dorsal striatum during initiation of movement, acceleration, and resting on cylindrical treadmill (Howe and Dombeck, 2016). The pattern recorded was then simulated with ChR2 optogenetic

stimulation of the dopaminergic neurons. Their findings suggested DA neurons may not be all that are required to initiate or accelerate movement. However, DA neuron input to the dorsal striatum may be required for motivation and modulation of movement. This study suggests application of time-precision firing in the transplanted DA neurons for PD treatment may increase its efficacy.

Dopaminergic neurons derived from human embryonic stem cells have been successfully transplanted in rodent and non-human primate PD models (Kriks et al., 2011). Using optogenetics with eNpHR3.0 to interrogate the mechanism of efficacy, transplanted DA neurons were found to release dopamine to the striatum and enhance medium spiny neuron excitatory post-synaptic potentials (EPSPs) through modulation of glutamatergic transmission. This is similar to physiologic dopaminergic neuron function (Steinbeck et al., 2015). A more recent study using *Drosophila* larvae demonstrated that enhancing DA neuron activity promoted recovery of PD symptoms (Qi et al., 2017). Therefore, utilization of an excitatory opsin (*i.e.*, ChR2) to activate transplanted DA neurons may enhance this treatment's efficacy through increasing the release of dopamine (Figure 5D).

Optogenetics has also been used to understand the therapeutic mechanism of DBS for PD. In a study using transgenic mice expressing ChR2 under Thy1 promoter, the mechanism of DBS was determined to be through activation of afferent neurons projecting from layer 5 of the cortex to the subthalamic nucleus (Gradinaru et al., 2009). One caveat to this study was that all layer 5 neurons contained ChR2, and since not all layer 5 neurons project to the subthalamic nucleus, it was not clear whether the behavior improvement was exclusively through the subthalamus projecting fibers

or fibers projecting elsewhere. Moreover, the region of the cortex providing therapeutic benefit was unclear. To clarify this role, Sanders and Jaeger (2016) injected adeno-associated virus constructs containing wheat germ agglutinin-Cre recombinase (WGA-Cre) in the subthalamic nucleus to retrogradely express Cre recombinase (Cre) in the projections to the subthalamic nucleus followed by injecting a Cre-dependent hChR2-EYFP construct in layer 5 of primary motor cortex (M1). This resulted in hChR2 expression in M1 layer 5 neurons projecting to the subthalamic nucleus. Optogenetic stimulation of afferent STN in M1 resulted in improved bradykinesia and hypokinesia, which clarified that these neurons mediate DBS efficacy in PD.

Optogenetics has also been used to study the neural circuitry involved in PD and levodopa-induced dyskinesia (LID). In a study by Kravitz et al., ChR2 was used to either activate the direct (D1) or indirect (D2) pathway using ChR2 (Kravitz et al., 2010). They found stimulation of the indirect pathway with ChR2 in mice causes bradykinesia, freezing, and decreased locomotion whereas stimulation of the direct pathway rescued these deficits. Optogenetics has also been used to study dyskinesia, which is an undesirable side effect from levodopa therapy. Hernandez et al. showed simultaneous stimulation of the medium spiny neurons of the direct and indirect pathway induced dyskinesia with levodopa (Hernandez et al., 2017). Moreover, a different study showed striatal cholinergic neurons also play an important role in regulating LID (Bordia et al., 2016). Interestingly, optogenetic inhibition of subthalamic nucleus was shown to be effective in treating LID in rat PD models (Fahn, 2000; Yoon et al., 2016). Collectively, these studies demonstrate optogenetics application to understand

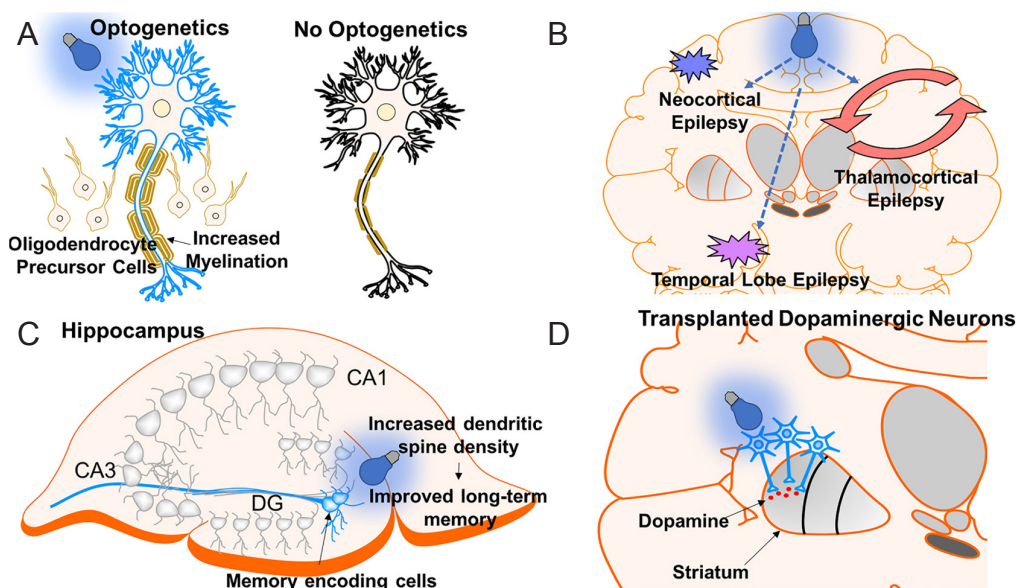


Figure 5 Application of optogenetics in other neurological disorders.

(A) Enhancing neuronal activity with optogenetics has been shown to increase myelination and oligodendrocyte precursor proliferation. (B) Optogenetics has been used to treat neocortical, thalamocortical, and temporal lobe epilepsy in rodent models. (C) Activating dentate gyrus memory engram cells in AD mouse models with optogenetics has been shown to improve long-term memory by increasing dendritic spine density. (D) Optogenetics is capable of controlling dopamine release into the striatum from transplanted dopaminergic neurons in Parkinson's disease.

current therapies in PD and to dissect pathological and physiological movement circuitry. Moreover, optogenetics has potential application for transplanted DA neurons to stimulate their release of dopamine.

Challenges and Future Direction

A major obstacle in the application of optogenetics is the translation from bench to patient, specifically the delivery of optogenetic tools in patients. It is an invasive treatment, requiring implantation of an optic fiber into brain tissue to supply light to activate opsins in deep regions. This implantation poses two potential risks: 1) overheating from light can induce damage to tissue, and 2) direct introduction of foreign objects into brain may cause tissue scarring and can become the source of infection by introducing microbes into the CNS.

To ensure successful clinical optogenetics application one must not overlook the opsin expression level in the targeted neuron. Several factors must be taken into consideration: 1) a non-invasive method to express opsins in specific targeted neurons, 2) a method to evaluate opsin expression level in specific targeted neurons, and 3) the potential risk of opsin, a foreign protein antigen, as autoimmune agent in patients. Currently, animal studies use viral transfection and transgenic mice. However, these techniques may raise technical and ethical issues for human application.

Despite the abovementioned challenges, progress has been made in optogenetics translational studies. Optogenetics has been safely and effectively applied to awake non-human primate rhesus macaques (Han et al., 2009, 2011). Clinical trials in phases I and II using adeno-associated virus injection have successfully demonstrated its safety in patients (Bartus et al., 2013; Murphy and Rabkin, 2013; Simonato et al., 2013). Another remarkable translational effort was the first patient dosed with optogenetics for retinitis pigmentosa in a clinical trial conducted by RetroSense Therapeutics, which was reported in 2016. Similar to retinitis pigmentosa, there are many other diseases which do not have effective treatments or have treatments that are intolerable. Optogenetics has not only helped us discover the pathophysiology of many neurological diseases, but has also opened the door to use this as a treatment option.

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